Skeletal Muscle Compliance and Composition in Resistance Trained and Non-Trained Men

by

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Skeletal muscle structure and function are inextricably bound as the mechanics of force generation and transmission are linked by the structures that connect muscle proteins to bone. Muscle force generating capacity is commonly cited as the hallmark of muscle function, but musculotendinous stiffness, at the whole muscle level, is an aspect of muscle that can either enhance or diminish performance. **Purpose:** The purpose of this study is to identify and understand musculotendinous properties in young adult males and compare those results to the musculotendinous properties in young adult women while providing further justification for the use of digital palpation in clinical settings. Therefore, our primary hypothesis is that activity will do more to affect stiffness than biological sex. The effect of stiffness on rate of torque development was evaluated as a determiner of performance between trained and untrained individuals. **Methods:** In the present study, resistance trained (RT) young men (n = 8) and non-trained (NT) young males (n = 3) were recruited. B-mode ultrasonography (US) was used to measure muscle composition and thickness. Active stiffness at the patellar tendon (PT) and vastus lateralis (VL) was measured during active contraction with US

and digital palpation. Digital palpation was only used for passive stiffness measurements. **Results:** RT men displayed a greater passive stiffness at the VL ($p = .01$) and visually displayed greater passive stiffness at the PT but did not portray statistical significance ($p = .07$). Additionally, active stiffness in the VL ($p = .61$) and PT ($p = .78$) were not different between groups. Absolute rate of torque development also showed no significant increase in the RT group ($p = .94$). Each volunteer's rate of torque development was then fitted to their personal maximum voluntary isometric contractions (MVIC). With this adjustment, RT individuals presented a decrease in their rate of torque development (RTD) but there was still no significance ($p = .19$). **Conclusion:** Activity does not do more than biological sex to affect stiffness. Overall, this study shows that the contributions to muscle performance is complex and cannot be limited to a single variable.

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Table of Contents

List of Figures

List of Tables

Background

Non-invasive clinical assessments of musculotendinous mechanical properties tend to use manual palpation, the practice of using a practitioner's hands to diagnose a disease or illness. Most typically, manual palpation of musculotendinous tissues is used to detect altered muscle stiffness or laxity. However, these techniques are subjective and associated standards are subject to variation by practitioner and level of expertise. More objective assessment tools such as ultrasound require dedicated clinical space, are less portable and typically require a large monetary investment. However, recent advances in the objective assessment of tissue mechanical properties may obviate these concerns. Myotonography, or "digital palpation" is a method by which muscle stiffness can be measured through the use of a mechanical probe (MyotonPro, Estonia). This device measures the biomechanical properties of the underlying tissue by delivering a pulse and recording the resulting deformation (1). Digital palpation and ultrasound (US) measurements can be compared to provide a thorough investigation of muscle stiffness and composition while also presenting a convenient and relatively inexpensive alternative to manual palpation techniques. For example, when taking the vastus lateralis (VL) into consideration, digital palpation can be used to measure passive stiffness since no movement is required. US would then be placed onto the muscle to elicit an image through which muscle thickness, echogenicity, and subcutaneous adipose tissue thickness could all be acquired. The combination of these variables could provide a comprehensive evaluation of muscle stiffness and composition. In addition, muscle stiffness and composition have been compared between male and female individuals to determine sex related differences in this context, but indirectly (1). Given

the musculotendinous stiffness results found in women, further analysis in males is implied (Mongold et al, in prep). Thus, there is a need to determine the muscle mechanical properties in males, while also establishing the differences, if any, between sexes.

Digital palpation has been used in experiments to determine muscle stiffness and composition. To validate digital palpation and explore its potential connection to muscle contractile performance, several studies have been performed using the MyotonPro (1–3). Sohirad et al. compared digital palpation to a piezoelectric accelerometer and found the MyotonPro to be capable and reliable in quantifying the stiffness of the Achilles tendon. However, it is worthy to note that the MyotonPro did present statistically different data when an increased amount of skin laying over the tendon was present. This may indicate that the data collected from these individuals may reflect more than musculotendinous stiffness (1). In conjunction, Davidson et al. also found the MyotonPro to be reliable when measuring the stiffness of the thenar and perineal muscles (2). To gain reputability in the use of the MyotonPro in muscles other than those in the lower leg, Chen et al. highlighted digital palpation's ability to quantify the stiffness of the vastus lateralis (VL) and patellar tendon (PT). Since the VL and PT are vital contributors to daily activities, this study was aimed at confirming an effortless method that measured these aspects and found the MyotonPro to be reliable in its measurements (3). To complement the results found by the MyotonPro, US imagining is used to determine the muscle composition of the VL and PT elongation as found in Malliaras et al. (4). The strength and torque of the VL is quantified using the Biodex System 3 Dynamometer. After a series of maximal voluntary isotonic contractions, the

fatigue of isotonic power and isometric contractile properties are assessed (5). Contractile elements, on the molecular level, can be further studied and understood through immunohistochemistry (IHC). IHC allows different fiber isoforms to be identified within skeletal muscle, which increases and assists in the understanding of why the VL behaves in the manner that it does (6).

Muscle is divided into several levels before getting down to its molecular components. First, the whole muscle contains fascicles each composed of 20 to 60 muscle fibers. These muscle fibers are wrapped by the sarcolemma and contain myofibrils. Each myofibril is composed of thousands to 100s of thousands of small muscle cells known as sarcomeres. The sarcomere contains filamentous proteins actin and myosin which comprise thin and thick filaments, respectively. Within each sarcomere, the components actin, myosin, troponin, and tropomyosin all contribute to the shortening of the sarcomere through cross-bridge cycling and generates force. This is the process by which muscle contracts using actin and myosin. Upon the release of neurotransmitters from an efferent neuron, acetylcholine binds to a receptor within the neuromuscular junction. This action initiates an action potential through the muscle allowing calcium to be released from the sarcoplasmic reticulum. Calcium then binds onto the troponin and causes a conformational shift in tropomyosin, exposing myosin binding sites on actin. The myosin head then binds to actin and performs a power stroke, pulling on actin. After the completion of the power stroke, ATP binds onto myosin and releases it from actin. The hydrolysis of ATP by myosin allows it to reset for the next cross-bridge cycle (7). These components and proteins of the sarcomere

may be altered by various metabolic processes, which could affect stiffness at the whole muscle.

There are a few other molecular elements that contribute to the stiffness of the muscle and one of those is titin. Titin is the largest protein found in the body, spanning from the Z-disc to the M-line within a sarcomere. To regulate stiffness, posttranslational modifications (through phosphorylation) occur to various sites within the I-band regions N2-B and PEVK allowing the protein to become more compliant or stiff. Titin then behaves much like a spring and alters the elasticity of the sarcomere. It has been suggested that an increase in titin stiffness helps maintain structural integrity of exercised muscle tissue and could improve performance (8). Titin has also been suggested to be calcium sensitive. When calcium binds to titin, it increases the binding of titin to the thin filament and shortens the length of titin. This action may increase the stiffness of the sarcomere because less of the titin is able to stretch, essentially making it more taut (9).

Another element that could explain the stiffness seen at the whole muscle is collagen. It has been found by Nkechinyere and colleagues that estrogen decreases tendon stiffness by directly inhibiting lysyl oxidase and decreasing collagen cross linking, which leads to the assumption that women are more prone to injury (10). What was interesting is that collagen synthesis was found to be increased, despite the decrease in tendon stiffness. Collagen, being a part of the extracellular matrix, is thought to be a contributor to passive stiffness (11–14). Despite the abundant research indicating that an increase in collagen concentration increases the stiffness of the tendon, estrogen still presents a significant role. Further, Tas et al. demonstrated a significantly higher PT

stiffness in males than in females, potentially indicating higher rates of collagen synthesis in males. In addition, PT stiffness was lower in obese individuals, indicating a potential difference between resistance trained and non-trained individuals (15). In men, resistance training has been observed to increase the cross-sectional area of the quadriceps muscle and PT tendon stiffness (16). These data suggest that tendon mechanical and material properties are related to loading history. In support of these findings, we recently compared muscle stiffness, assessed through multiple approaches (US and digital palpation) in healthy young adult females with varying physical activity levels. We found enhanced stiffness measured through both US and digital palpation in resistance trained volunteers when compared with their sedentary counterparts (Mongold et al, in prep). However, stiffness measured via digital palpation did not correlate with US based measures, and only digital palpation predicted key indices of muscle contractile function, suggesting this approach may not only improve ease of use and access, but also provide additional clinical insight when compared with more traditional measures of tissue stiffness.

Digital palpation has demonstrated its utility in various clinical settings. It has been able to accurately quantify the stiffness and tone of various muscles (1–3). However, muscle stiffness and tone explain only one aspect of VL musculotendinous properties. When paired with US and Biodex measures, muscle composition and function are also quantified (4,5). Through the compilation of stiffness statistics from digital palpation, muscle composition from US, and muscle function from the Biodex, a wholistic and complimentary body of data can be used to explain contractile performance achieved. Previous studies have shown in men and women that resistance

trained individuals have enhanced stiffness in the VL and PT (13,17,18). Digital palpation also affirms this increased stiffness in young women (Mongold et al, in prep). However, digital palpation has not been used to compare the musculotendinous properties of resistance trained individuals to sedentary individuals in young men. Further, these properties have not been compared between young men and young women. Based on studies used to determine the passive stiffness of the VL and PT, men have shown a higher passive stiffness than women, but that is still to be confirmed in digital palpation. Therefore, further research is required to uncover whether the same results are seen in men as those seen in women and if there will be distinguishable measures in the musculotendinous properties between men and women through digital palpation.

Introduction

The composition and mechanical properties of muscle tissue play a vital role in locomotion and manual tasks. Specifically, physiological cross-sectional area largely determines force generating capacity and can alter physical function (19). In addition to muscle size, muscle and tendon compliance have been shown affect function, including rate of torque development, power development and even jump height. Beyond these performance considerations, musculotendinous stiffness is associated with an increased risk of muscle strains and tendon tears (20). In comparison, a decrease in muscle strength and tendon stiffness has been associated with poor balance recovery and joint stability in older adults (21). However, various stimuli including disease, age, and physical activity all alter stiffness and impact muscle composition (12,22,23). These findings suggest a possible relationship between stiffness properties and muscle composition in response to similar stimuli. However, while previous studies have demonstrated a link between physical activity (resistance exercise training) and tendon stiffness, they have not interrogated the potential role of biological sex on these factors. Further, we are not aware of any studies comparing multiple approaches to assess tendon and muscle stiffness in the same cohort. Therefore, we propose to assess muscle and tendon stiffness in young adult males, separated by training status, via multiple modalities to compare with an existing and complementary dataset in females.

To specifically assess stiffness, two measures will be used, ultrasound (US) and digital palpation. US has long been the gold standard for determining skeletal muscle architecture and has been proven to reliably measure aponeurosis displacement and tendon elongation during locomotion, jumping, cycling, and single joint rotational tasks

(24). A newer approach, known as digital palpation, will be compared to US as a resource and time-efficient alternative. B-mode US uses soundwaves outside the audible spectrum to create 2d images based on variations in tissue density under the probe. This non-invasive imaging technique has a myriad of clinical applications, but for our purposes, it can be used to visually track tissue displacement during active muscle contraction. By measuring tissue load and displacement concurrently, tissue stiffness can be assessed (24–26). Digital palpation, by contrast, measures tissue stiffness under resting conditions through surface compression by eliciting an impulse onto the muscle and tendon through a probe (~2mm in diameter). Based on the oscillations received from the viscoelastic properties of the tissue, the MyotonPro uses the ratio of the resistive force and the deformation of the tissue to characterize the stiffness (8, 9). When compared to shear wave elastography (another US based approach to stiffness measurement), digital palpation has proved to be reliable and valid (26). However, it has not been demonstrated that digital palpation is sensitive to sex and habitual physical activity-based differences in tissue stiffness, nor has it been used to predict stiffness-based variation in physical function.

The purpose of this study is to identify and understand musculotendinous properties in young adult males while providing further justification for the use of digital palpation in clinical settings. The stiffness and composition data acquired from this study would then be compared to the data found in young and old women to determine any sex and age-related differences.

Methods

For this study, 11 healthy, young men have been recruited. Passive and active musculotendinous stiffness and composition of the vastus lateralis (VL) and patellar tendon (PT) were evaluated based on the criteria explained below. After screening, 8 volunteers were been placed into Resistance Trained (RT) group and 3 volunteers were placed in the Non-Trained (NT) group.

Volunteers:

To categorize each individual subject, all volunteers underwent a health screening interview before being integrated into the study. The variables that were assessed were medical history, orthopedic limitation, endocrine disease, myopathies, dietary/smoking habits, and resistance training history. If a volunteer exhibited any impairment that results in the inability to perform the tasks of this study, they were excluded. Volunteers in the RT group had to have been actively participating in habitual lower body resistance training and have had at least a 1-year history of training at least 3 times per week. To be designated to the NT group, individuals were required to have not participated in any formal resistance training in the last year but are allowed to be recreationally active. Lastly, if approved, participants were instructed to not participate in any exercise 48 hours before the study.

Data Collection:

All volunteers visited the lab on one occasion and all measurements will take place on the Biodex System 3 Dynometer (Biodex Medical Systems, Shirley, NY). The

volunteer was seated with the dominant leg flexed at 90° to allow an extension of 180°. Knee extensor (KE) mechanical characteristics were measured during passive and active conditions. In addition, muscle composition was measured during the passive conditions of this study.

Muscle Morphology. To attain VL muscle composition measurements, three images were taken with US (Philips iE33; Philips, Andover, MA). These images were then used to acquire subcutaneous adipose tissue thickness (SAT), echogenicity (intramuscular fat), and muscle thickness. These aspects assist in determining what the muscle is made up of and how it may be contributing to performance and stiffness.

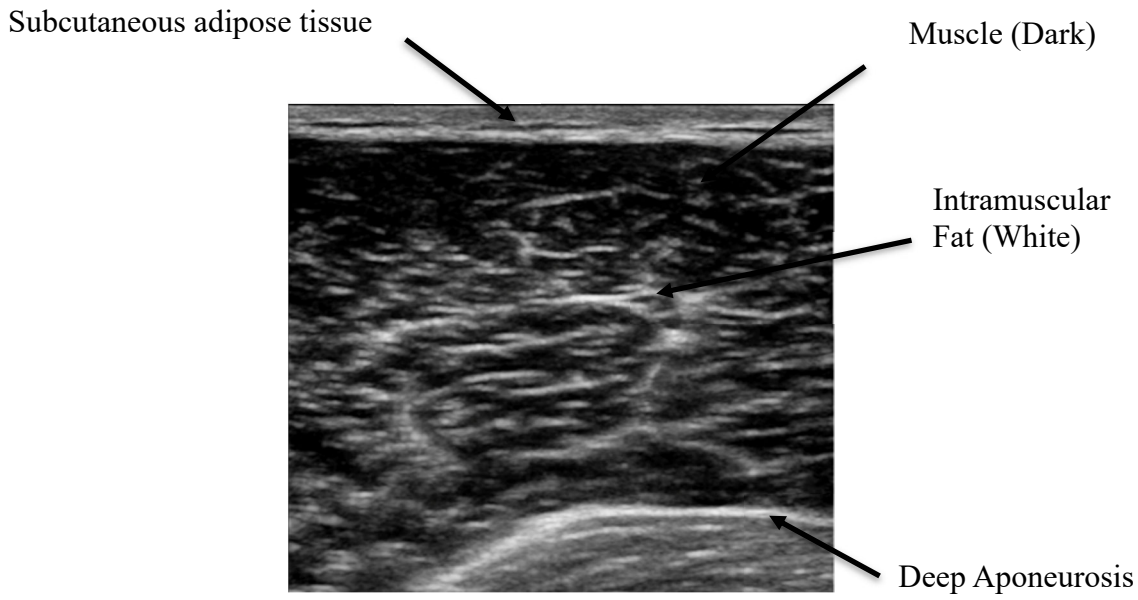

Figure 1. Image portraying the location of the subcutaneous adipose tissue (top left), muscle (top right), echogenicity (middle right), and deep aponeurosis (bottom right) on an ultrasound image.

Passive Stiffness Measures: All of the passive measures were obtained before any voluntary contraction took place. Passive stiffness measurements were assessed using digital palpation (Myoton Pro, Myoton Estonia) in the same location as the US

images. Similarly, the MyotonPro was used to attain the passive stiffness measurements of the PT. To acquire passive stiffness, the MyotonPro elicits a small pulse onto the surface of the skin. Based on how much the tissue displaces based on the initial pulses, a stiffness value is calculated by the MyotonPro.

Active Stiffness Measures: After completing passive stiffness measurements, a US probe holder was mounted to the distal thigh. While the probe is mounted, participants completed three maximum voluntary isometric contractions (MVIC), each lasting five seconds with one minute between contractions. For all the contractions, an analog signal was converted to digital with an A/D converter (CED, UK) torque was acquired from the Biodex. A stripe of surgical tape was placed on top of the PT to mark the location where the measurement was taken. The hypoechoic tape blocked US waves from being transmitted into the body leading to a shadow-like display. This shadow was then used as maker for displacement during analysis. The data was analyzed through Spike2 software (CED, UK) in real time and allowed the volunteers to be encouraged while performing maximal and submaximal contraction efforts during MVIC through visual feedback. US video acquisition and MyotonPro measurements was taken simultaneously at the VL and PT during contractions. To obtain active stiffness at the VL and PT, the displacement of the deep aponeurosis and the displacement of the tip of the patella from the hypoechoic tape were respectively measured through the use of US video acquisition while they completed ramped isometric knee extensions at 25%, 50%, and 100% MVIC.

After the conclusion of these data collections, the probe was removed and attached to the flexed knee. While secured to the knee, US video acquisition was acquired while the volunteer completed ramped isometric contractions at 25%, 50%, and 100% MVIC. The probe was then removed, and the volunteer performed an additional set of ramped isometric contractions at 25%, 50%, 75%, and 100% MVIC while MyotonPro measurements were taken at the PT during each contraction. These measurements determined the mechanical properties of the PT.

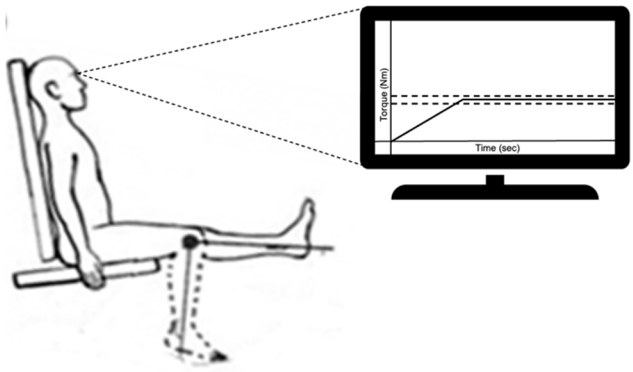

Figure 2. Image portraying the position of the volunteer and the visual feedback received during MVICs.

Ultrasonography:

US images and videos were taken with a B-mode imaging device and a linear-array US probe transducer during each set with specific settings that are appropriate for attaining muscle composition, aponeurosis displacement, and PT elongation. The US images were analyzed through ImageJ software and muscle composition were assessed through the use of computer-aided gray-scale analysis, also known as echogenicity. Echogenicity was used to determine the amount of intramuscular fat (white spots within

the muscle on the US image). A greater echogenicity indicates a greater intramuscular fat concentration.

Tissue Tracking Analysis:

Using Ultratrack software, aponeurosis displacement and tendon elongation US videos were processed by tracking the deep aponeurosis frame by frame of moving images. To attain these measurements, a pre-contraction segment was placed onto the image taken by the US. The pre-contraction segment was determined by placing one endpoint along the deep aponeurosis on the rightmost edge of the acquired video and a second endpoint was placed to the left of the first endpoint before any contraction had taken place. Therefore, the straight line between these two points was designated as the pre-contraction segment. These two points, as well as the distance between them, was tracked during the contraction. The aponeurosis displacement was measured by taking the difference of the displacements for the endpoints. As for the elongation of the PT, one endpoint was placed at the edge of the surgical tape and the second endpoint was placed on the distal edge of the patella before any contraction had taken place. PT elongation was calculated by subtracting the pre-contraction segment length (taken at steady-state) from the mid-contraction length. Likewise, Ultratrack video was used to determine the tendon elongation of US videos. All of the acquired data from Ultratrack was exported into MATLAB to be further analyzed using a custom program to obtain the measurement of displacement during the transition from passive to active conditions.

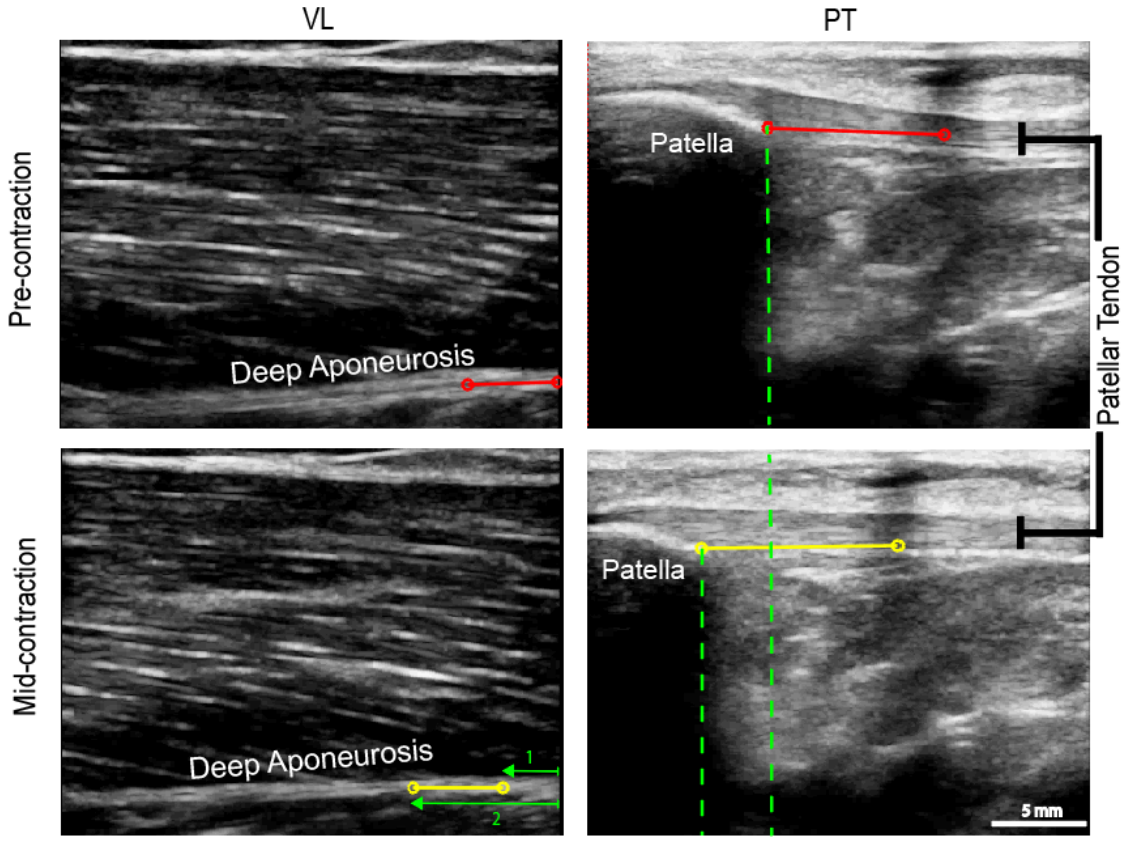

Figure 3. Image of acquired videos at the VL (left) and PT (right) in Ultratrack during tissue tracking. Red segment displays two arbitrary points placed before contraction. Yellow segment displays the same two arbitrary points mid-contraction. Green arrows and lines denote the total displacement.

To attain the force of the PT, the KE torque was divided by the length of the PT moment arm (4). The length of the PT moment arm was given as a fixed value of 44.7mm from previous studies (1, 2). Using the attained variables, stiffness was calculated using the following equation:

$$Stiffness = \frac{\Delta Force\ (N)}{\Delta Tissue\ deformation\ (mm)}$$

This approach was used with the acknowledgment that it does not measure true Young's Modulus as tissue cross section, and thus tension, is not included. The stiffness

of the aponeurosis was identified by US and calculated using the aponeurosis displacement during 25%, 50%, and 100% MVIC contractions. Previous studies have reported that the rate of torque development during 100% MVIC resulted in unreliable data and were not used to calculate aponeurosis stiffness (Mongold et al, in prep). Instead, displacement measurements relied solely on 50% MVIC contractions. However, in contrast, tendon stiffness by US proved to be reliable using all 25-100% tendon elongation measurements.

Muscle Function Analysis:

Raw torque data was acquired from isometric knee extensions through the auxiliary output of the dynamometer and recorded through a Micro 1401 analog-to-digital converter using Spike 2 software. As stated previously, data was collected live and displayed on a television monitor in front of the volunteer, which allowed for visual feedback during contractions. During data collection, the volunteer was instructed to contain their KE effort by staying in between two displayed bands representing ± 5% of the target torque for a duration of 5s. All raw torque data was processed into MATLAB and then analyzed using a custom code to identify the peak torque and rate of torque development (RTD) during MVICs. To determine the peak torque, the three MVICs were averaged. Peak RTD was calculated by finding the maximal instantaneous rate of change in torque over time. Similarly, the RTD was average based off the three MVICs.

Statistics:

Two-way ANOVA (sex/training status) was used to examine group differences. Where sex by training interactions were observed, post-hoc tests were used to distinguish differences between means. Levene's Test was used to assess equality of variances. Significance was deemed as having a $p \leq 0.05$. Pearson correlations was used to assess the relationship between morphological, composition, and stiffness-based measures. A stepwise multiple regression analysis was used to evaluate the predictability of passive stiffness, active stiffness, and relative rate of torque development. The importance of a regression analysis lies in its ability to predict the value of a specific variable to the value of another variable. So, an association between two variables can be evaluated. All data was analyzed using SPSS Statistics from IBM. The output of this analysis provides an R^2 value and then the rank included variables based on their ability to improve prediction.

Results

Participants. RT and NT men and women were similar in height, weight, and BMI. Various other relevant participant characteristics can be found in table 1.

Variables	Men (n = 11)	Women (n = 7)	p-value
Height (cm)	183.99 (8.82)	163.02 (2.83)	-
Weight (kg)	82.62 (13.25)	63.19 (6.13)	-
BMI (kg/m^2)	24.29 (2.50)	23.78 (2.22)	-
Muscle Thickness (cm)	2.39 (.44)	2.12 (0.31)	0.23
Corrected Echogenecity (AU)	23.78 (14.16)	58.00 (13.72)	0.02*
SAT - VL (cm)	0.26 (0.23)	0.80 (0.23)	0.02*
Passive Stiffness VL (N/m)	389.55 (76.23)	307.29 (22.62)	0.14
Passive Stiffness PT (N/m)	860.42 (149.09)	831 (117.57)	0.61
Active Stiffness VL (N/mm)	268.90 (76.58)	154.69 (87.09)	0.8
Active Stiffness PT (N/mm)	1023.81 (724.53)	458.03 (236.80)	0.23
$RTD_{Absolute}$ (N*m/s)	2265.80 (793.75)	1079.52 (337.76)	0.02*
$RTD_{Relative}$ (%MVIC/s)	6.62 (1.77)	6.31 (2.18)	0.96

*Table 1. Combined male and female participant characteristics shown as mean (standard deviation). SAT = subcutaneous adipose thickness. RTD = rate of torque development. * indicates significant difference between groups.*

Muscle Morphology. US-based measures displayed differences between men and women. Subcutaneous adipose tissue at the VL (SAT-VL) (p = .02) and corrected echogenicity (p = .02) in the VL were lower in the men when compared to women. However, VL muscle thickness was not greater in women when compared to men (p = .23).

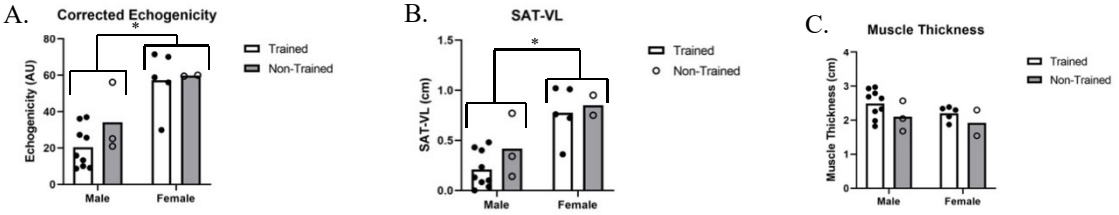

*Figure 4. Visual representations comparing the differences between RT and NT men and women in corrected echogenicity (A), SAT-VL (B), and muscle thickness (C). SAT = subcutaneous adipose thickness. * indicates significant difference between groups.*

US-Based Active Stiffness. Although there were no significant differences found between men and women in the PT (p = .23) or VL active stiffness measurements (p = .80), men do display a greater mean in the PT and VL.

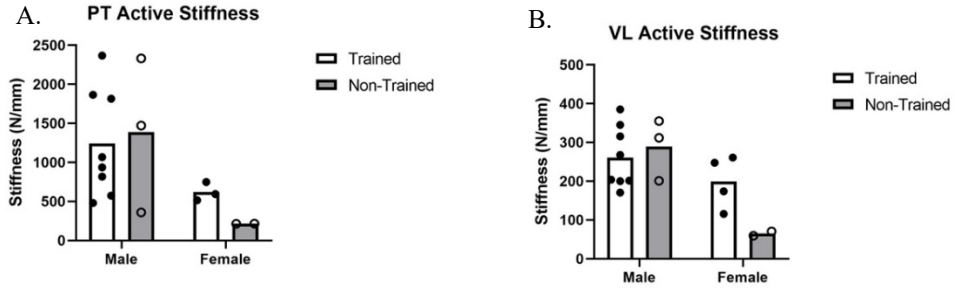

Figure 5. Visual representations comparing the differences between RT and NT men and women in PT active stiffness (A) and VL active stiffness (B). No significance was found between groups in both measured variables.

Myoton-based Passive Stiffness. While the VL was relaxed, MyotonPro passive stiffness measurements were taken. There was no significance between the men and women groups in the PT (p = .61) or VL (p = .14).

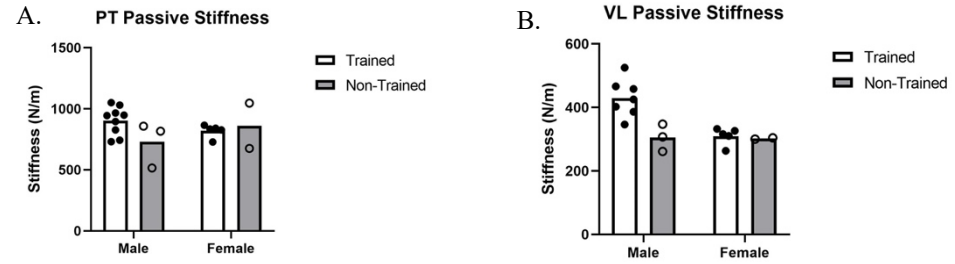

Figure 6. Visual representations comparing the differences between RT and NT men and women in PT passive stiffness (A) and VL passive stiffness (B). No significance was found between groups in both measured variables.

RTD measurements. Males displayed a higher absolute RTD development then women. ($p = .02$). However, when that was normalized to their MVIC, those differences disappeared ($p = .96$).

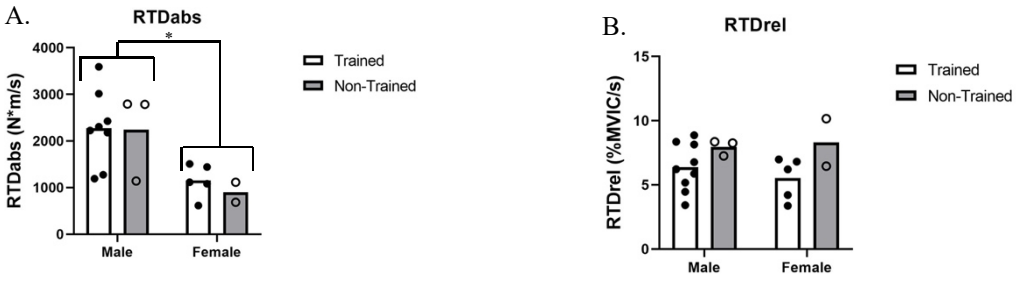

*Figure 7. Visual representations comparing the differences between RT and NT men and women in absolute rate of torque development production (A) and relative rate of torque development production (B). Significance was found in the adjusted rate of torque development comparison. * indicates significant difference between groups.*

Participants. RT and NT men were similar is height, weight, and BMI. Various other relevant participant characteristics can be found in table 2.

Variables	Resistance Trained (n = 8)	Non-Trained (n = 3)	p-value
Height (cm)	183.99 (6.8)	181.82 (15)	-
Weight (kg)	82.34 (8.7)	83.38 (24.7)	-
BMI (kg/m^2)	24.12 (2.3)	24.76 (3.4)	-
Muscle Thickness (cm)	2.49 (.35)	2.1 (.44)	0.19
Corrected Echogenicity (AU)	20.34 (11.4)	34.11 (19.2)	0.15
SAT - VL (cm)	0.2103 (.18)	0.4167 (.32)	0.18
Passive Stiffness VL (N/m)	429.7 (59.7)	305 (43)	0.01*
Passive Stiffness PT (N/m)	903.8 (115.7)	730.3 (186.7)	0.07
Active Stiffness VL (N/mm)	261.2 (79.5)	289.4 (79.6)	0.61
Active Stiffness PT (N/mm)	1241 (688.2)	1388 (989.7)	0.78
$RTD_{Absolute}$ (N*m/s)	2276 (801.5)	2238 (948.7)	0.94
$RTD_{Relative}$ (%MVIC/s)	6.364 (1.8)	7.956 (0.6)	0.18

*Table 2. Male participant characteristics shown as mean (standard deviation). SAT = subcutaneous adipose thickness. RTD = rate of torque development. * indicates significant difference between groups.*

Muscle Morphology. US-based measures revealed no differences between groups in men. When comparing the RT and NT groups, VL muscle thickness was not greater in the RT group when compared to the NT group (p = .19). In addition, SAT (p = .18) and corrected echogenicity (p = .15) in the VL both displayed greater mean differences in the RT but did not show statistical significance.

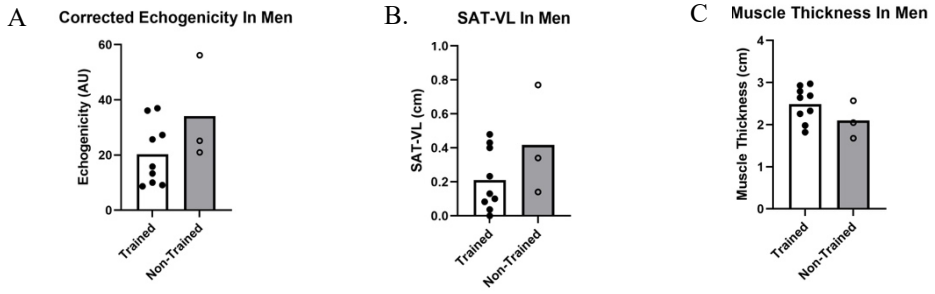

Figure 8. Visual representations comparing the differences between RT and NT men in corrected echogenicity (A), SAT-VL (B), and muscle thickness (C). SAT = subcutaneous adipose thickness. No significance was found between groups in all measured variables.

US-Based Active Stiffness. There were no differences found between RT and NT groups in the PT (p = .78) or VL active stiffness measurements (p = .61).

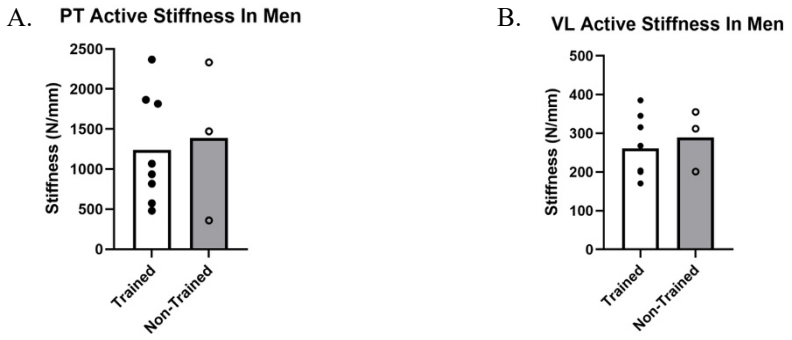

Figure 9. Visual representations comparing the differences between RT and NT men in PT active stiffness (A) and VL active stiffness (B). No significance was found between groups in both measured variables.

Myoton-based Passive Stiffness. While the volunteers had relaxed their VL, MyotonPro passive stiffness measurements were taken. In the PT, RT men had increased stiffness when compared to NT men, but there was no significance (p = .08). In the VL, RT men had increased stiffness when compared to NT men (p = .01).

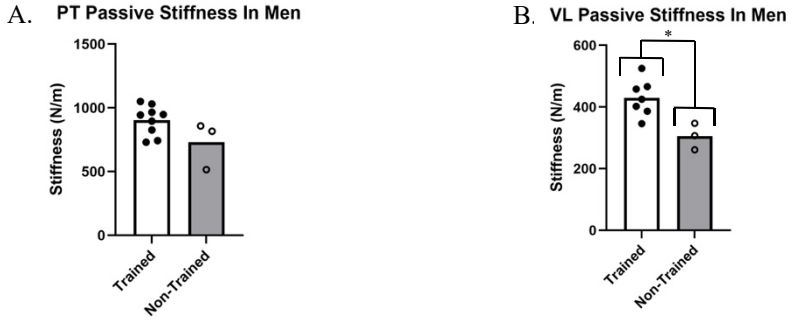

*Figure 10. Visual representations comparing the differences between RT and NT men in PT passive stiffness (A) and VL passive stiffness (B). Significance was found in the VL passive stiffness variable. * indicates significant difference between groups.*

RTD measurements. No differences were present between RT and NT men in their absolute RTD measurements (p = .94). However, when adjusted for MVIC, RT men presented a decrease in their RTD but there was no significance (p = .19).

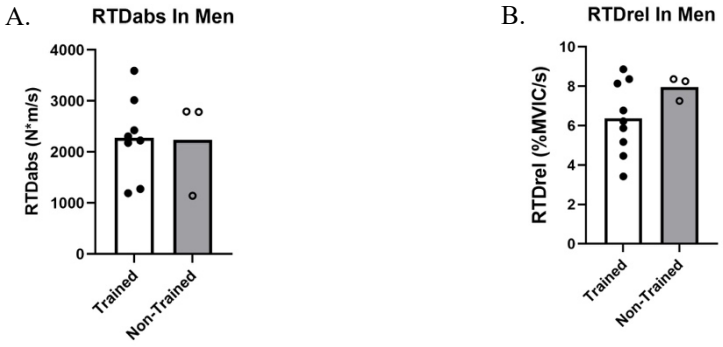

Figure 11. Visual representations comparing the differences between RT and NT men in absolute rate of torque development production (A) and relative rate of torque development production (B). No significance was found between groups in all measured variables.

Participants. RT and NT women were similar is height, weight, and BMI. Various other relevant participant characteristics can be found in table 3.

Variables	Resistance Trained (n = 5)	Non-Trained (n = 2)	p-value
Height (cm)	163.20 (2.93)	162.55 (3.61)	-
Weight (kg)	65.97 (4.59)	56.25 (2.47)	-
BMI (kg/m^2)	24.77 (1.74)	21.28 (.007)	-
Muscle Thickness (cm)	2.2 (.21)	1.92 (.54)	0.32
Corrected Echogenicity (AU)	57.3 (16.74)	59.78 (.41)	0.85
SAT - VL (cm)	0.77 (.27)	0.85 (.14)	0.73
Passive Stiffness VL (N/m)	309.4 (27.31)	302 (2.83)	0.73
Passive Stiffness PT (N/m)	819 (52.96)	861 (263.04)	0.71
Active Stiffness VL (N/mm)	199.5 (67.83)	65.14 (7.97)	0.06
Active Stiffness PT (N/mm)	619.9 (118.13)	215.3 (1.42)	0.02*
$RTD_{Absolute}$ (N*m/s)	1152 (354.39)	900.9 (304.54)	0.42
$RTD_{Relative}$ (%MVIC/s)	5.51 (1.63)	8.31 (2.62)	0.13

*Table 3. Female participant characteristics shown as mean (standard deviation). SAT = subcutaneous adipose thickness. RTD = rate of torque development. * indicates significant difference between groups.*

Muscle Morphology. US-based measures displayed no differences between groups in women. When comparing the differences between RT and NT, VL muscle thickness was not greater in the RT group when compared to the NT group (p = .19). SAT (p = .18) and corrected echogenicity (p = .15) in the VL did not decrease in the RT group.

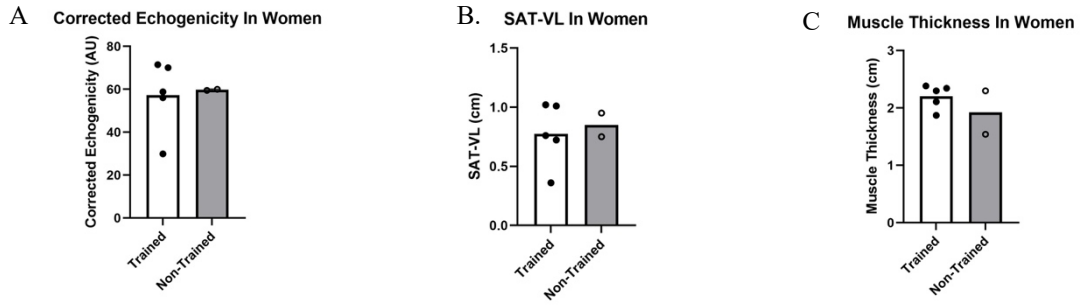

Figure 12. Visual representations comparing the differences between RT and NT women in corrected echogenicity (A), SAT-VL (B), and muscle thickness (C). SAT = subcutaneous adipose thickness. No significance was found between groups in all measured variables.

US-Based Active Stiffness. Compared to the male group, the female group displayed less variance. With that, RT active stiffness trended toward being greater in the VL compared to NT but was not significant (p =.06). However, the RT group displayed significantly greater active stiffness in the PT (p = .02).

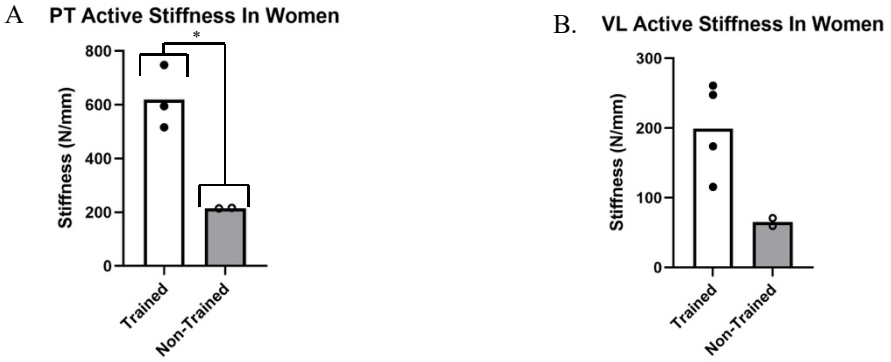

Figure 13. Visual representations comparing the differences between RT and NT women in PT active stiffness (A) and VL active stiffness (B). Significance was found in the VL active stiffness variable.
** indicates significant difference between groups.*

Myoton-based Passive Stiffness. MyotonPro passive stiffness measurements were taken while participants relaxed their VL. There was no significance in the PT (p = .71) or the VL between groups (p = .73).

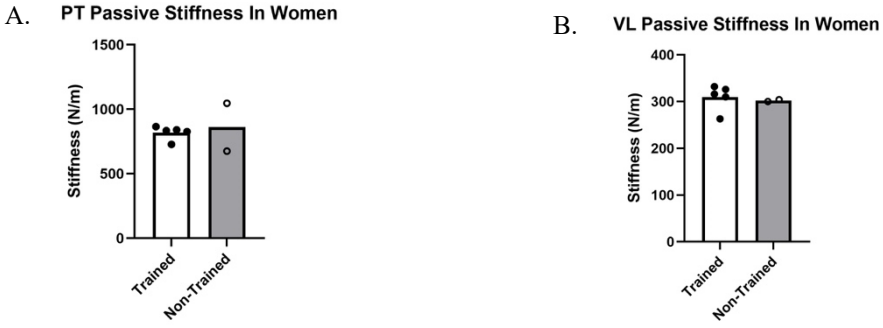

Figure 14. Visual representations comparing the differences between RT and NT women in PT passive stiffness (A) and VL passive stiffness (B). No significance was found between groups in both measured variables.

RTD measurements. No differences were present between RT and NT women in their absolute RTD measurements (p = .42). However, when adjusted for MVIC, RT women trended toward a decrease in their RTD but there was no significance (p = .13).

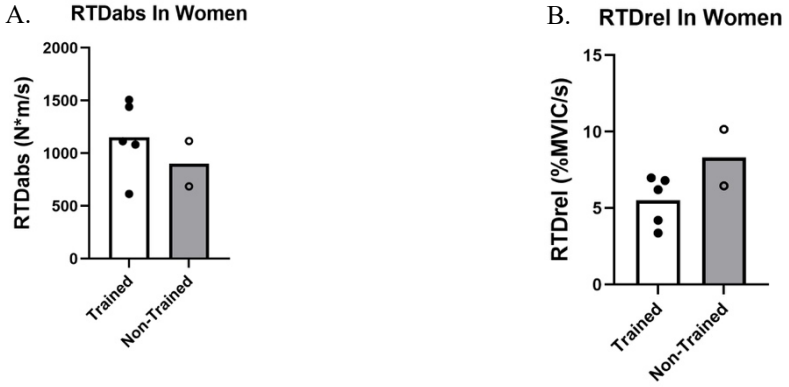

Figure 15. Visual representations comparing the differences between RT and NT women in absolute rate of torque development production (A) and relative rate of torque development production (B). No significance was found between groups in all measured variables.

Correlations. To examine the associations between variables and evaluate muscle performance as a function of passive and active stiffness, correlative measures must be considered. Figure 16 visually displays all performance to stiffness correlations and Table 4 provides all R^2 and p-values. When all groups are placed together, no correlations were deemed significant. However, when observing the associations of individual groups, a positive correlation between the relative RTD and PT passive stiffness was significant in the non-trained male group ($p = .02$). A positive correlation between relative RTD and VL passive stiffness was also significant in the trained male group ($p = .03$).

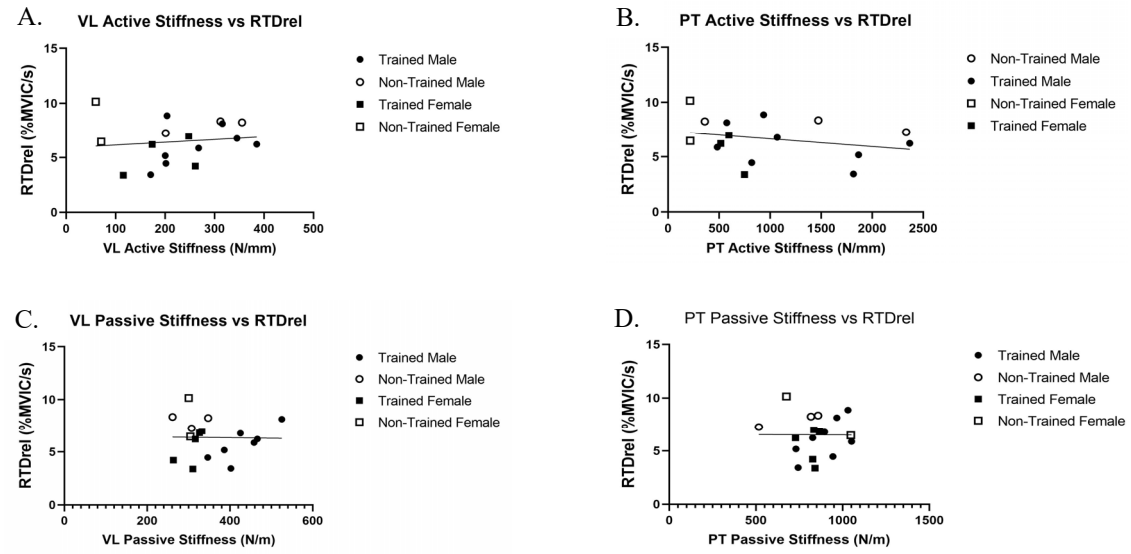

Figure 16. Correlations comparing the associations between active stiffness at the VL vs RTDrel (A), active stiffness at the PT vs RTDrel (B), passive stiffness at the VL vs RTDrel (C), and passive stiffness at the PT vs RTDrel (D).

Correlations	Trained Male	Non-Trained Male	Trained Female	Non-Trained Female
PT Passive VS RTDrel	0.3767	0.9993	0.01081	1
P-value	0.1055	0.0164*	0.8679	-
PT Active VS RTDrel	0.1544	0.6033	0.7291	1
P-value	0.3356	0.4337	0.3485	-
VL Active VS RTDrel	0.173	0.8757	0.1991	1
P-value	0.3054	0.2294	0.5538	-
VL Passive VS RTDrel	0.6466	0.01541	0.4424	1
P-value	0.0293*	0.9208	0.2206	-

*Table 4. Correlative R^2 values along with p-values. * indicates significant difference between groups.*

Discussion

To understand the implications and benefits of human performance, understanding musculotendinous properties is vital. One of those musculotendinous attributes that could be a predictor of muscle performance is stiffness. The primary purpose of this study was to identify and understand musculotendinous properties in young adult males. Then those results can be compared to those found in woman to determine the differences, if any, between biological sex. Therefore, our primary hypothesis is that activity will affect stiffness in males. Stiffness was then evaluated on rate of torque development as a determiner of performance between trained and untrained individuals.

When comparing the variables in this study, many differences can be portrayed between men and women. First, women were found to portray greater SAT at the VL ($p = .02$) and a greater corrected echogenicity ($p = .02$). However, there were no real differences observed between muscle thickness which could be because each individual's muscle growth is dependent on habit and the nutrients consumed on a daily basis. Men also visually displayed higher mean active stiffness values in the PT ($p = .23$) and VL ($p = .80$) which could have contributed to their significantly higher absolute rate of torque development when compared to women ($p = .02$). No differences between sexes were observed in PT ($p = .61$) or VL ($p = .14$) passive stiffness.

Stiffness has often been thought to positively contribute to muscle performance in that higher musculotendinous stiffness leads to a greater rate of torque development (29). During resting conditions, RT men displayed a significantly greater passive stiffness at the VL ($p = .01$). Similarly, RT men also visually displayed greater passive

stiffness at the PT but did not portray statistical significance ($p = .07$). With these increases in passive stiffness, it could then be assumed that the rate of torque development would be increased in RT men when compared to NT men. However, no significant increase in absolute rate of torque development was observed in the RT group ($p = .94$). To further justify these observations, each volunteer's rate of torque development was evaluated relative to their personal MVIC so that the relative rate of torque development could be generated. With this adjustment, RT individuals trended toward a decrease in their RTD, but there was still no significance ($p = .19$).

Within women, PT active stiffness was significantly greater in the trained group ($p = .02$) and trended toward a greater active stiffness in the VL in the trained group as well ($p = 0.06$). However, no differences were found in passive stiffness between training groups in the VL ($p = .73$) and PT ($p = .71$). The lack of significance could be attributed potentially to the lack of differences seen in echogenicity ($p = .85$) and SAT at the VL ($p = .73$). Since MyotonPro measurements are taken on the surface of the skin and adipose tissue has demonstrated to absorb applied force upon impact (30), it can be inferred that the pulses elicited by the MyotonPro may have been attenuated. Further research should be conducted, as the MytonPro may have not been able detect passive stiffness due to increased adiposity. As seen in the men, an increase in active stiffness would have been expected with an increase in the rate of torque development. However, there were no differences between training groups in the absolute rate of torque development ($p = .42$). When normalized to their peak torque, the non-trained group trended toward a greater relative RTD ($p = .13$).

RTD is the speed at which force can be generated. The lack of relative and absolute RTD differences between RT and NT groups in men and women can be attributed to multiple factors. First, force transmission through tendons is found to be rapid and may make it difficult to measure (31,32). Further, the measurement of short tendons, like the PT, further heightens the difficulty to measure force and may also explain the lack of relationship between these two groups. Second, volunteers in this study were asked to perform maximum voluntary isometric contractions to quantify rate of torque development measurements. Under these fixed isometric conditions, research has indicated at the sarcomere level that force rise time is slower than in kinetic conditions and can lead to minimal contribution from the tendon (33). Similarly, it has been revealed that once the distal and proximal ends of a sarcomere have been clamped, the skeletal muscle-tendon unit remains unchanged (34). Therefore, under isometric conditions, force generation is limited to the contractile elements of the sarcomere rather than the elastic properties of the tendon. As the muscle lengthens, force is generated through cross-bridge cycling during active contraction. The resistance to that contraction is what is known as active stiffness and should contribute to the rate of torque development (35,36). Given that the present data showed no difference between groups in active stiffness at the VL or the PT, it could account for the absence of relative and absolute RTD differences.

Echogenicity is a measure of muscle composition by which the contractile tissue is estimated relative to the non-contractile muscle and subcutaneous adipose tissue by US (37) and is correlated with muscle strength (38). A higher echogenicity is equated to increased intramuscular fat. Although not significant, the RT group does appear to have

lower corrected echogenicity values when compared to the NT group. Further, the RT group visually displayed less subcutaneous adipose tissue (SAT) at the VL then the NT group. Even without significance, this measurement is expected, as resistance training is known to decrease fat mass through various metabolic processes, overall energy expenditure, and appetite (39). SAT is of importance as SAT thickness may have a profound influence on digital palpation outcomes (40). Complementary to less fat, resistance training also increases muscle thickness (41) and was also visually shown to be greater, but not significant in the RT group. However, no correlation has been established between muscle thickness and stiffness (42), leading to the assumption that muscle thickness may not be the sole determiner of stiffness. With the accumulation of these variables, RTD was still not found to be greater in the RT group.

Another purpose of this study was to justify the use for the MyotonPro digital palpation device. In the present study, passive stiffness at the VL was significantly greater in the RT group. In addition, passive stiffness at the PT also visually appeared greater in the RT group however no statistical significance was found. Given these results, and the evidence presented by various other groups (1–3), the MyotonPro does provide accurate measures of stiffness under passive conditions. But these results should be taken with caution. Under normal physiological conditions, increased blood flow is sent to the contracted muscle. The increased amount of blood provides the muscle with the proper nutrients it needs to perform its intended function. To accommodate for the increase in blood, vasodilation of the necessary circulatory vessels induces a state of hyperemia in the muscle (43). This hyperemic state may be what is

being measured under the passive conditions. However, this does not decrease the MyotonPro's ability or validity to measure passive stiffness.

The limitations of this study must be recognized to provide future direction. The original objective of this study was to recruit a total of 20 individuals (10 resistance trained and 10 nontrained). Our sample size (8 resistance trained and 3 nontrained) was small. Even though our results were informative, more participants would potentially allow for more definitive conclusions as well as greater differences between groups. Another limitation to this study was that the MyotonPro appeared to have a threshold for measuring stiffness in the RT group. The digital palpation device sometimes displayed "too much noise" if the PT or VL was considered too stiffness to be measured. This was especially evident under active conditions when the muscle was contracted, and tendon was taut.

To further our understanding of the alterations in musculotendinous structure, composition, and function, the stiffness and composition data acquired from this study can then be compared to the data found in young and old women to determine any sex and age-related differences. Further, the results compared to trained versus non-trained individuals would assist toward the question of identifying the mechanisms related to the combination of atrophy and aging (sarcopenia) seen in older adults in future studies.

Conclusion

Overall, this study was able to take a closer look into the properties of the musculotendinous unit. Although, stiffness did not directly influence rate of torque development it was compelling to observe the differences between training groups in the passive stiffness in men. In addition, it was also interesting to see that activity did affect active stiffness in women, indicating that stiffness could be activity dependent. Therefore, resistance training is still a very viable intervention that both men and women should partake in because it could result in the increased passive or active stiffness of the VL and PT. An increase in these variables would be beneficial toward injury prevention but may not lead to performance enhancements. What can be concluded from this study is that activity does not do more than biological sex to affect stiffness. It could also be concluded that the musculotendinous unit is extremely complex, and its performance cannot be limited to a single variable. Further research should be conducted to determine more definitive answers toward the variables presented in this study.

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