Applied physiological investigations of the structure and mechanics of the muscle-tendon complex in conjunction with associated neuromuscular performance

by

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B. S., The University of Tulsa, 2013
M.S., East Stroudsburg University of Pennsylvania, 2015

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Food, Nutrition, Dietetics and Health
College of Health and Human Sciences

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Abstract

The primary objective of the investigations presented here were to examine muscle-tendon complex variables with corresponding neuromuscular performance variables. In total, three completed studies are contained within this body of work, employing noninvasive methods of ultrasound (US) and myotonometry (MYO), as well as postural stability (BAL) to explore the roles and relationships of the medial gastrocnemius (MG) and Achilles tendon (AT).

Study 1 was developed to identify intra-rater reliability in measurements of MG thickness (MGT), cross-sectional area (CSA), and echo intensity (EI) using two US imaging techniques (B-mode and panoramic) compared with measurements of the same variables between each technique. Findings supported that B-mode and panoramic US imaging are reliable techniques for assessing MG MGT, CSA, and EI (ICC: 0.699-0.999; SEM%: 0.839-5.324%). Good correlations were found between B-mode and Panoramic (r = 0.791-0.892, p = 0.013-0.001), demonstrating that the two techniques might be interchangeable when assessing MGT and EI. No differences were observed between the techniques (B-mode and Panoramic) when measuring EI (p = 0.174-0.828) and MGT (p = 0.185).

Study 2 was designed to explore gender differences in the free AT structure (CSA, tendon thickness [TT], and EI) utilizing B-mode and panoramic US imaging techniques. Results of this investigation demonstrated good to excellent reliability for imaging (ICC = 0.840-0.985, SEM% = 1.96-12.01%). Additionally, only tendon quality (measured as EI) was observed to be gender-dependent (p = 0.001).

Study 3 was designed to explore the relationship between structural (CSA, TT, MGT, EI) and passive mechanical properties (stiffness and elasticity) of the musculotendinous junction of the MG and AT; and how those properties may be related to postural stability performance in

young adult males. Findings supported that select MG structures were associated with passive mechanical properties of the AT with significantly high correlations found between MG CSA and AT elasticity ($r = 0.773$, $p = 0.015$), MGT and AT elasticity ($r = 0.717$, $p = 0.030$). Additionally, a significantly high negative correlation was observed between TT and MG EI ($r = -0.770$, $p = 0.015$). A single muscle-tendon complex property was associated with one postural stability variable, MGT and Anterior-Posterior Index (API) demonstrated a significantly high, negative relationship ($r = -0.747$, $p = 0.021$).

Finally, a culminating project has been developed to identify mechanical properties (stiffness and elasticity) of the muscle-tendon complex of the knee extensors to provide a timeline of mechanical decay in conjunction with neuromuscular performance across a series of eccentric contractions, and whether age influences the onset of mechanical and neuromuscular alterations. This study will include US imaging and MYO of the rectus femoris (RF) muscle and the patellar tendon (PT) prior to, during, and after an acute bout of 10×10 maximal, voluntary eccentric contractions on an isokinetic dynamometer at a speed of $60°\cdot s^{-1}$. In addition to muscular structure and passive mechanics, this study will also incorporate surface electromyography (EMG) of the RF to identify and record electrical activity and neuromuscular characteristics (peak torque [PT;Nm], rate of torque development [RTD;$N\cdot m\cdot s^{-1}$], and electromechanical delay [EMD;ms]) throughout the acute eccentric contractions. Pilot data collected and analyzed following a short, acute drop jump exercise with healthy, college age males and females demonstrated a significant interaction for time*tissue, with pre-exercise RF stiffness (N/m) increasing following exercise ($p = 0.027$). Pilot data also demonstrated PT stiffness was greater than RF stiffness ($p = 0.001$).

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I would like to put this acknowledgement into context in order to express my genuine gratitude. I have never been an outstanding student. I would rather play sports or engage in creative activities and art. I would attribute my distain of basic education courses to my dyslexia. So, if you were to tell me as a child that I would be on the precipice of completing a terminal academic degree, I doubt little Lauren would believe you. Completing this dissertation and finally earning my PhD is very much an unbelievable achievement for me. There are a few individuals who have assisted me in pursing and achieving the completion of this research and hence my doctoral degree. All that I have seen or accomplished would not have been possible without these individuals. I would like to express my deepest gratitude to the following people:

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Dedication

I would like to dedicate this culminating work to my parents and my fiancé.

To Mom and Dad, you have always been my source for inspiration and dedication. Throughout my life you both, in different ways, offered never ending support in all of my endeavors. You truly have the deepest understanding of what an immense personal challenge pursuing my Ph.D. is for me. I will always be eternally grateful for your patience, support, and love through my personal and professional endeavors.

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Most Grateful and Lovingly,

Lauren

Chapter 1 - Introduction

At a glance, tendons may seem to be just passive fibrous tissues that connect muscle to bone. While indeed tendons do connect muscle to bone, they are certainly not passive structures. The infancy of our knowledge on tendon physiology began in 1938, with physiologist A. V. Hill's publication of his three component model of musculotendinous behavior that elucidated the role of elastic energy in human movements[1]. The model consisted of a contractile component (CC) that exerts active force during shortening, a series elastic component (SEC) that allows for the storage and transfer of elastic strain energy, and finally a parallel elastic component (PEC) that stores elastic energy in parallel to the contractile components of a muscle. For several decades, this model went unchanged and served as the foundation of exploration into the muscle-tendon complex, relationships between muscles and robust energy storing tendons, and isolated tissue response to loading and unloading[2–8]. Until recently, Hill's model served as the framework for investigations into the muscle-tendon complex. Over the last three decades, a large protein housed within the contractile component was identified, titin, and helped enhance Hill's model to further explain elastic strain energy storage and transfer. This protein runs somewhat obliquely within the sarcomere of the muscle, and has been identified as a source of passive force that plays a central role in length-dependent activation by transmitting the stretch signal to the contractile elements of the muscle[9] (Figure 1).

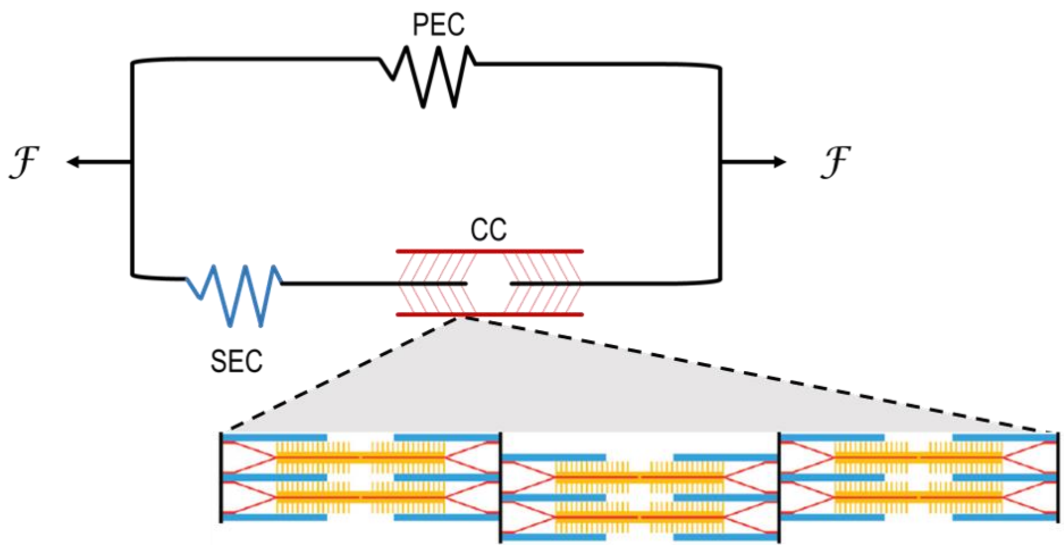


Figure 1.1. Hill's Muscle Model with the addition of the contractile component inclusion of titin (red > < attached to thick filament). CC: contractile component, SEC: series elastic component, PEC: parallel component, F: directional force.

Additionally, titin-based elastic forces can support high Ca2+ dependent tension development[10]. This effect of increased titin stiffness may keep the thick myosin filaments centered during passive stretch and re-center the filaments as the muscle is relaxed after an active contraction[9,10]. Current physiological explorations are continuing to explore titin as a passive modulator of contraction. However, examinations on the role of titin are limited to *ex vivo* animal model methods, which may increase the cost and level of difficulty for researchers[9]. Additionally, it is unclear what, if any, role titin plays in musculoskeletal injuries. Therefore the clinical relevance is ambiguous[9,10].

While there have been enhancements in the understanding of the storage and transfer of elastic strain energy within the CC, the SEC that consists of tendons composed of highly elastic collagen proteins, remains the primary biomechanical component that transmits contractile forces to the skeletal system to generate joint movement[2,11–13]. Tendons exhibit viscoelastic properties that affect muscle function, functioning as a mechanical spring-like structure. There are a number of daily activities and locomotor functions that require this spring-like function. During jumping, the tendons can act as power amplifiers, storing the energy of muscular work slowly, then releasing it quickly to power a rapid increase in the body's kinetic energy[14,15]. Tendons play a different role in walking and running, where fluctuations in mechanical energy are recycled via elastic energy storage and recovery to reduce muscular work and improve metabolic economy[13,16].

The architecture and organization of predominantly collagen proteins of tendons is what allows tendons to efficiently store and return energy during locomotion. The predominant stiff collagen proteins organized in parallel make tendinous tissue well suited to uniaxial tensile strain needed for efficient energy transfer [17]. Organized in a hierarchical fashion (Figure 2), the resilient collagen composition contributes to the viscoelastic mechanical properties of large energy storing tendons (i.e. patella and Achilles) that significantly support muscle force production in the spring-like behavior previously mentioned[17].

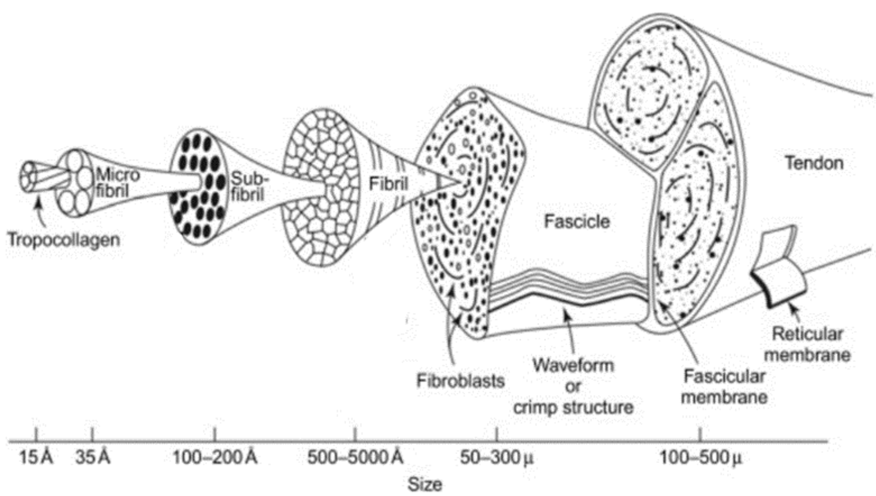


Figure 1.2.Tendon Hierarchical organization.

Tendon elasticity is a critical determent of muscle performance, allowing for variations of tendon elongation and compression under load[17,18]. The mechanical property of elasticity is quantified by tissue stiffness, strain, and hysteresis. Alterations in one of these mechanical properties ultimately influences the other. *Stiffness* describes the relationship between the force exerted on the tissue, mechanical and tensile, and results in changes in length[19]. This property is directly related to the *strain* property of tendon which is the deformation or alteration in structure under a force or stress[19,20]. Increased tissue stiffness would reduce the strain or deformation of a tissue. As mentioned, the *stress* would be the tensile force applied to the tendon[2,19] (Figure 3A). *In vivo*, this stress is produced by the specific tension of the tendon's associated musculature, musculature contraction, as well as ground-reaction forces[6,21–23]. The mechanical property of *elasticity* is calculated from the stress and strain of the tendon, and its ability to resume normal or resting shape and length after being stretched or compressed[24,25] (Figure 3B.). Tissue stress is the force applied to a tissue divided by the tissue's cross-sectional area, where tissue strain is the measurement of deformation or displacement of the tissue in response to the stress. Taken together, a tendon with high stiffness would have a decrease in strain for a certain stress. Additionally, a highly stiff tendon that resists a greater magnitude of strain would require a greater amount of stress to result in the same amount of deformation as a less stiff tendon. Tendon *hysteresis* is an additional mechanical property indicative of the tendon's ability to transfer energy to the contractile and PEC[21] (Figure 3C.). This is represented as a shift in the length-tension curve following a single period of loading and unloading and often evaluated following a series of loading and unloading. Often a tissue's ability to dissipate energy occurs through increased heat surrounding the tissues. However, the highly efficient tendinous tissue that stores and delivers approximately 90% of all mechanical energy, hysteresis is considered evidence of energy transfer, not dissipation[21,26,27].

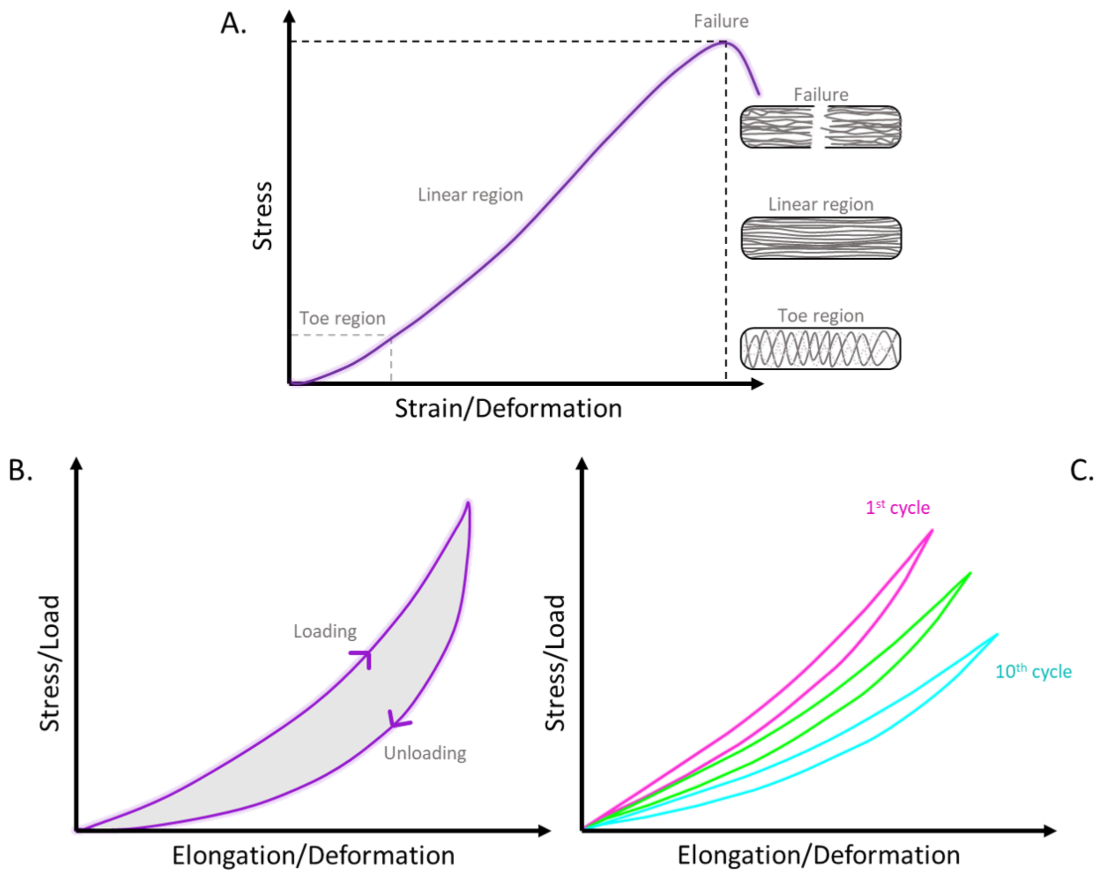


Figure 1.3. A) demonstration of Stress-Strain curve specific to tendon, B) representation of energy dissipation, C) visual demonstration of hysteresis.

Alterations in the tendinous mechanical properties can influence the speed of contractile elements, resulting in profound effects on muscle force, power, and overall work as calculated from force and movement measured in Newton-meters (Nm)[28]. In very rapid movements, elastic mechanisms can amplify muscle power by storing the force of muscle contraction slowly and releasing it rapidly enhancing force economy and improving muscle work capacity[15,16,29]. When energy must be dissipated rapidly, such as in landing from a jump, energy stored rapidly in elastic elements can be released more slowly to stretch muscle contractile elements, reducing the power input to muscle and possibly protecting it from damage. Muscles absorb, or dissipate, mechanical energy by producing force while shortening and lengthening, thus providing a controlled means of reducing kinetic or potential energy of the body[30]. This function is important for activities such as deceleration, downhill walking or running, or when landing from a jump. Energy absorption by muscles and tendons is also a part of the cyclic exchange of energy that occurs during walking as well as running[13,21,22]. The elastic mechanisms investigated since Hill's original publication have helped to provide details on the cooperative function between muscles and tendons. It is well known how series elastic components, more specifically tendons, can store and transmit energy to maintain muscular concentric contractions with relatively low metabolic cost. However, the role of tendons in potentially altering the timing and performance of muscle fascicles during eccentric contractions resulting in energy buffering in young as well as older individuals remains unclear[16,18,27,30,31]. Similarly, while it has been demonstrated that older adults experience age-related alterations to tendinous tissue as well as muscular performance during eccentric contractions there is a dearth of knowledge on the relationship between aged tendinous tissue and eccentric contraction performance[32–37].

Active muscle fiber lengthening occurs regularly throughout everyday tasks, and becomes more frequent during physical activity or athletic events. Eccentric contractions occur in muscles when the external force acting on them is greater than the force they produce leading to a lengthening of the muscle-tendon complex[38,39]. These contractions are high strain contractions that can result in soreness or tissue damage when performed at high intensities and high repetitions, but eccentric contractions also positively affect tendon structure and mechanical properties and improve muscular strength[40–45]. Eccentric contractions play a crucial role in the production and control of movement and contribute to energy efficiency. The benefits of eccentric training are also being increasingly recognized for improving exercise tolerance as well as improving tendon structure and function. During these contractions, muscle fascicles are near isometric with the musculotendinous junction (MTJ) performing the majority of the lengthening[18,46]. It is not until the MTJ reaches its terminal length and energy storing capacity that the muscle fascicles begin to shorten due to the recoil of the MTJ and then subsequently lengthening as kinetic energy is dissipated into heat. The timing of events between the MTJ and muscle fascicles would suggest that tendons can delay muscle fascicle lengthening and energy dissipation by transiently absorbing impact energy and then slowly releasing energy to do work on muscle fascicles[16,18,30,46].

The cooperative relationship between tendon and muscle fascicle would suggest that the mechanical characteristics of the series-elastic component have some influence on neuromuscular timing or performance. Faude and Donath presented a well-articulated definition of neuromuscular performance as the ability of the neuromuscular system to functionally control and drive movements by integrating, coordinating, and using sensory feedback, reflex activity, central motor drive, muscle recruitment pattern, muscular excitation-contraction coupling and energy availability [47]. It is commonly accepted that a well-developed capacity of the neuromuscular system is highly relevant to fitness and health throughout our lives. Some aspects of neuromuscular performance can be assessed non-invasively through surface electromyography (EMG) at the level of the muscle. Work by Griffiths in felines demonstrated that paw-shake contractions resulted in high EMG activity accompanied by high force tendon stretching, and conversely ear-scratching results in much lower EMG activity that did not lead to any significant tendon lengthening [48]. This led to the conclusion that the neural recruitment of fewer motor units would mean lower force production, and less tendency to stretch the tendon [48]. Unique neuromuscular performance characteristics have been identified during maximal eccentric quadriceps contraction, and EMG activity is markedly lower during maximal voluntary eccentric quadriceps contraction in humans compared with that of fast concentric contractions [49–51]. The decrease in EMG activity during the high strain eccentric contractions may, in part, may be due to the fact that the majority of muscle-tendon complex lengthening during these eccentric contractions is taken up by the tendon which delays muscle fascicle activity and ultimately upon recoil returns energy to the muscle decreasing metabolic cost and work of the muscle [51–53]. Eccentric contraction induced tendon lengthening is much different than the tendon lengthening that occurs during concentric contractions. This is partly because some tendinous length change during concentric contractions is considered passive due to changes in joint angle. A larger part of the difference between tendon length changes during eccentric versus concentric contractions is due to the higher strain forces present during eccentric contractions [54,55]. These higher strain forces are needed to lengthen the tendon beyond an isometric length, which is achieved under isometric contraction [54,55]. Lengthening the tendon beyond the isometric length is essential for elastic energy storage [51,55]. This means that muscles cannot produce a sufficient amount of strain on to the tendon to allow the tendon to reach an elastic energy storing capacity concentrically [51].

Specific neuromuscular variables of maximal strength have been assessed during investigations into lengthening contractions, age-related muscle alterations, and to a lesser degree muscle-tendon complex characteristics. Maximal muscle strength characteristic such as peak torque or peak force are primarily associated with muscular architecture. To differentiate, peak torque is the assessment of muscular strength about an axis of rotation thus the torque units of measurement include force and distance (N·m), peak force does not account for distance and therefore is measured as units of Newton. Torque and force are commonly measured during isometric contractions, however isokinetic contractions allow for assessments of torque throughout a range of motion [56,57]. Isokinetic torque has been seen to fluctuate throughout the full range of motion and is influenced by changes in joint angle, while isometric torque increases to a plateau of torque production [57]. In isokinetic dynamometer studies, maximal voluntary torque developed when MTJs were eccentrically loaded by joint flexion were similar to or only slightly greater than that of an isometric contraction [58]. However, when muscles are supra-maximally activated by electrical stimulation, muscle fibers develop forces during lengthening that are 70-100% greater than the force developed during isometric contraction [49,51]. It has been proposed that the lower voluntary forces result from a neural mechanism that acts to limit force via inhibitory feedback via the sensory group I and II afferents including sensory Ib afferents from Golgi tendon organs that reduces muscle recruitment during maximal lengthening events [49]. While a slight reduction in EMG signal activity during eccentric loading might support this neural mechanism, another alternative explanation also exists. Eccentric loading during maximal voluntary contractions stretches the tendon with almost no lengthening of the muscle fascicles resulting in the muscle fascicles performing an isometric contraction [50]. During eccentric maximal voluntary contractions (MVCs) of the knee extensors, this quasi-isometric behavior of muscle fascicles has been observed for the majority of knee range of motion with the vastus lateralis muscle fascicles shortening at approximately the last 20% of knee flexion [59]. Due to the fibers essentially remaining quasi-isometric, the muscle produces no more force than it would in a contraction in which the joint does not move [51]. The isometric behavior in muscle fascicles during lengthening contractions has been identified in the isolated gastrocnemius muscle as well as the tibialis anterior observed during stair negotiation [48,50].

Rapid strength characteristics seem to be more influenced by muscle-tendon complex mechanical properties. Examination of rapid strength has allowed for the ability to identify electromechanical delay (EMD) which has been recognized to be highly influenced by series-elastic components [60,61]. EMD is the time lag between onsets of muscle activation as detected by EMG and muscle force production. Specific to the knee extensors, the onset of muscular activation is set 3 standard deviations (SD) above the baseline EMG signal and the point of force production is identified at 7.5 Nm [62]. Since the majority of EMD is determined by the time taken to stretch the series-elastic component, differences in the elastic tendon compliance can greatly alter the timing of neuromuscular activation and force or torque production[61,62].

Rate of torque (or force) development (RTD; Δtorque/Δtime) is another neuromuscular characteristic that can be examined during rapid strength assessment. Similar to EMD, it has been reported that mechanical properties of the series-elastic component may influence RTD. Rate of torque is an important marker of neuromuscular function that examines torque production from onset of torque as 0ms all the way up to peak torque [49,63]. This rapid strength measurement can be examined at different time intervals of a muscle contraction such as the early (0-50 ms) and later phase (100-200 ms) [64]. Early RTD is primarily associated with initial motor unit recruitment and firing rates and is also a reflection of intrinsic muscle properties (i.e., fiber type composition, calcium kinetics) [63,65]. Later RTD represents the cooperative mechanical or structural components of the muscle with neural activation and considers other structural factors including tendon stiffness and muscle pennation angle [66–69]. Both of the latter factors are negatively affected by age [64,68,70]. Following previous hamstring muscle strain (injury), there is a lower early phase RTD (50ms) and peak torque that is seen to a greater extent during eccentric contraction than concentric contraction [71]. When measured between 20% to 80% of peak torque values, RTD has also been found to be higher in the knee flexors of older women who do not have a history of falling as compared to older women who have experienced falls within a single year [72].

The lack of evidence for the time course of elastic tendinous mechanical changes may, in part, be due to the available instruments for an *in vivo* assessment. Traditionally, *in vivo* assessments of tendons have relied heavily on imaging techniques utilizing diagnostic ultrasound (US), magnetic resonance imaging, and computed tomography in conjunction with devices measuring muscle force and torque production such as load cells and dynomometers[21,22,73–75]. While the incorporation of non-invasive imaging for tendinous tissue exploration has been greatly additive, the ability to specifically isolate series-elastic characteristics to determine these structures specific contributions to locomotion has not been possible. However, more recently a new hand-held myotonometry device has been identified as a reliable, accurate and sensitive method for providing objective, non-invasive measurements of specific tissue. This device enables measurement of not only muscles, but tendons as well [76–78]. This handheld device produces a mechanical impulse to the skin overlying the target structure. The oscillation of the tissues underneath the probe permits calculation of the viscoelastic properties of the tissue. This device is able to analyze tissue tone, stiffness, creep, stress-relaxation time, elasticity as the proportional inverse to tissue decrement [79].

Continuing research has provided details on the role and function of the highly elastic tendinous tissue during muscle contractions. It has been identified that the mechanical properties of series-elastic component may be influential on some neuromuscular characteristics. Furthermore, there is evidence to support the possibility that the tendon can delay energy transfer to muscle fascicles during eccentric contractions to allow, potentially acting in a preventative manner against muscular injury or disruption. With the understanding that the muscle-tendon complex works cooperatively, noninvasive examination of structural and mechanical alterations for isolated muscle related to those of the isolated tendon have been limited by available technologies. Further examining the separate components of the muscle-tendon complex may help to determine specific tissue contributions to contraction and exercise performance. Additionally, thus far the duration of the tendinous tissue buffering high strain energy for the muscle has not been fully explored. Similarly, the role of the elastic tendinous structure and mechanics during exercise in older adults requires continued examination. Finally, given that neuromuscular characteristics are known to play a protective role regarding muscle during eccentric contractions, the relationship between the tendinous tissue buffering of energy and the protective neuromuscular performances remain unexplored.

Chapter 2 - Manuscripts

2.1 An ultrasound investigation into reliability and correlations of the medial gastrocnemius muscle using B-Mode and Extended-View imaging.

2.1.1. Introduction

Ultrasound (US) imaging is a non-invasive, real time method to evaluate muscular morphology, and is widely used in many medical specialties [80]. Such assessments have included measurements of muscle size [81–83] and muscle quality[83,84]. Specific muscle size and muscle quality variables of muscle thickness and echo intensity (gray-value) of muscle, US has been able to detect structural difference among healthy participants[81,83–87] as well as muscular changes caused by neuromuscular disorders[88,89]. Initially, US imaging allowed for a single static image referred to as B-mode imaging that then progressed to capturing assimilations of 2-D cross-sectional consecutive images, called panoramic US imaging (extended field of view)[80,90]. Panoramic US imaging is a novel extended field of view imaging technique that enables visualization of larger muscles that was first found to be a valid muscular assessment tool more than a decade ago and has been employed extensively for the quantification of muscle[80,83,84,91,92]. Some clinicians and researchers favor the panoramic US imaging for the ability to assess cross-sectional area, fatty infiltration and echo intensity of muscles asserting that these measurements cannot be measured with B-mode US[93]. However, not all US systems include extended field of view or panoramic functions. Additionally, because panoramic US image capturing requires the imaging probe to be passed over the contours of limbs for the assessment of the targeted muscle(s), areas of the body that consist of tight cylindrical shapes (lower leg, forearm e.g.) may lead to difficulties in capturing clean, consist panoramic images[94].

Previous investigations utilizing both panoramic and B-mode US imaging have demonstrated that echo intensity strongly reflects fibrous tissue content and intramuscular adiposity[95,96]. Rate of force development[97], lower extremity strength[98], and sit-to-stand ability[99] have all been correlated with measures of echo intensity assessed via US for young and older adults. In addition to muscle quality characteristics of fibrous tissue content and intramuscular adiposity, echo intensity has been suggested to reflect other details of muscle composition such as glycogen and water content[100]. Combined with measurements of muscle thickness and cross-sectional area, the US assessment of echo intensity can provide information specific to muscle size and muscle quality or composition that may provide important health-related information for clinicians and researchers[101].

While there is evidence demonstrating that B-mode and panoramic US imaging are valid and reliable techniques for assessing characteristics of muscle size and quality[80,81,83,84,91–93], a comparison of these two imaging techniques is so far absent. Due to the potential lack of the panoramic function of some US devices and the existence of difficulties in obtaining clear panoramic images for analysis due to greater curvature of some limbs within certain participants, evaluating both B-mode longitudinal and panoramic imaging should be assessed to understand if both methods have the potential of providing similar information and reliability for measurements of muscle size and quality. Therefore, the aim of this investigation was to obtain B-mode longitudinal images and panoramic images from the right medial gastrocnemius of female participants and analyze the images for variables of muscle size and quality including muscle thickness, cross-sectional area, and echo intensity for comparison in reliability in correlation of like variables from the two imaging methods (B-mode longitudinal and panoramic).

2.1.2. Methods

Participants

Twelve healthy females (mean ± SD age = 19.42 ± 1.16 years; height = 164.57 ± 8.79 cm; mass = 62.65 ± 11.22 kg) volunteered to participate in this investigation. This study was approved by the University Institutional Review Board, with all participants completing a health history questionnaire and signing an informed consent document prior to participation. None of the participants reported current/ongoing neuromuscular conditions or musculoskeletal injuries of the lower extremities within 6 months prior to testing. Participants were excluded if they engaged in structured aerobic and/or anaerobic exercise more than three days a week. Additionally, all participants were asked to refrain from engaging in any vigorous exercise of the lower extremity within 24 hr their laboratory visits.

Procedures

Each participant visited the laboratory on two occasions, separated by 2-4 days, at approximately the same time of day (± 2 h). For each trial, participants were placed in an unloaded position, laying prone on a padded examination table with the low leg extended and the right ankle held in neutral (90°) position. All US images were gathered from the right medial gastrocnemius (MG). Within this position, longitudinal and panoramic images were obtained. Two B-mode still frame images were collected for longitudinal scans. All US assessments were performed after participants had rested for 5 min to allow for any fluid shifts to stabilize[102,103].

Ultrasound Imaging

US images were obtained using a B-mode US imaging device (GE Logiq E, Milwaukee, WI, USA) and a linear-array probe (Model 12L) of the right MG. US images of the MG were optimized for image quality, including gain (50 dB), depth (6 cm), and frequency (10 MHz)[104], and were set prior to testing and held constant between participants and across trials[84]. To ensure all measurements were taken from a consistent region of interest within the MG, US probe placement was determined at 1/3 the low leg circumference at 10cm distal to the tibial tuberosity and was maintained for every measurement[105]. A non-toxic permanent marker was used to indicate the probe position at the posteromedial 1/3 of the low leg circumference between visits (Figure 4A). The US probe was kept perpendicular to the skin, and a generous amount of water-soluble transmission gel was applied to both the probe and participants' skin to provide acoustic coupling without depressing the dermal surface.

For each of the panoramic US scans (extended field of view), the primary investigator moved the probe manually at a slow and constant rate along the surface of the skin from the lateral to the medial aspect of the gastrocnemius muscle using the LogiqView function (GE LogiqE), which is a special function on the US imaging device. To support image continuity and quality, a foam guide was positioned bisecting the common probe placement mark (Figure 4B). Panoramic and longitudinal US images were scanned at the same level of the low leg as described above and the same experienced sonographer (L. E. P.) performed all assessments as well as image analysis. All US images were obtained with the participants in the prone position on a cushioned examination table. Ankle joint was held stationary in a neutral (90°) position by the researcher conducting the scans.

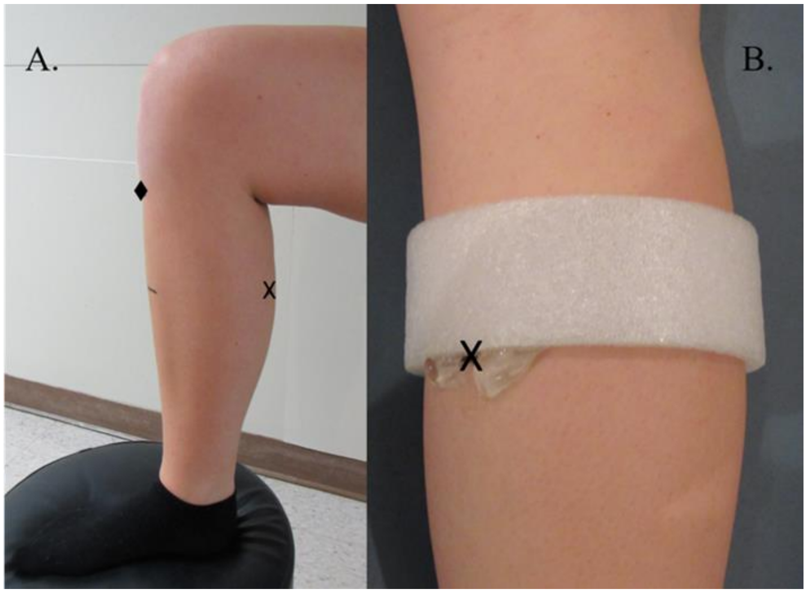


Figure 2.1. A) Identification of MG (×) with the tibial tuberosity identified by ♦ B) demonstration of foam guide placement to steady panoramic ultrasound.

Image Analysis

All US images were analyzed utilizing a third-party image analysis software (ImageJ; Version 1.47v, National Institutes of Health, Bethesda, MD, USA). Prior to analysis, each image was scaled individually from an area in pixels to cm with the straight-line function using a known distance of 2 cm. The superficial and deep fascia on every longitudinal US image were then identified and stationary lines were superimposed to extend the trajectory of both anatomical structures.

Longitudinal images were analyzed for following variables: MG thickness, the distance between the superficial and deep fascia [MGT; cm (Figure 5A.)]. MG echo intensity [EI_{Long}; AU (Figure 5B.)] was assessed from a 1×1 cm^2 region of interest (ROI) placed approximately in the center of the MG within the longitudinal US images. The 1×1 cm^2 ROI was constructed using the rectangle function and shift key within ImageJ and was measured off of the visible US image scale of known distance (2 cm). The center of the MG was approximated based on the location of the central probe landmark contained in the US images, the center of the ROI rectangle was aligned with this landmark and positioned approximately equal distance between the superficial facia and the top of the ROI rectangle and the deep fascia and the bottom of the ROI rectangle.

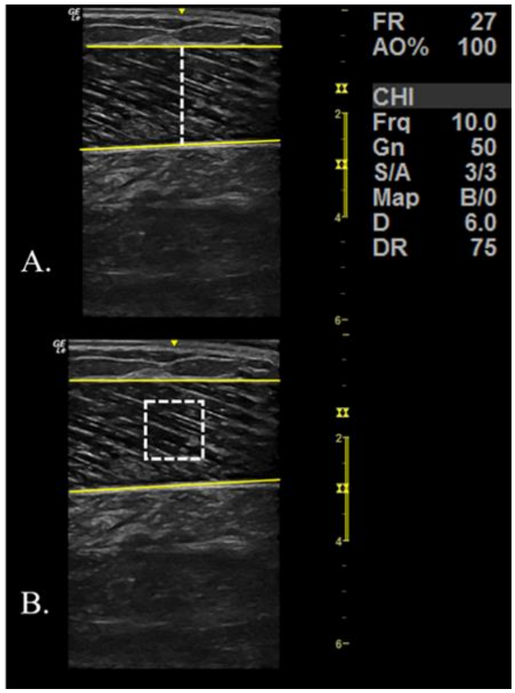


Figure 2.2. A) Longitudinal US images with the superficial and deep fascia identified by the two single yellow lines, and the identification of MGT measurement with straight white dotted line B) demonstration of the assessment of EI_{Long} with the placement of the ROI 1 × 1 cm^2 white dotted box.

The Panoramic US images were analyzed for MG cross-sectional area [CSA; cm^2 (Figure 6A)], echo intensity [EI_{Pan}; AU (Figure 6A)], 1 × 1 cm^2 ROI echo intensity [EI_{ROI}; AU (Figure 6B)], and muscle thickness [MGT_{Pan}; cm (Figure 6C)]. Echo intensity values were assessed by gray-scale analysis using the standard histogram function with values calculated in arbitrary units (AU) on a scale of 0-255 (black = 0, white = 255)[83]. Each variable gathered from longitudinal and panoramic US images were measured once from two separate US images, and the average of those two measurements were used for statistical analysis.

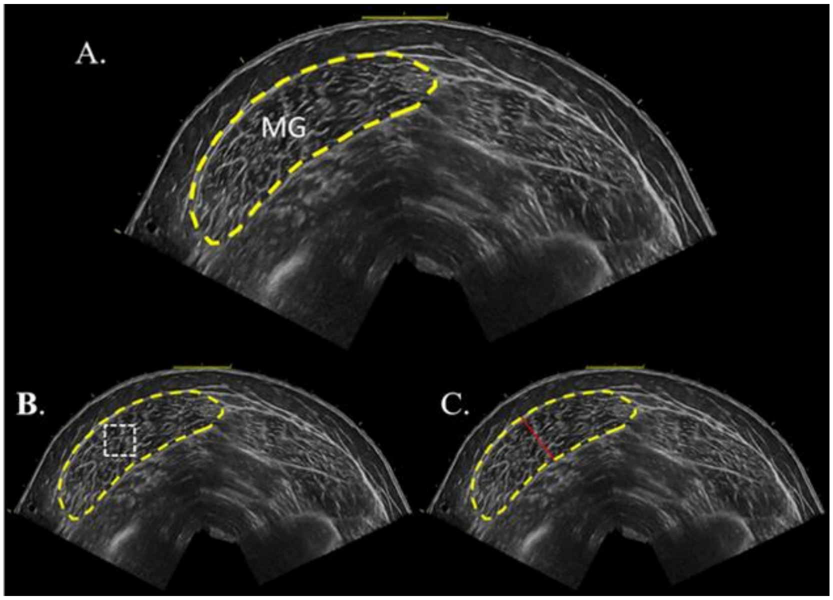


Figure 2.3. A) Representation of the panoramic US images with the MG identified along with the boundary from which MG CSA and EI_{Pan} were measured B) visual demonstration of the measurement of EI_{ROI} with the ROI identified by the dotted white square C) visualization of the assessment of MGT_{Pan} marked by the single red line extending to opposite sides of the MG boundary.

Statistical Analysis

One-way repeated-measures analyses of variance (ANOVAs) were employed to examine the means of like variables for systematic variability. Like variables were identified as those of echo intensity (EI_{Long}, EI_{Pan}, and EI_{ROI}) and muscle thickness (MGT and MGT_{Pan}). The intra-class correlation coefficient (ICC) indicating relative consistency (test-retest reliability), the standard error of measurement (SEM) representing absolute consistency and the minimal difference (MD) needed to be considered real were calculated for the participants across the variables and across the two separate laboratory visits. Both the SEM and MD were expressed as absolute values and percentages of the mean.

The ICC model "2,*k*" by Shrout and Fleiss [106] was used to calculate the test-retest reliability. Model 2,*k* is a two-way random factor model that utilizes scores signifying the average of the *k* (number of trials) scores and uses random and systematic error to support generalization to other laboratories and testers[107,108]. The ICC (2,*k*) was calculated with the equation presented by Shrout and Fleiss[106] and the standard error of measurement (SEM) and minimum difference (MD) values were based on recommendations by Weir[108]. The SEM and MD for model 2,*k* were calculated with the equations produced by Hopkins[109] and Weir[108], respectively. Implemented guidelines for translating ICC values consisted of the following, excellent reliability = ICC $\geq$ 0.90, good reliability = ICC 0.75-0.9, moderate reliability = ICC 0.50-0.74, and poor reliability = ICC < 0.50[108,110]. The calculations for ICC, SEM, and MD were performed using a custom-written spreadsheet (Microsoft Excel, Microsoft, Redmond, WA, USA)[83,84].

Pearson product-moment correlation coefficients (*r)* from mean data were calculated separately to examine the relationships between like variables (MGT, EI_{Long}, CSA, $EI_{Pan,}$ MGT_{Pan}, EI_{ROI}). Correlations were considered high when $|r| \geq 0.7$, moderate when $0.5 \leq |r| < 0.7$, low when $0.1 \leq |r| \leq 0.5$, and null when $|r| < 0.1$[111]. Level of significance was identified as a *p-value* of $\leq$ 0.05. Correlation analyses were performed using SPSS Statistics Version 27.0 (IBM, Armonk, NY, USA). An ɑ level of $p \leq 0.05$ was considered statistically significant for all analyses.

2.1.3. Results

The means and SD values for longitudinal and panoramic variables can be found within Table 1. There were no significant differences observed between the means of the variables captured in longitudinal and panoramic US images ($p > 0.05$). There was no significant difference for MGT and EI measured within B-mode and panoramic images (p = 0.174-0.828).

Table 2.1. Mean (SD) values for MGT, EI_{Long}, CSA, EI_{Pan}, EI_{ROI} of participants' medial gastrocnemius.

	MGT (cm)	EI_{Long}	MGT_{Pan} (cm)	EI_{Pan}	EI_{ROI}	CSA (cm^2)
Longitudinal	1.932 (0.177)	54.223 (14.098)				
Panoramic			4.989 (10.613)	57.867 (10.188)	57.604 (11.551)	10.618 (1.427)

MGT = muscle thickness measured from longitudinal images; EI_{Long} = echo intensity measured from longitudinal images; MGT_{Pan} = muscle thickness measured from panoramic images; EI_{Pan} = echo intensity measured from panoramic images; EI_{ROI} = echo intensity measured from panoramic images using the placement of a 1cm×1cm square region of interest; CSA = cross-sectional area measured from panoramic images.

Additionally, no systematic variability was found among any of the muscle quality variables across the two laboratory visits (p = 0.064-0.542). Reliability statistics for the longitudinal and panoramic US variables can be seen in summary in Table 2. Reliability analysis revealed excellent consistency for EI_{Long}, EI_{Pan}, CSA, and EI_{ROI} with ICCs ranging between 0.929-0.999 and SEM% values between 0.839-7.949% while measurements of MGT and MGT_{Pan} demonstrated good consistency with ICCs 0.856 and 0.699 with SEM% of 5.324% and 9.857%, respectively.

Table 2.2. Reliability statistics for US variables for the medial gastrocnemius in the longitudinal and panoramic imaging.

Longitudinal			Panoramic		
MGT (cm)	*p*-value	0.508	**MGT_{Pan} (cm)**	*p*-value	0.103
	$ICC_{2,k}$	0.856		$ICC_{2,k}$	0.699
	SEM (cm)	0.102		SEM (cm)	0.182
	SEM %	5.324		SEM %	9.857
	MD (cm)	0.273		MD (cm)	0.393
	MD %	14.243		MD %	21.338
EI_{Long}	*p*-value	0.419	**EI_{Pan}**	*p*-value	0.307
	$ICC_{2,k}$	0.957		$ICC_{2,k}$	0.929
	SEM (AU)	4.252		SEM (AU)	3.982
	SEM %	7.949		SEM %	6.783
	MD (AU)	11.791		MD (AU)	10.518
	MD %	22.042		MD %	17.919
			EI_{ROI}	*p*-value	0.542
				$ICC_{2,k}$	0.999
				SEM (AU)	0.483
				SEM %	0.839
				MD (AU)	1.410
				MD %	2.450
			CSA (cm2)	*p*-value	0.064
				$ICC_{2,k}$	0.930
				SEM (cm^2)	0.549
				SEM %	5.262
				MD (cm^2)	1.204
				MD %	11.541

MGT = muscle thickness measured in a longitudinal image; EI_{Long} = echo intensity measured from a 1x1cm region of interest centered in a longitudinal US image; CSA = cross-sectional area measured in a panoramic US image; EI_{Pan} = echo intensity measured traditionally within the CSA boundary of a panoramic US image; MGT_{Pan} = muscle thickness measured from superficial to deep fascia centered within the CSA muscle boundary of panoramic US image; EI_{ROI} = echo intensity measured from a 1x1cm region of interest within the center of the CSA muscle boundary of panoramic US image.

Pearson's Product Correlation results are displayed within Table 3. Positive correlations were found between all three echo intensity variables (EI_{Long}, EI_{Pan}, and EI_{ROI}). Echo intensity measured in the longitudinal US images (EI_{Long}) demonstrated a positive relationship with EI_{Pan}

(r = 0.791, R^2 = 0.625, p = <0.001) and with EI_{ROI} (r = 0.805, R^2 = 0.648, p = <0.001). The echo intensity variables assessed in the panoramic US images (EI_{Pan} & EI_{ROI}) were also in positive agreement with an r = 0.937, R^2 = 0.878, p = <0.001. Additionally, EI_{ROI} was negatively correlated with MGT_{Pan} (r = -0.619, R^2 = 0.383, p = 0.032). Variables of muscle thickness (MGT and MGT_{Pan}) were positively correlated with each other (r = 0.892, R^2 = 0.796, p = <0.001). A strong positive correlation was observed between CSA and MGT. Regarding CSA, there was a significantly high positive correlation between CSA and MGT (r = 0.867, R^2= 0.752, p = <0.001) while there was a significantly moderate positive correlation between CSA and MGT_{Pan} (r = 0.693, R^2 = 0.480, p = 0.013).

Table 2.3. Pearson's Product Correlation Matrix (*r*) for longitudinal and panoramic ultrasound variables.

		Longitudinal	
		MGT (cm)	**EI_{Long} (AU)**
Panoramic	**MGT_{Pan} (cm)**	0.892*	-0.424
	EI_{Pan} (AU)	-0.402	0.791*
	EI_{ROI} (AU)	-0.486	0.805*
	CSA (cm^2)	0.867*	-0.335

* significant correlation ($p \leq 0.05$)

2.1.4. Discussion

The results of the present study indicated that there was no systematic variability among ultrasound measurements obtained using two different US imaging techniques (p > 0.05), with the ICC and SEM% values ranging from 0.699-0.999 and 0.035%-0.327%, respectively (Table 2). Additionally, significant positive relationships were observed between like variables of echo intensity and muscle thickness, while only EI_{ROI} was negatively correlated with MGT_{Pan}, both of which are panoramic variables (Table 3). Muscle CSA was positively correlated with both MGT and MGT_{Pan}.

The current findings are comparable to the reliability of previous US studies examining the muscle characteristics of the medial gastrocnemius utilizing longitudinal and panoramic imaging, reporting ICCs of 0.720-0.991[80,84,103,112,113]. Specifically with the variables of MGT obtained from the longitudinal images, the current ICC value of 0.856 are in good agreement with studies examining MGT of the right MG of young children with an ICC of 0.977[113] and MGT of the MG poststroke patients with ICC values ranging from 0.967-0.973[112]. Similarly, the present study reports comparable values for CSA and EI_{Pan} to a previous investigation utilizing panoramic US, reporting ICCs values for CSA and EI of male participants MG of 0.720-0.914 and SEM% 3.680-5.830, respectively[84] and CSA ICC value of 0.991[80]. However, there appears to be a paucity of literature exploring the reliability of both longitudinal and panoramic imaging in health, college ages females. Since the participant demographics of the current study (healthy college ages females who are recreationally active) varies greatly from previous reliability studies reporting values for MGT of the MG, a direct comparison of results may not be appropriate. Taken together consideration of previous literature, the high ICCs (≥0.699) and relatively low SEM% values (≤0.035) observed in the present study demonstrated that US imaging via B-mode may be identified as a reliable alternative assessment technique to extended-field US imaging for measuring characteristics commonly associated with muscle size and quality.

The comparative analysis of like variables in the present investigation has demonstrated no significance difference in the variables among the two US imaging techniques. Of specific interest is the echo intensity variable that was obtained with the different US imaging techniques. Recent investigations have associated echo intensity with muscle quality and strength performance[95–100]. The current investigation employed a novel approach to image analysis for echo intensity, applying a 1×1 cm^2 ROI that was positioned approximately mid-muscle in both longitudinal and panoramic images. The comparative results demonstrated that this approach produced no significant difference in the value of echo intensity for EI_{Long} with EI_{Pan} ($p = 0.174$) and EI_{Long} and EI_{ROI} ($p = 0.189$). Additionally, when comparing echo intensity values analyzed in the more traditional method within the boundary of muscle fascia in a panoramic image (EI_{Pan}) with that of the echo intensity gained via the 1×1 cm^2 ROI placed approximately mid-muscle of the same panoramic image (EI_{ROI}) there was no significant difference ($p = 0.828$). This is of interest since echo intensity is a gray-scale assessment of tissue, US images that contain darker tissues results in a lower echo intensity and lighter tissues results in higher echo intensity[114]. Within transverse plane US images or panoramic images, muscles have a speckled appearance due to reflections of perimysial connective tissue which is moderately echogenic[114]. It might be suggested that employed the non-traditional methodology for assessing echo intensity within longitudinal US images, may have resulted in greater variance of gray-scale. Unlike transverse plane US images, longitudinal images reflections of the perimysial connective tissue results in linear, pinnate or triangular structures[114]. The alteration in US image orientation may lead to more visualization of the lighter colored perimysial connective tissue resulting in a higher echo intensity value. However, if this were the case one would expect differing values for echo intensity when the two US imaging techniques are compared.

2.1.5. Conclusion

Our findings demonstrate that both longitudinal imaging and panoramic imaging techniques for US assessments of characteristics of muscle size and quality of the MG (MGT, CSA, and EI) are reliable. Additionally, our results support that when the imaging techniques are compared for like variables of the MG, there is no significant difference in the values of echo intensity and muscle thickness. This study may help support the employment of longitudinal US imaging for the assessment of the clinically important variables of muscle thickness and echo intensity should the available US device lack the panoramic function or when assessing areas of the body that have a higher curvature.

2.2. Ultrasonographic study of the free Achilles tendon properties and gender.

2.2.1. Introduction

Ultrasound (US) imaging is a non-invasive, real-time method to evaluate soft tissue morphology, and is widely used in many medical specialties[80,115]. Widely used to assess tendinous tissue structure (i.e. length, thickness, cross-sectional area) and mechanical properties (i.e. stiffness, elasticity, strain), US can provide information that is relevant for researchers and clinicians alike[22,116–119].

It has been recognized that given the length and the three muscle structure of the associated triceps surae group, the Achilles tendon structure and pattern of strain differ throughout the length of the tendon[115,115,120–122]. A portion of the investigations that seek to identify the mechanical properties of the Achilles tendon utilizing US tend to focus on the musculotendinous junction (MTJ), or the muscle-tendon aponeurosis, for the purpose of tracking the convergent of tissues at the MTJ under contractions[2,24,31]. However, the free Achilles tendon is clinically relevant since Achilles tendinopathy and rupture commonly occur at the level of the free Achilles tendon and does not share the same properties as the MTJ[121,123–126]. Identified as the distal most portion of the Achilles tendon, the free tendon extends from the bony insertion of the posterior calcaneus to the most distal fascicles of the soleus muscle[127]. With Achilles tendinopathy and ruptures typically occurring 2-6 cm proximal to the calcaneal insertion, such injuries specifically affect the free tendon portion of the Achilles. Despite the clinical importance of the free Achilles tendon, there are only a few studies that specifically focus on baseline, resting images of the free tendon[127–130]. The purpose of this investigation was to provided additional data specific to the structure of the free Achilles tendon utilizing non-invasive US. Additionally, while free Achilles tendinopathy seems to affect males and females equally in the general population[131,132], degenerative or spontaneous Achilles tendon ruptures have been observed in more male individuals than females[124,132,133]. Through the assessment of transverse and longitudinal panoramic US images of the free Achilles tendon cross-sectional area, tendon thickness, echo intensity, and length (respectively), it is anticipated that no gender differences will exist for the structures of the free tendon in recreationally active young adults.

2.2.2. Methods

Participants

Twenty-three healthy, young adults (mean ± SD: 12 females: age = 19.4 ± 1.2 years, weight = 62.65 ± 11.21 kg, height = 164.57 ± 8.79 cm; 11 males: age = 20.7 ± 1.3 years, weight = 78.02 ± 15.89 kg, height = 166.01 ± 27.9 cm) volunteered to participate in this investigation. This study was approved by the University Institutional Review Board, with all participants completing and signing an informed consent document, and health history questionnaire prior to participation. None of the participants reported current or ongoing neuromuscular conditions or musculoskeletal injuries of the lower extremities within 6 months prior to testing. Participants were excluded if they engaged in structured aerobic and/or anaerobic exercise more than three days a week or if structured exercise exceeding three hours a week. Additionally, all participants were asked to refrain from engaging in any vigorous exercise of the lower extremity within 24 hours of their laboratory visit

Procedures

All participants had one laboratory visit, and based on availability 10 participants were asked to visit the lab a second time, separated from the first visit by 2-4 days at approximately the same time of day (± 2 h). Participants were placed in an unloaded position, laying prone on a padded examination table with their right low leg extended with knee extension and hip flexion at approximately 0-5° and the right ankle held in neutral (90°) position. All US images were gathered from the right Achilles tendon. Transverse and extended-field-of-view US images were obtained from all participants. All US assessments were performed after participants had rested for a minimum of 5 minutes to allow for any fluid shifts to stabilize[102,103].

Body Composition

Participants' height and weight were measured using a stadium scale and recorded in centimeters (cm) and kilograms (kg), respectively. Shank length was collected from all participants by measuring the distance between medial malleolus to lateral condyle[134] in centimeters. Low leg girth was also measured in cm for all participants using a fabric girth tape measure placed at the level of the greatest muscular protrusion in the lower leg with participants knee and ankle positioned at 90°.

Ultrasound Imaging

Prior to imaging, the osteotendinous junction (OTJ) was palpated and a non-toxic permanent marker was used to identify the OTJ on the skin. From this mark, a fabric measuring tape was placed and extended up the posterior low leg and held in place so researchers could place a mark on the skin at the level of 4cm superior to the OTJ mark (Figure 7).

Figure 2.4. OTJ identified with × with the free Achilles tendon measured 4cm proximal from OTJ marked with ●.

This was done to identify the level of the free Achilles tendon as a superficial guide for the transverse B-mode US imaging, ensuring only minimal fascicles of the distal most portion of the soleus muscle would potentially be included in imaging[127]. All US images were obtained using a B-mode US imaging device (GE Logiq E, Milwaukee, WI, USA) and a linear-array probe (Model 12L). To support image quality, transverse images were optimized with gain (50 dB), depth (3.5 cm), and frequency (10 MHz) set prior to image collection and held constant between participants[135–138]. Additionally, extended field of view US images were obtained from the OTJ to the gastrocnemius muscle. The extended-field-of-view images were captured by using the LogiqE function on the US device with the imaging probe maneuvered slowly and continuously along the surface of the skin following the linear trajectory of the Achilles tendon. A water-based gel medium was generously applied to every participants' posterior low leg to enhance US image quality. All US imaging were scanned and analyzed by the same experienced sonographer (L. E. P.).

Image Analysis

All US images were analyzed using a third-party image analysis software (ImageJ; Version 1.47v, National Institutes of Health, Bethesda, MD, USA). Prior to analysis each image was scaled individually from an area in pixels to cm with the straight-line function using a known distance of 1 cm. Image analysis was conducted from two transverse B-mode US images and two extended field of view US images, the average of the two variant images were used for statistical analysis.

Transverse images were analyzed for the free tendon thickness (TT; cm), cross-sectional area (CSA; cm^2), and echo intensity (EI; AU) (Figure 8). Free tendon TT was using the straight-line function that was initiated at the most superficial point of the visible free tendon and drawn to the deepest aspect of the free tendon, care was taken to ensure only tendinous tissue was included for measurement. The free tendon CSA was assessed using the polygon selection function by selecting a region of interest (ROI) of the free tendon that included as much of the tendinous tissue as possible without any surrounding fascia or aponeurosis. Additionally, utilizing this ROI, EI was assessed by gray-scale analysis using the standard histogram function. The EI values in the ROIs were calculated in arbitrary units (AU) on a scale of 0-255 (black – 0, white = 255).

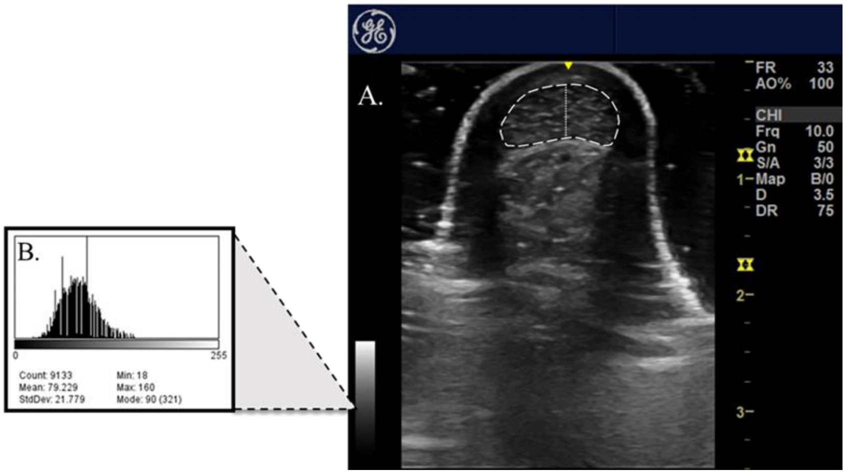


Figure 2.5. B-mode transverse US image A) identifies the ROI, represented by the long dash white line, encompassing the free tendon for the measurement of CSA and evaluation of EI; vertical line represents the TT measurement assessment from the superficial to the deep aspects of the free tendon B) demonstration of the histogram function assessing the gray-scale of the ROI to determine EI.

The extended field of view images were analyzed for free tendon length (TL; cm) (Figure 9). Using the segmented line function, this measurement was initiated just superior to the bony attachment at the OTJ with continuously linked segments made to the point of visible aponeurotic attachment from the soleus to the Achilles tendon[127]. For this measurement, the segmented line was placed within the image of the free tendon and followed the slight curvature of the superficial aspect of the free tendon.

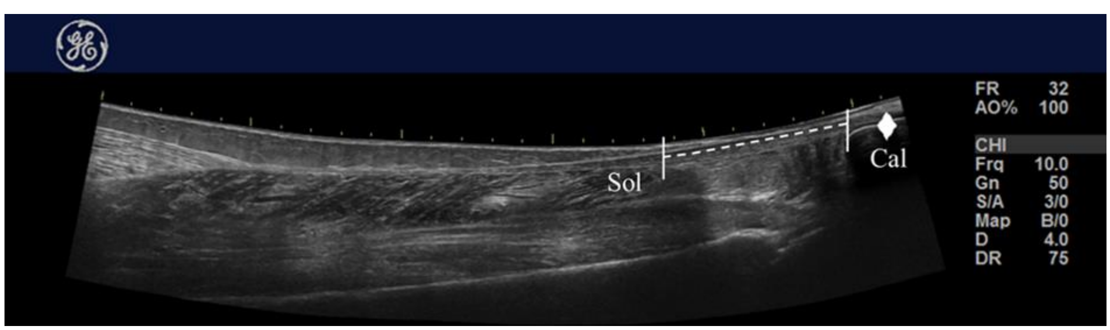


Figure 2.6. Extended field of view US image with the calcaneus (Cal) and the soleus (Sol) identified. The ♦ indicates the OTJ. The dashed line represents the measurement of the free tendon from just superior to the OTJ to the beginning of the soleus aponeurosis extending to the AT.

Statistical Analysis

Independent samples t-test were used to analyze differences in demographic and body composition characteristics. Separate 1-way analyses of variances (ANOVA) were employed to examine differences for gender by each free tendon structural variables (TT, CSA, EI, and TL). Pearson product-moment correlation coefficients (r) from mean data were calculated separately to examine the relationship between free tendon variables and body composition variables. Correlations were considered high when $|r| \geq 0.7$, moderate when $0.5 \leq |r| < 0.7$, low when $0.1 \leq |r| \leq 0.5$, and null when $|r| < 0.1$[111]. Interactions, main effects, and correlation analyses were performed using SPSS Statistics Version 27.0 (IBM, Armonk, NY, USA). Reliability was assessed using data from 10 participants with the intra-class correlation coefficient (ICC) model 2,k to calculate test-retest reliability. This model is a two-way random factor model that utilizes scores signifying the average of the k (number of trials) scores and used random and systematic error to support generalization to other laboratories and testers[83,84,108]. Standard error of measurement (SEM) and minimum difference (MD) values were based on recommendations by Weir[108]. Implemented guidelines for translating ICC values consisted of the following, excellent reliability = ICC ≥ 0.90, good reliability = ICC 0.75-0.9, moderate reliability = ICC 0.50-0.74, and poor reliability = ICC < 0.50[108,110]. The calculations of ICC, SEM, and MD were performed using a custom-written spreadsheet (Microsoft Excel, Microsoft, Redmond, WA, USA). An α level of $p \leq 0.05$ was considered statistically significant for al analyses.

2.2.3. Results

The means and standard deviations (SD) for body composition and free tendon variables have been organized by gender and can be found in Table 4 and Table 5, respectively. There was a significant main effect for body weight (males weight = 78.05±15.89 kg, females weight =

62.70±11.22 kg; $p = 0.013$). There were no significant differences for TT, TL, or CSA and body composition variables of height, shank length, and low leg girth between the two genders ($p > 0.05$).

Table 2.4. Body composition means (±SD) for female and male participants.

	Ht (cm)	Wt (kg)	SL (cm)	LLC (cm)
Females	164.57±8.79	62.70±11.22	39.75±2.55	36.21±2.16
Males	166.02±27.90	78.02±15.89*	39.73±2.80	37.00±4.19
Marginal Means	165.30	70.36	39.74	36.61

Ht= height; Wt= weight; SL = shank length; LLC = low leg circumference; * = *p-value* ≤ 0.05

Table 2.5. Free Achilles tendon variable means (±SD) among female and male participants.

	TL (cm)	CSA (cm^2)	TT (cm)	EI (AU)
Females	4.83±0.98	0.35±0.06	0.40±0.04	72.92±8.03
Males	4.56±1.02	0.39±0.10	0.43±0.06	49.76±5.82*
Marginal Means	4.70	0.37	0.42	61.34

Lt= free tendon length; CSA = free tendon cross-sectional area; Tk = free tendon thickness; EI = free echo intensity; * = *p-value* ≤ 0.05

One-way ANOVA analysis demonstrated free tendon EI was significantly different between the two genders ($p = 0.001$), with male participants demonstrating lower (darker) echo intensity compared to females who exhibited a higher (lighter) EI. Reliability statistics for the free tendon variables can be seen in summary in Table 6. Reliability analysis revealed excellent consistency for TL, CSA, TT, and EI with ICCs ranging between 0.84-0.985 and SEM reported as a percent (SEM%) values between 1.96-12.01%.

Table 2.6. Reliability statistics for free Achilles tendon variables from 10 participants.

TL (cm)	*p*-value	0.558
	$ICC_{2,k}$	0.840
	SEM (cm)	0.583
	SEM %	12.01
	MD (cm)	1.160
	MD %	23.89
CSA (cm^2)	*p*-value	0.476
	$ICC_{2,k}$	0.967
	SEM (cm^2)	0.016
	SEM %	4.48
	MD (cm^2)	0.044
	MD %	12.31
TT (cm)	*p*-value	0.451
	$ICC_{2,k}$	0.901
	SEM (cm)	0.023
	SEM %	5.71
	MD (cm)	0.045
	MD %	11.17
EI (AU)	*p*-value	0.609
	$ICC_{2,k}$	0.985
	SEM (AU)	1.550
	SEM %	1.96
	MD (AU)	4.590
	MD %	5.82

TL= free tendon length; CSA = free tendon cross-sectional area; TT = free tendon thickness; EI = free echo intensity; $ICC_{2,k}$ = intra-class correlation coefficient, model 2,*k*; MD = minimum difference to be considered real, expressed as absolute values and percentages of the mean; SEM = standard error of measurement expressed as absolute values and percentages of the mean.

Pearson's Product Correlation results are displayed within Table 7. There were no significant correlations between the free tendon variables and body composition for the females ($p > 0.05$). However, there was a significant correlation between free tendon TT and low leg girth ($r = 0.639$, $R^2 = 0.408$, $p = 0.034$) as well as TL and low leg girth ($r = 0.610$, $R^2 = 0.3721$, $p = 0.047$) for male participants.

Table 2.7. Pearson's Product Correlations (*r*) values for free Achilles tendon variables and body composition organized by gender.

	TL	**CSA**	**TT**	**EI**
Females				
Ht	0.157	0.328	0.166	-0.312
Wt	0.375	0.205	0.179	-0.068
SL	0.088	0.373	0.160	-0.227
LLC	0.217	0.259	0.186	-0.110
Males				
Ht	0.117	0.597	0.279	0.195
Wt	0.516	0.536	0.332	0.392
SL	0.573	0.450	0.301	0.037
LLC	0.610*	0.452	0.639*	0.001

Ht = height; Wt = weight; SL = shank length; LLC = low leg circumference; TL = tendon length; CSA = cross-sectional area; TT = tendon thickness; EI = echo intensity; * = *p-value* ≤ 0.05

2.2.4. Discussion

The primary findings of this study revealed that gender influences the free Achilles tendon tissue quality assessed by echo intensity values, with males demonstrating a significantly lower EI value (darker) free tendon on US assessment (Table 5; females: 72.92±8.03, males: 49.76±5.82). The secondary finding identified through this investigation demonstrated, that while low leg circumference was not different between males and females, low leg circumference for the male participants was the only body composition measurement that correlated with free Achilles tendon size, while none of the body composition variables were associated with the free tendon variables for females (Table 7). Additionally, despite mean body weight being significantly difference among participants, it was not associated with free Achilles tendon structure or quality (Table 4 & Table 7).

Gender Influence on Tendinous Tissue

To our knowledge, there has yet to be an exploration of the influence on gender on the free Achilles tendon in humans considering EI as a variable of tendon quality. The primary discussion on the relationship of gender and tendinous structure and mechanical properties poses conflicting evidence[116,139–143]. Along with conflicting evidence, the variations of the tendon examined, variables focused on, and methodology of assessing those variables make navigating previous literature challenging. Evaluating tendon stiffness and Young's elastic modulus have been the primary mechanical properties, while tendon cross-sectional area, thickness, and length have been the primary structural properties explored in previous literature. In some investigations, it was found that female participants exhibited lower Achilles tendon and patellar tendon stiffness compared to males[116,140]. Specific to the Achilles tendon, Kubo, Kanehisa, and Fukunaga found that women exhibited significantly lower stiffness, Young's elastic modulus, and hysteresis of the tendinous tissue compared to men, leading the authors to conclude that women had more compliant tendinous structure[116]. It should be noted that female participants in the mentioned study had significantly smaller body composition measures compared to the male participants. Specifically, the women were shorter in height and lower leg length and had a lower body mass, where the current investigation found only mean body weight was different among participants[116].

Similarly to the Kubo investigation, it was found the Achilles tendons of female participants were significantly less stiff by ~35% compared to male participants as well as being significantly shorter in length with a smaller cross-sectional area[116,143]. However, it was found that cross-sectional area of both gender groups was not associated with tendinous stiffness or Young's elastic modulus[143]. Additionally, Onambélé and colleagues found that females had significantly thinner patellar tendons compared to male subjects (88.6±5.4 vs. 112.5±5.9 mm^2, respectively) while both genders demonstrated no significant difference in patellar tendon length (females 48.9±2.7, males 46.5±2.0 mm)[140]. While the current study also found no significant difference in tendon length similar to the above investigations, the current study focused on the free Achilles tendon while the aforementioned Onambélé study involved the patellar tendon[140].

In contrast to the previous studies above, instead of gender; muscular strength, body composition, and tendon mass density have been reported to influence tendinous structure and mechanical properties[139,141,142]. Subjects' tendon length, elongation and stiffness were associated with subject mass, height and shank length[139]. Within Morrison and colleagues study, maximum Achilles tendon force was significantly related to subjects' mass but not height or sex, although, this was a poor positive relation ($R^2 = 0.01$)[139]. It has also been observed that while stiffness appears to be significantly less in females compared to males, it is muscular strength that influences this difference and not sex[139,141]. Maximum ankle torque, and thus the maximum Achilles tendon force, was significantly correlated with tendon stiffness ($R^2 = 0.19$)[139], while maximal voluntary isometric plantar flexion torque was significantly, positively corelated with Achilles tendon elongation ($R^2 = 0.15$)[141]. While the current investigation did not incorporate direct measures of plantar flexor strength, lower leg circumference was collected from all participants. Within the present study, lower leg circumference was positively correlated with free Achilles tendon length and thickness for only the male participants (Table 7; $r = 0.610$; $r = 0.639$; $p \leq 0.05$ respectively). Anthropometric measurements of limb circumference are readily used to estimate muscular strength, where limb circumference exhibits a positive correlation with muscular strength[144]. The correlation of lower leg circumference, as an indirect measure of muscle strength, with tendon length and tendon thickness may elude to the evidence that has demonstrated greater muscular strength in males than females[139,141]. However, free Achilles tendon length and thickness were no significantly different between the female and male participants in the current investigation, nor were there any body composition variables correlated with tendon variables for the female participants (Table 7). Another consideration that may impact tendon mechanical properties is tendon mass density as opposed to gender[142]. Utilizing 20 donor patellar tendons, 10 males and 10 females, it was observed that the mass density of the tendinous tissue was not significantly different between female and male tendons (1.68 g/cm^3 vs. 1.52 g/cm^3, respectively) with ultimate tensile strength and strain energy density being positively correlated with the mass density of the samples[142]. While there have been conflicting conclusions on the influence of gender on tendinous tissue properties, there seems to be agreement in previous evidence that does demonstrate females have lower mean tendinous stiffness compared male subjects and donors[116,139–143]. It should be stated that while several authors have examined the Achilles tendon, these investigations have been specific to the proximal MTJ and not the free Achilles tendon as in the current study[24,116,139,141,143,145]. Additionally, the present investigation was focused on structural characteristics and did not explore direct or indirect measurements of tendon mechanical properties.

Echo Intensity of Tendinous Tissue

The results within the present investigation demonstrated a gender interaction for echo intensity in which male participants had a darker free Achilles tendon (lower EI value; 49.76±5.82 AU) than the female participants (72.92±8.03 AU). Recently, the measurement of grayscale or echo intensity of tendinous tissues has been explored as a non-invasive, inexpensive assessment of tendon quality within the clinical setting. Several studies have sought to demonstrate echo intensity as a valuable metric in assessing and tracking clinical progress of tendinopathies[117,146–148]. Compared to the reliability found within the current examination, similar ICC and SEM values for the measurement of echo intensity in healthy Achilles tendons have already been reported in the literature (ICC = 0.94, SEC = 2.19)[149]. Within ex vivo animal models, echo intensity has been shown to have a linear relationship to tendon strain (tendon deformation under a given force) and a nonlinear relationship to stress[118,150,151]. Previous *in vivo* studies have found that in the case of tendinopathies or partial tendinous tears, echo-intensity is lower or the tendinous tissue appears darker in US images[117,146–148]. This darkening of the tendinous tissue has also been associated with increased tendon strain and decreased stiffness of the Achilles tendon[148]. When compared to the few investigations using healthy participants, the EI values for females in the current study (72.92±8.03 AU) are similar to those reported in the patellar tendon (86.8 AU) and the Achilles tendon (89.85 AU). However, the EI values for males in the current study are far below those previously reported for healthy participants (49.76±5.82

AU) [152]. There may be various explanations for the significantly lower EI values of the male participants in the current study, and since none of the male participants reported having any tendinous tissue injury the occurrence of tendinopathy is unlikely. Increased body mass has been associated with lower EI values in asymptomatic tendinopathy patients and in the uninvolved side of patients living with insertional Achilles tendinopathy[146,148]. While there was no interaction for gender by body mass in this current investigation, the mean body mass of the male participants was significantly greater than the female participants (Table 4). Additionally, given that EI has been observed to have a linear relationship with longitudinal and transverse tendinous strains[150,152], and previous literature has found that male tendinous tissue exhibits significantly greater stiffness[116,139–143], while producing greater muscular stress or force[139,141] the deficit in the EI values of male participants could be directly related to the mechanical properties of the free Achilles tendon. Further investigations are needed to decipher the exact relationship that may exist between tendinous tissue structure and mechanical properties with EI in the human model.

Acknowledgements

To our knowledge, this is the first investigation that utilized echo intensity as a measure of tendon quality in healthy, young adults exploring gender differences of the free Achilles tendon. It has been noted that the use of US can be heavily operator-dependent and may not directly reflect true structural or mechanical properties of tissues[148,153,154]. Although, the inter-rater reliability (ICC = 0.840-0.985, SEM% = 1.96-12.01, MD% = 5.82-23.89) from the current study does demonstrate the US variables as reliable, only one experienced investigator performed US image collection and analysis. Specifically addressing EI, as mentioned above the majority of *in vivo* human investigations utilizing EI as an assessment of tendinous tissue are associated with tendinopathies or tendon injury, limiting the resources for reference values of EI in healthy, recreationally active young adults.

2.2.5 Conclusion

The present investigation found that free Achilles tendon echo intensity was significantly decreased in healthy, young adult males when compared to healthy, young adult females, supporting that echo intensity values may be sex-dependent. Additionally, lower leg circumference often used as an estimate of muscular strength was positively related to free Achilles tendon length and thickness only for the male participants. The findings from this study along with previous literature may demonstrate that greater triceps surae muscular stress is applied to the free Achilles tendon in male participants resulting in lower echo intensity values.

2.3. Clinically relevant examination of distal Achilles tendon musculotendinous junction and medial gastrocnemius properties with postural stability.

2.3.1. Introduction

The Achilles tendon (AT), which is the singular tendon of the two gastrocnemius muscles along with the soleus muscle, is the strongest and largest tendon in the human body[155,156]. The cooperative function between this muscle-tendon complex exhibits a spring-like characteristic during walking, running, and jumping[14,15,157]. Additionally, the muscle-tendon complex can influence joint flexibility as both tissues are known to adapt, structurally and mechanically, to a reduction or an increase in mechanical loading leading to decreases or increases in specific tension and joint stiffness[145,145,158,159]. It is suggested that adaptations of tendon mechanics and structure occur slower than those of muscle morphology and function with increased mechanical loading and training which may be one aspect of tendinous injury[124,132,145]. The stiffness (ability to resist deformation) and elasticity (ability to return to original state following deformation) of muscle and tendon represents tissue mechanical properties on the longitudinal axis[2]. Commonly, mechanical properties of the AT are most often assessed for stiffness and elasticity through measurements of shear wave elastography which can be very costly[121]. Additionally, tendon elongations during isometric contraction are imaged via ultrasound (US), where elongations to tendon force estimations gathered from force plates or dynamometry measurements of joint force or torque can be employed to determine stiffness and the elastic modulus[21,121]. Some researchers have called for a cost-effective simplification to the approach for measuring tendinous mechanical properties and to seek out methods that allow for the isolation of tendon and muscle properties separately *in vivo* to better understand each tissue and support clinically relevant outcomes[121,160,161]. A novel portable device, MyotonPRO, has recently been employed in place of the more complicated and expensive methods for assessing tissue mechanics. Numerous researchers have assessed the MyotonPRO as a reliable and valid device for measuring tissue tone, elasticity, stiffness, creep, and time to relax[162–166]. Additionally, this device has been able to detect changes in tendinous stiffness and elasticity following a series of post activation potentiation activity as well as reduced transverse stiffness in tendinopathic patients compared to control participants[167,168].

As mentioned, US has been readily used not only for determining tendon elongation in the presence of contraction, but it has been widely used to assess muscle size and architecture[84,93,103,169,170]. As a cost effective alternative to computed tomography and magnetic resonance imaging, Brightness-mode (B-mode) and panoramic US have allowed for the examination of muscle and tendon architecture *in vivo* assessing changes associated with exercise interventions, neuromuscular diseases, and aging[91,171,172]. Specific to muscle imaging, diagnostic images of muscle cross-sectional area, fascicle length, pennation angle, and thickness have been associated with muscular strength[173–175]. Moreover, US has been able to provide details of tendon structure in addition to the above mentioned tendon elongation[40,129,130,137,153]. Clinically, US has been able to demonstrate that limitations in mobility and functionality linked to changes in muscle size and structure along with alterations in tendon size and structure associated with tendinopathy and increased compliance negatively impacting the series elastic component resulting in impaired strength and rapid movements [34,87,128,132,145,176].

The lower limb musculature is often thought to play key roles in standing, with lower leg muscular strength aiding in postural stability[177–179]. Postural stability is a complex task that results in the integration of sensory and motor function of the neuromuscular system, with stability decrements stemming from peripheral and central nervous system changes and/or musculoskeletal system alterations[180]. Musculoskeletal deficits of both tendon and muscle have been linked to decreased balance performance and perturbation recovery. Decreases in low leg muscular strength and tendon stiffness have been found to influence balance recovery stepping[36]. Additionally, it has been proposed that increases in low leg muscular strength and tendinous stiffness could improve postural correction through enhanced energy transfer through the series elastic component[178,181,182] .

Combining the above observations, the present study sought to utilize the clinically relevant MyotonPRO device to assess passive mechanical properties of stiffness and elasticity of the muscle-tendon complex and examine the relationships between the passive mechanical properties with the structural characteristics gained from US. Furthermore, given the importance of lower leg muscle-tendon complex on postural stability, this investigation also aimed to explore the association of muscle-tendon complex passive mechanical properties and structural properties with postural stability performance.

2.3.2. Methods

Participants

A total of 10 healthy, young adult males (mean±SD: age = 20.7±1 year, mass = 75.11±13.33 kg, height = 175.75±8.26 cm) volunteered to participate in this investigation. This study was approved by the University Institutional Review Board, with all participants completing and signing an informed consent document and health history questionnaire prior to participation. All participants reported no current or ongoing neuromuscular, musculoskeletal, or musculotendinous conditions or injuries specific to the lower extremities within six months prior to testing. Participants were excluded from the investigation if they engaged in structured aerobic and/or anaerobic exercise more than three days a week of if structured exercise exceeded three hours a week. All participants were asked to refrain from participating in any vigorous lower body exercise within 24 hours of their laboratory visit.

Procedures

Participants reported to the laboratory for a single testing day. Upon arrival to the laboratory participants were asked with which leg/foot they would kick a ball with in order to identify their dominant limb[183]. All participants indicated they would use their right leg/foot, as such their right limb was used for further investigation. A brief familiarization procedure followed, this included an introduction to the balance device where participants performed a single trial of 20-s single leg balance on the dynamic platform of the devise set to allow moderate mobility of the platform. Following familiarization, participants were asked to lay prone on a padded examination table with their right low leg extended with knee extension and hip flexion at 0°, their right ankle was held in neutral (90°) position by researchers. All ultrasound (US) images were gathered from the right Achilles tendon (AT) and medial gastrocnemius (MG) with transverse images collected from the AT and MG, panoramic images were obtained of the MG only. US imaging took place following a minimum of 5 minutes of quiet rest in the prone position to allow for any fluid shifts to stabilize[102,103]. Proceeding US imaging, participant positioning was maintained for myotonometric measurements, collected from the proximal AT and the MG muscle belly. Postural stability assessments were performed approximately 20 minutes after participants arrived at the laboratory and were conducted at the completion of the soft tissue evaluations.

Ultrasound Imaging

US images were obtained using a B-mode US imaging device (GE Logiq E, Milwaukee, WI, USA) and a linear-array probe (Model 12L) of the right low leg. Images were optimized for image quality including gain (50 dB), depth (6 cm for MG; 3.5 cm for AT), and frequency (10 MHz) and were set prior to testing and held constant between participants[84,104]. To ensure all measurements were taken from similar regions of the MG and proximal AT, the MG muscle belly and the AT musculotendinous junction were located on each participant prior to imaging. For identification of the MG muscle belly, a mark on the skin with a non-toxic permanent marker was placed on participants' skin at the level of 1/3 the low leg circumference at 10 cm distal to the tibial tuberosity and was maintained for every measurement of the MG[105]. The osteotendinous junction (OTJ) was palpated and a mark was placed used to identify the OTJ on the skin. From the OTJ mark, a fabric measuring tape was placed and extended up the posterior low leg and held in place and a mark was made on the skin at the level of 6 cm superior to the OTJ[163], this served as the location for US and MYO assessment of the AT (Figure 10A & 10B).

This was done to approximate the location of the distal musculotendinous junction (MTJ) which consists of the convergence of the tendon collagen fibers with the MG, lateral gastrocnemius, and soleus muscle fibers or aponeurosis[127]. Following the application of a water-based medium, the distal MTJ, with the convergence of tendon, aponeuroses, and muscle fibers, was confirmed by US. The US probe was kept perpendicular to the skin, and a generous amount of water-soluble transmission gel was applied to both the probe and the participants' skin to provide acoustic coupling without depressing the dermal surface.

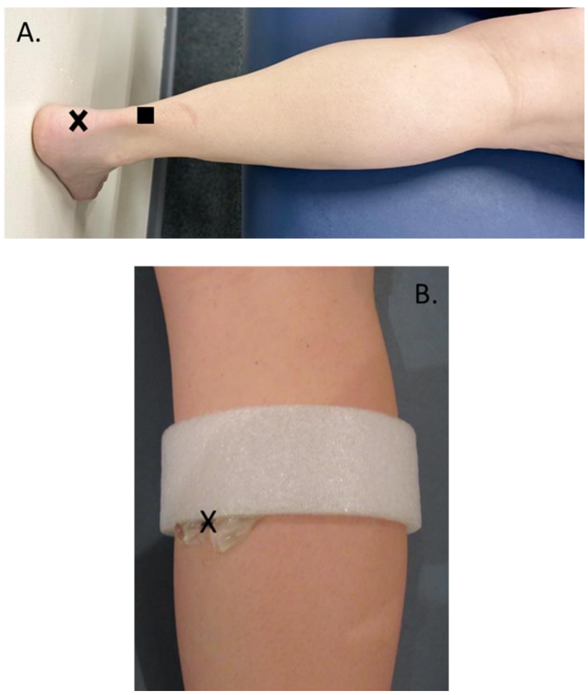


Figure 2.7. A) Identification of the OTJ (×) and the distal MTJ measured 6cm proximal to the OTJ marked with ■ B) demonstration of the placement of the foam guide along the MG to aid US panoramic image collection.

Panoramic US images were collected of the MG at the muscle belly mark on participants' skin. Transverse images were obtained from the proximal AT, again at the level of the distal MTJ mark on participants' skin. For the panoramic US imaging, the US probe was maneuvered manually at a slow and constant rate along the surface of the skin from the lateral to the medial aspect of the gastrocnemius muscle using the LogiqView function (GE LogiqE), which is a special function on the US imaging device[83,84]. To support image continuity and quality, a foam guide was positioned bisecting the MG muscle belly mark on participants' skin (Figure 10B). To support imaging reliability, all US images were obtained by the same experienced sonographer (L. E. P.). If non-uniform defects of the MG or AT could clearly be identified on US imaging, participants were informed and subsequently those images were not used for further analysis. All US images were obtained with participants in the prone position identified previously, with the ankle joint held stationary at neutral (90°) by the researcher conducting the scans. Two of each image variation were obtained for every participant.

Image Analysis

All US images were analyzed utilizing a third-party image analysis software (ImageJ; Version 1.47v, National Institutes of Health, Bethesda, MD, USA). Prior to analysis, each image was scaled individually from an area in pixels to cm with the straight-line function using a known distance of 1cm for MG images and AT images.

Panoramic US images were analyzed for MG cross-sectional area (CSA_{MG}; cm2), MG echo-intensity (EI_{MG}; AU), and MG thickness (MGT; cm) (Figure 11A).

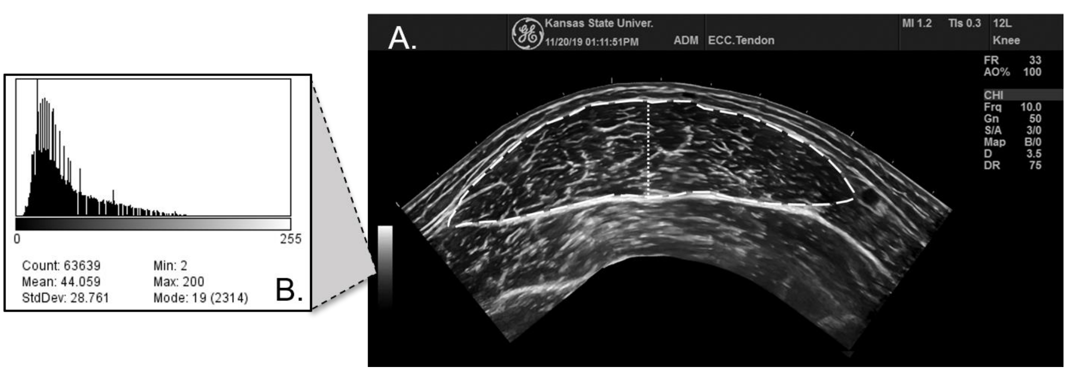


Figure 2.8. Panoramic US image of the MG with CSA ROI boundary identified by the white long dash line and the measurement of MGT identified by the short square dash line B) example of the histogram related to the assessment of the EI captured from the ROI.

The transverse images of the proximal AT were analyzed for AT thickness (TT; cm), AT CSA (CSA_{AT}; cm^2), and AT EI (EI_{AT}; AU) (Figure 12A).

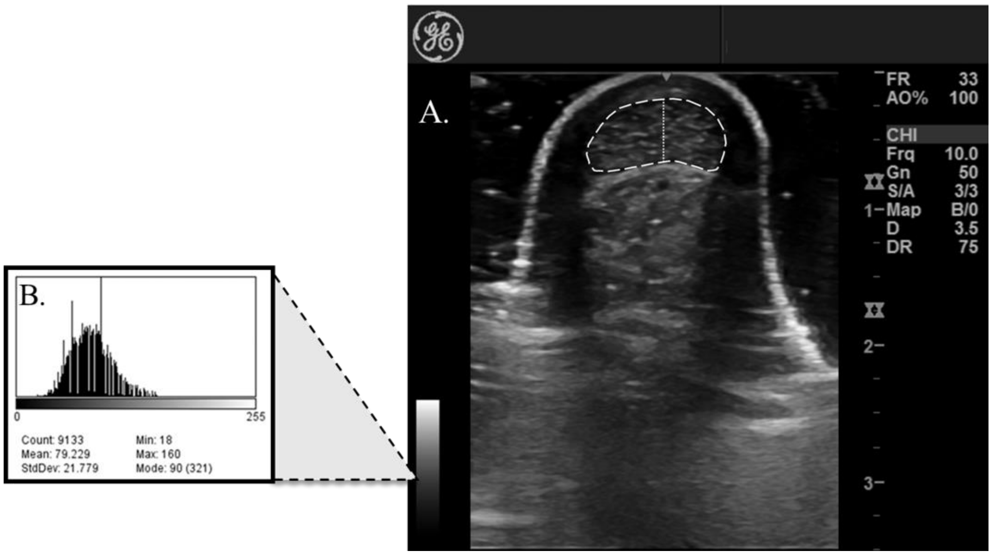


Figure 2.9. Transverse US image of the distal MTJ with CSA ROI boundary identified by the white long dash line and the measurement of TT identified by the short square dash line B) example of the histogram related to the assessment of EI captured from the ROI.

Thickness measurements were gathered using the straight line that was placed at the approximate center of the superficial aspect and drawn to the deep aspect of the MG and AT on imaging. All CSA measurements utilized the polygon selection function by selecting a region of interest (ROI) of the AT and MG that included as much of the tendinous and muscular tissue, respectively, without any surrounding fascia or aponeurosis. From this same ROI, EI was assessed by gray-scale analysis using the standard histogram function (Figure 11B & 12B). The EI values in the ROIs were calculated in arbitrary units (AU) on a scale of 0-255 (black = 0, white = 255). Two measurements were obtained for every variable from two panoramic and two transverse images, and the average of these measurements were used for statistical analysis.

Myotonometry

We utilized the non-invasive MyotonPRO (Myoton AS, Tallinn, Estonia) to assess tissue stiffness and elasticity. This device is a small, handheld and digital palpation device which delivers a controlled preloaded 0.18 N initial compression of superficial tissues followed by additional 15-ms impulses of 0.40 N of mechanical force[162]. The subsequent impulses induce a damped or decaying natural oscillation in the acceleration signal which is analyzed for passive mechanical tissue properties[162,166,167]. With participants positioned in the same manner as ultrasound imaging collection, myotonometric assessments were gathered from the MG and AT utilizing the marks on participants' skin previously explained (Figure 13A & 13B).

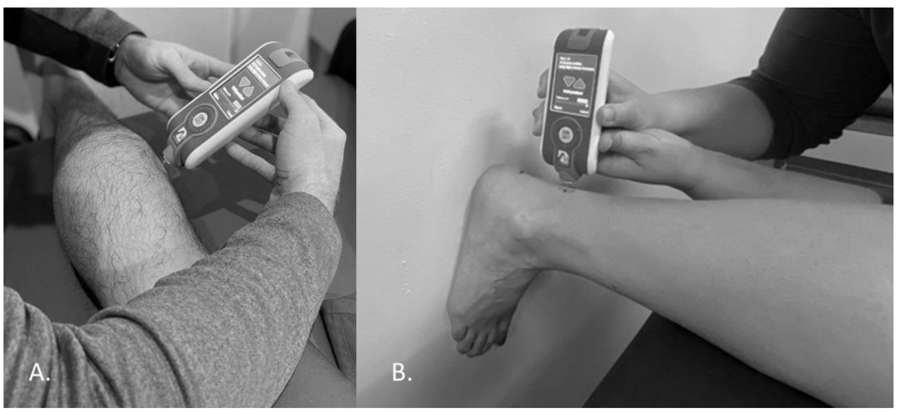


Figure 2.10. A) placement of MyotonPRO on the MG for measurements of stiffness and elasticity B) placement of MyotonPRO on the AT for measurements of stiffness and elasticity.

The passive mechanical properties of interest in this investigation were stiffness and elasticity. Tissue stiffness can be characterized by the resistance of soft tissue to an external force and the ability of that tissue to restore its initial shape, measured in Newtons per meter (N/m)[167]. Tissue elasticity is the quantity of tissue deformation for a given stress or force applied, and is traditionally defined as the slope of the stress-strain curve (Young's elastic modulus)[2,184]. Increases in the traditional Young's elastic modulus indicate the tissue being assessed is less elastic or less compliant[185]. The MyotonPRO characterizes tissue elasticity by the inverse of the logarithmic decrement of the dampened oscillations, and is expressed in arbitrary units (AU)[162,164,167]. Similar to the interpretation of the Young's elastic modulus, the smaller the decrement value, less mechanical energy is dissipated resulting in the tissue being classified as having higher elasticity[167,186]. Following depression to the preloaded 0.18 N, the device delivered five additional oscillations to the previously marked ROI on the AT and MG. This was repeated twice for each tissue on all participants, the average values for stiffness and elasticity were used for statistical analysis. Participants were asked to remain as still and relaxed as possible during myotonometric assessments with their right ankle held in neutral (90°).

Postural Balance

Single leg postural stability was assessed utilizing the Biodex Balance System SD (Biodex Medical Systems, INC, Shirley, NY, USA) which quantifies participants' ability to maintain dynamic postural stability on an unstable multiaxial platform. The testing platform of this device consists of an integrated spring resistance system that allows for the platform to be adjusted from static, stationary to passively dynamic allowing for 20° of mobility[187]. Spring resistance levels range between 1 (most stable) to 12 (least stable). Prior to testing, participants were asked to remove their footwear and step onto the locked, stable balance platform and take a comfortable single leg stance position on their dominant limb with slight knee flexion (~5-10°), looking straight ahead with their hands on their hips[187–190]. We then proceed to make minor adjustments to participants foot placement using the coordinates on the platform's grid and their center of pressure was centralized using the onboard screen as a guide[191]. The platform grid coordinates for heel and great toe placements were used to record final foot placement for all participants, and participants were asked not to move their right foot once final placement was confirmed. All participants completed 3 trials of 20-s of dynamic balance conditions on the right leg in which the platform moved freely at a spring resistance of 6. Participants were instructed to maintain an even, level platform throughout all balance trials, and maintaining a forward gaze with hands on their hips (Figure 14). The onboard screen was obstructed during testing so no visual ques supported participant performance. The opposing limb was kept out of contact from the right leg, and participants were asked to maintain opposing knee flexion of approximately 90°. Between each trial, participants were allowed to use their left limb for support but kept their right foot positioned on the platform. System software calculated each participants' dynamic balance performance of the anterior/posterior index (API), medial/lateral index (MLI), and overall stability index (OSI) for all three trials. The device then calculated the average of all the trials which was then used for statistical analysis. Postural stability scores are numeric values without units of measurements. Lower postural stability scores indicate less movement of the dynamic platform, indicating improved stability.

Figure 2.11. Demonstration of balance posture assumed by all participants during all postural stability trials.

Statistical Analysis

Separate paired-samples t-tests were performed on like variables of structure means of (TT-MGT and CSA_{AT}-CSA_{MG}), tissue quality (EI_{AT}-EI_{MG}), and passive mechanics ($Elast_{AT}$-$Elast_{MG}$ and $Stiff_{AT}$-$Stiff_{MG}$) of the AT and the MG. Pearson Product Correlation Coefficients (r) were used to examine the relationship between AT, MG characteristics and the balance variables. Correlations were considered high when $|r| \geq 0.7$, moderate when $0.5 \leq |r| < 0.7$, low when $0.1 \leq |r| \leq 0.5$, and null when $|r| < 0.1$[111]. Level of significance was identified as a *p-value* of ≤ 0.05. All analyses were conducted using SPSS Statistics Version 27.0 (IBM, Armonk, NY, USA).

2.3.3. Results

A single participant had clear AT defects upon US imaging, when informed of this the participant identified a history of tendinopathy but had been asymptomatic for approximately one year prior to the current study. For this reason, this participant's data was not included in further analysis. As such, a total of 9 healthy, young adult males (mean±SD: age=20.7±1 year, mass=74.54±14.00 kg, height=164.33±30.87 cm). The means and standard deviations of all tissue variables has been provided in Table 8.

Table 2.8. Means (±SD) for Achilles tendon and medial gastrocnemius tissue characteristics as measured via ultrasound imaging and myotonometric evaluation.

	CSA (cm^2)	**Thickness (cm)**	**Echo-intensity (AU)**	**Stiffness (N/m)**	**Elasticity (AU)**
Distal MTJ	0.279 (0.051)	0.32 (0.04)	47.46 (6.59)	1190.78 (144.07)*	0.741 (0.103)
Medial Gastrocnemius	13.64 (4.31)*	2.128 (0.350)*	48.72 (10.98)	369.27 (43.37)	0.832 (0.135)

* = p-value ≤ 0.05; CSA = cross-sectional area (cm2)

Comparison of like variable means of the AT and MG demonstrated that structural and passive mechanical values of the separate soft tissues are significantly different (CSA_{AT}-CSA_{MG} p = 0.001; TT-MGT p = 0.001; $Stiff_{AT}$-$Stiff_{MG}$ p = 0.001), while tissue quality assessed via gray-scale of echo-intensity exhibited and elasticity no significant difference between the tissue types (p = 0.792; p = 0.088). Pearson Product Correlation Coefficient results for AT and MG tissue properties as well as tissue properties and balance performance variables can be seen in Tables 9 and 10, respectively.

Table 2.9. Pearson Product Correlation (*r*) demonstrating relationship between Achilles tendon and medial gastrocnemius characteristics.

	CSA_{MG} (cm^2)	MT (cm)	EI_{MG} (AU)	$Stiff_{MG}$ (N/m)	$Elast_{MG}$ (AU)
CSA_{AT} (cm^2)	-0.019	0.022	-0.258	0.537	0.129
TT (cm)	-0.114	0.063	-0.770*	0.447	-0.558
EI_{AT} (AU)	0.398	0.117	0.075	0.529	0.185
$Stiff_{AT}$ (N/m)	-0.186	-0.252	-0.16	-0.052	-0.438
$Elast_{AT}$ (AU)	0.773*	0.717*	0.479	-0.083	0.33

***** = p-value ≤ 0.05

CSA_{AT} = cross-sectional area of the Achilles tendon; TT = tendon thickness; EI_{AT} = echo-intensity of Achilles tendon; $Stiff_{AT}$ = stiffness of Achilles tendon; $Elast_{AT}$ = elasticity of Achilles tendon; CSA_{MG} = cross-sectional area of medial gastrocnemius; MT = muscle thickness; EI_{MG} = echo-intensity of medial gastrocnemius; $Stiff_{MG}$ = stiffness of medial gastrocnemius; $Elast_{MG}$ = elasticity of medial gastrocnemius

Significantly high, positive correlations were demonstrated for $Elast_{AT}$ (the inverse of the logarithmic decrement) with CSA_{MG} and MGT (r = 0.773, p = 0.015; r = 0.717, p = 0.030, respectively) while MGT and CSA_{MG} were also highly correlated (r = 0.724, p = 0.027). A high, negative correlation presented between TT and MG_{EI} (r = -0.770, p = 0.015). Finally, a significantly high, negative relationship was found between MGT and API (r = -0.747, p = 0.021).

Table 2.10. Pearson Product Correlation (*r*) for tendon and muscle characteristics with balance performance measures.

	OSI	API	MLI
CSA_{AT} (cm^2)	-0.418	-0.233	-0.263
TT (cm)	-0.552	0.059	-0.638
EI_{AT} (AU)	0.243	0.139	0.125
$Stiff_{AT}$ (N/m)	-0.167	0.047	-0.321
$Elast_{AT}$ (AU)	0.17	-0.359	0.621
CSA_{MG} (cm^2)	-0.151	-0.511	0.387
MT (cm)	-0.153	-0.747*	0.521
EI_{MG} (AU)	0.095	-0.135	0.375
$Stiff_{MG}$ (N/m)	-0.249	0.118	-0.442
$Elast_{MG}$ (AU)	0.273	-0.293	0.475

***** = p-value ≤ 0.05

CSA_{AT} = cross-sectional area of the Achilles tendon; TT = tendon thickness; EI_{AT} = echo-intensity of Achilles tendon; $Stiff_{AT}$ = stiffness of Achilles tendon; $Elast_{AT}$ = elasticity of Achilles tendon; CSA_{MG} = cross-sectional area of medial gastrocnemius; MT = muscle thickness; EI_{MG} = echo-intensity of medial gastrocnemius; $Stiff_{MG}$ = stiffness of medial gastrocnemius; $Elast_{MG}$ = elasticity of medial gastrocnemius; OSI = overall stability index; API = anterior-posterior index; MLI = medial-lateral index

2.3.4. Discussion

The paired-samples analysis demonstrated that the muscular and tendinous tissues did not differ in terms of elasticity and echo intensity properties which may be due to the mix of muscular fascicles and aponeurosis with collagen fibers observed at the MTJ where tendinous variables were collected[126,130,163]. Interestingly, AT thickness had a significantly high, negative relationship with echo intensity of the MG. With incorporation of the novel MyotonPRO device, the findings from this investigation demonstrated that only the passive mechanical property of elasticity in the AT ($Elast_{AT}$; the inverse of the logarithmic decrement) correlated with two size characteristics of the MG (CSA_{MG} and MGT). Additionally, a single muscular property, thickness, was associated with postural stability performance only pertaining to the API value. To the knowledge of investigators, this was the first study to explore the relationship between these properties obtained via myotonometric and US imaging of the muscle-tendon complex along with postural stability performance.

Muscle and Tendon Variables

Anatomically, the proximal portion of the Achilles tendon converges with the aponeurosis of the MG as opposed to the more distal aspect of the Achilles tendon which shares connection with the MG as well as the soleus and the lateral gastrocnemius[73,121]. It is widely understood that strain patterns throughout the Achilles tendon are regionally specific, accounting for the line of stress from the contraction of the three muscles of the triceps surae as well as a mild twisting structure of the Achilles tendon[75,115,121,126,160,192]. Muscular material and structural properties are often associated with Achilles tendon structure. For example, MG elastic modulus determined by shear wave US was found to positively correlate with the more distal aspect of the AT but there was no significant association with MG elasticity and the proximal AT[160]. Additionally, the proximal AT has been observed to have a larger CSA than the mid to distal tendon, and under ramped contraction, larger CSA reductions were observed in the proximal AT as opposed to the mid or distal tendon, inferring larger longitudinal elongations in the proximal region of the AT compared to the mid and distal regions[121]. In the current study, it was found that larger measures for MG thickness and CSA were highly correlated with increases in logarithmic decrement, which indicated lower elasticity of the AT (CSA_{MG}- $Elast_{AT}$ $r = 0.773$, $p = 0.0.15$; MGT- $Elast_{AT}$ $r = 0.717$, $p = 0.030$). As a viscoelastic property of tissue, elasticity is the measure of both deformation and force application, to which a higher value of elasticity would result in greater compliance of the tissue and excessive compliance is associated with decreased force production and ballistic activity[141,185,193]. The structural variables of muscular thickness and cross-sectional area are often linearly reflective of muscular strength, thus a decrease in tendinous elasticity would indicate greater muscular stress be applied to elongate or deform the tendinous tissue[21,26,73,173,174,194]. Decreases in elasticity as measured via Young's elastic modulus (higher Young's elastic modulus value represents decreased tissue elasticity) have been reported following resistance training in young and old participants, further demonstrating that increases in muscle hypertrophy and strength assessed in previous studies influence tendinous adaptation[24,25,145,185,195]. The relationship found in the current study between muscle size and tendon elasticity would seem to support previous investigations into the relationship between muscle size and strength with tendinous adaptations were tendon elasticity is traditionally measured employing the calculation of Young's elastic modulus[22,25,185,196]. The average values for elasticity as the inverse of the logarithmic decrement found in the current study of recreationally active males (0.741±0.103) are similar to those found in the control group of highly trained uninjured middle aged badminton players (dominant limb: 0.64±0.22) from a recent investigation into structural and mechanical properties of the Achilles tendon between post-operative tendon rupture repair badminton players[197]. Additionally, the elasticity values in the present study are similar to those obtained of the Achilles tendon in healthy, young adult females (0.8±0.1)[198].

With regard to EI, it is often used to assess the quality of muscle. It has been observed that a lower EI muscle value, observed as a darker tissue on US images, is associated with increased muscular strength[97,99,199–202]. Within the current study, a high, negative correlation was observed between AT thickness and MG EI (r = -0.770, p = 0.015), demonstrating that at lower values of muscle EI, the AT would be thicker. As an indirect variable of muscle, this lower EI would imply an increase in the number of muscle fibers and a decrease in intramuscular fat resulting in a more efficient or stronger muscle which may increase the stress applied to the tendinous tissue. Increased AT thickness within a tendinopathic population results in an increase in tendon stiffness outside of the optimal threshold for elastic energy storage and transfer and if not addressed could lead to tendinous tissue failure resulting in partial or full rupture[124,127,132,148]. In the absence of any known or observed tendinopathy among participants in the current study, increased AT thickness is observed as a response to habitual loading and limb dominance[115,203]. The association between lower EI and thicker AT in the present study may reflect an optimal state for the tendinous tissue to store and transfer elastic energy under increased muscular performance of the dominant limb assumed from the lower EI values of the MG. This relationship between MG EI and TT would also seem to support the correlations found between muscle size variables with decreased tendon elasticity as mentioned above. While the methodologies of the current study were non-invasive and may be seen as indirect measures of muscle strength and tendon behavior, the relationships found between muscle and tendon within the current study would seem to support previous research that has identified muscle stress or strength are determinates of tendon structural and mechanical adaptation[24,25,145,185,195,196].

Tissue Characteristics and Postural Stability

It is generally thought that the muscles of the lower limbs may play a key role in standing where calf muscles specifically shorten and lengthen to control sway and calf muscle strength observed to determine, in part, postural stability[177,178,204,205]. Additionally, dorsiflexion range of motion as a result of musculotendinous stiffness of the plantar flexors has been found to significantly influence dynamic balance to where decreased dorsiflexion leads to poor balance performance[206]. Decreased Achilles tendon stiffness and elastic modulus have also been associated with poor tandem and single-leg balance tasks in middle-aged and older adults[177]. Within the current investigation, only one muscle-tendon complex variable was observed to correlated with postural stability performance. Medial gastrocnemius thickness presented as having a high, negative correlation with API (r = -0.747, p = 0.021), demonstrating that at greater MG thickness, participants had improved postural stability performance in the anterior-posterior plane. Along with the correlations found between MGT and MG CSA with $Elast_{AT}$, the correlation between MGT and API may demonstrate improved force control through an increase in intrinsic tension and neuromuscular efficiency[11]. It has been suggested that as muscular structure improves, rate of velocity and torque development are enhanced allowing for quicker adjustments with stability oscillations[178,207]. Additionally, MGT as a variable associated with muscular strength in healthy individuals may lead to an increase in specific tension[26,208]. An increase in specific tension would aid in increasing ankle joint stiffness and potentially limit active dorsiflexion which would decrease anterior-posterior corrections[180,206,209]. These are just postulations; continued investigations are needed to further explore the relationship between MGT and API.

Acknowledgements

A consideration that should be made for the current investigation would be that due to the small convenience sample of participants, the effect size of the results may not be large enough for significant applications to broader populations. Additionally, the ability to non-invasively assess tissue structure, quality, and passive mechanics is important to the safety of research participants and clinical patients. However, direct implications from non-invasive procedures should be considered carefully. It should also be noted that the tendinous region of interest utilized in this study is closely associated with the more distal aspect of the Achilles tendon MTJ, which would include the convergence of all three muscles of the triceps surae with the tendon[73,121]. This region of the Achilles tendon is distinctly different from the more proximal medial gastrocnemius-Achilles tendon MTJ, and the most distal aspect of the Achilles tendon which is often referred to the free Achilles tendon that is predominantly tendinous collagen fibers with no muscular fascicle or aponeurotic extension. Despite the interconnection of the triceps surae at the tendinous region of interest observed in this investigation, only characteristics of the MG were assessed given the superficial location compared to the soleus and the dominance of the MG in plantarflexion strength and performance as compared to the lateral gastrocnmeius[132,160].

Traditionally, tendon elongation, stiffness, and elastic modulus are measured utilizing US imaging of the MTJ during ramped contraction, shear wave elastography, and calculating Young's elastic modulus from CSA and length tendon measures at rest and following contraction[22,24,121,126,177,210]. With regard to the variables assessed via myotonometry, good to excellent reliability and validity have been reported for both muscular and tendinous tissue demonstrating that such variables can be used for inferences on tissue mechanics[162–166].

Moreover, within this investigation US imaging and MYO assessments were collected from participants in the typical non-weight bearing, rested position with the participants lying down[103,211,212]. Recently, in a study exploring the relationship between gastrocnemius muscle mechanical properties with postural control, myotonometric assessments were collected from participants in the standing position in order to account for the role of gravitational and ground forces acting on the body[213]. Also, our laboratory has explored capturing US images of medial gastrocnemius structural properties with participants in a supported single-leg, weight-bearing position and found measurements for muscle thickness, fascicle length, and pennation angle are reliable, correlate with postural stability, and vary from mean values obtained in the traditional, non-weight bearing position[214]. While fluid pooling is a concern with assessments obtained in the upright position, US and MYO assessments collected in such position may provide a better predictability and association with performance measures and activities of daily living performed upright.

2.3.5. Conclusion

In summary, the present study demonstrated that medial gastrocnemius size (CSA and MGT) are positively associated with Achilles tendon passive mechanics (logarithmic decrement). This finding supports previous evidence that demonstrated that resistance training decreases tendinous elasticity while increasing muscle architecture and size. Furthermore, tendon thickness, measured at the MTJ of the triceps surae, was influenced by medial gastrocnemius muscle quality (EI). In the absence of tendinopathy, this finding may support previous literature demonstrating that frequent and habitual exercise results in an increase in tendon thickness. Examination into postural stability have demonstrated that displacements in the center of mass or pressure have been associated with a decrease in muscle size, strength, and ballistic activation.

The evidence in the present may support past findings, since medial gastrocnemius thickness (MGT) was linked to postural stability in the anterior-posterior plane (API), where a thicker muscle improved API performance.

Chapter 3 - Culminating Project

3.1. Time-Course of Elastic Properties in Muscle and Tendon with Neuromuscular Activity during Repetitive Lengthening Contractions of the Knee Extensors in Young and Old Men

3.1.1. Statement of Purpose

While past and present investigations into the role of the series-elastic tendon have provided robust knowledge to the cooperative function of the muscle-tendon complex, there is still a need to isolate tendon function and performance from muscle function and performance during lengthening contractions. Therefore, the purpose of this study is to utilize a new myotonometry device in conjunction with EMG during maximal eccentric contractions in order to provide a timeline of tendon and muscle mechanical behavior during lengthening contraction in young and old adults. This study also seeks to explore the influence of muscle-tendon complex stiffness on maximal and rapid strength characteristics throughout a series of eccentric contractions, also in young and older adults.

3.1.2. Research Questions

This study has the potential to provide new information about the performance of tendon and muscle tissue separately while functioning as a single unit during lengthening contractions. The following questions were developed to guide this study in order to present new information regarding series-elastic characteristics and performance in relation to muscular and neuromuscular characteristics and performance under high strain contractions that are common in everyday activity.

- How does a series of maximal eccentric contractions influence mechanical properties of tendon and muscle tissue?
- What is the time-course of tissue mechanical property change throughout a series of eccentric contractions?
- What is the relationship between muscle-tendon complex mechanical property changes and maximal and rapid strength alterations throughout the course of repetitive eccentric contractions?
- How does age influence muscle-tendon complex mechanical property changes over a series of eccentric contractions?
- Is there an age-related difference to muscle-tendon complex mechanical property changes and maximal and rapid strength alterations during repeated acute eccentric contractions?
- How is recovery from repetitive lengthening contractions associated with tendinous stiffness and elasticity?

3.1.3. Hypotheses

- During repetitive lengthening contraction, the changes in mechanical properties of the tendinous tissue will occur at a different time-point than changes in mechanical properties of the muscular tissue.
- Following an initial increase in tendinous stiffness, continued completion of maximal voluntary eccentric contractions will lead to a decrease in stiffness.
- The onset of fatigue during a series of lengthening contractions will lead to a decrease in peak torque and an increase in EMD that coincides with increased tendinous tissue compliance.

- Over the course of repetitive lengthening contractions, a progressive deficit in early and late phase RTD will be observed.
- Age-related muscle-tendon complex stiffness alterations will be associated with decreased rapid strength characteristics.

The older participants will exhibit differential recovery pattern for muscle-tendon complex stiffness and rapid and maximal strength characteristics compared to younger adult participants.

3.1.4. Significance of Study

This study will enhance our understanding of the separate but simultaneous tendinous and muscular tissue performance during high strain contractions. Previous investigations into tendinous behavior and adaptations have been limited to quantifying changes of the musculotendinous junction which includes both muscular and tendinous tissue[73–75,145,194,210]. The ability to isolate the mechanical properties of the tendon during lengthening contractions has not been possible up until recently. Additionally, this investigation will provide information regarding the age-related alterations in muscle-tendon complex structure and mechanics and their influence on maximal and rapid strength variables which could help inform fall-risk research[36,181,215]. Finally, this study may also provide new insight into the role of elastic tendinous tissue on fatigue recovery related to repeated eccentric contractions.

3.1.5. Delimitations, Limitations, and Assumptions

Delimitations

The following criteria will be applied for this study:

- Approximately 20-30 healthy adult males are needed to complete this investigation

- Young participants will be between the ages of 18-30 years old, older participants will be between the ages of 60-70 years old.
- All participants will be identified as healthy, and free of any lower extremity injury, neuromuscular disease, or physical illness or ailment that might limit physical activity as identified via self-reporting through a health history questionnaire
- All participants must fall within the moderately active range for physical activity as identified within the Physical Activity Guidelines for Americans put forth by the U.S. Department of Health and Human Services. This consists of a total of 3 hours per week of moderate-intensity activity, which may include a combination of aerobic, anaerobic, and strength activities.
- At the time of the study or within 6 months prior, participants do not or have not consumed any ergogenic aid that might support or enhance physical performance or recovery.
- Participants will be asked to perform maximal voluntary contractions of knee extension.

Limitations

- Participants will respond to recruitment flyers, emails, and/or classroom visits by the investigator and chose to enroll on a volunteer basis. Therefore, the process of subject selection will not be truly random.
- Participants will serve as their own control with pre-test data being used for comparative analysis.

- By using voluntary contractions, it is not guaranteed that participants will provide 100% effort for every lengthening contraction.

Assumptions

- Subjects accurately and honestly answer questions pertaining to their current and past health status as well as physical activity level.
- Maximal effort will be given during each maximal contraction.
- The EMG variables detected at the level of the sensors accurately represent the behavior of the whole muscle.

3.2. Methods

Participants

A total of twenty to thirty participants will be asked to volunteer for this study. Both young adult males (18-30 years old) and older adult males (60-70 years old) will be recruited for this investigation. Inclusionary criteria will include no current or within 6 months of study any lower extremity musculoskeletal or neurological disorders, injuries, or surgeries. All participants will identify as moderately active as defined as 2.5-3 hours per week of moderate-intensity activity including various combinations of aerobic and anaerobic exercise with strength training[216]. Participants who are currently taking anti-inflammatory medications, prescription pain medication, or prescription medication for cardiovascular disorders will be excluded from this study. Additionally, participants who have restrictions on physical activity by a primary care physician or medical specialist will not be asked to participate in this study. Finally, participants will be asked to refrain from consuming ergogenic aids and/or recovery supplements for 8 hours prior to laboratory visits as well as 48 hours following the eccentric protocol intervention. Such supplements include creatine, caffeine, protein, or recreational medication such as erectile dysfunction medication that may enhance muscular performance or aid in recovery. All subjects will complete an informed consent, a health history and current health status questionnaire, and a physical activity questionnaire. This study has gained approval by the University's Institutional Review Board (IRB-10586).

Research Design

This study will require three separate visits to the laboratory. The first visit will consist of familiarization and collection of baseline information. Baseline data to be collected will include body composition measurements, 24-hour dietary recall, ultrasonographic and myotonometric of the quadriceps muscle and patellar tendon architecture and mechanical assessments, and maximal voluntary contraction (MVC) testing of the knee extensors and flexors. Following a 72-hour wash-out period, the second visit to the laboratory will involve another 24-hour dietary recall and the eccentric contraction protocol. The primary muscle of interest will be the rectus femoris (RF) and the primary tendon of interest will be the patellar tendon (PT). Forty-eight hours after the completion of visit two, participants will be asked to return to the laboratory in order to reassess 24-hour dietary recall, ultrasonographic and myotonometric variables of the RF and PT as well as perform MVC testing.

3.2.1. Instrumentation and Procedures

24-hour Dietary Recall

The Automated Self-Administered 24-hour Dietary Assessment Tool (ASA24 v. 2018; NIH, National Cancer Institute) will be employed to identify participants' dietary consumption immediately prior to each laboratory visit. This assessment will help to identify participants' habitual consumption of macro- and micro-nutrients. The macro-nutrient of specific interest for this study is protein. Ingestion of high protein supplements or foods may influence muscle-tendon complex structural and mechanical properties as well as muscular performance [217,218]. This tool will also assist in participant inclusion and exclusion, with dietary protein consumption exceeding 25% of total daily calories (whole food) resulting in exclusion from study. The ASA24 will also be used to identified participant consumption of foods that increase inflammation such as high-fructose corn syrup, artificial trans fats, refined carbohydrates, and alcohol[219–222]. The ability to evaluate participants consumption of these pro-inflammatory foods may go to support findings on participant recovery from the repetitive eccentric exercise protocol. Additionally, utilizing this assessment at each laboratory visit will help evaluate if participants' have refrained from consuming ergogenic aids or recovery supplements during the recovery period following the eccentric contraction protocol.

Ultrasonography

Muscle and tendon architecture will be assessed via portable diagnostic ultrasound (GE LogiqE; Waukesha, WI, USA). The quadriceps muscle group will be evaluated with the participant lying supine on a cushioned examination table with the hip and knee in a neutral position. Two separate imaging methods will be used to assess the rectus femoris. First, using the LOGIQ ultrasound imaging with a linear-array probe panoramic images of the rectus femoris will be captured. This consists of moving the probe manually at a slow and continuous rate along the surface of the skin from lateral to medial. A foam guide will be used to ensure that the image taken is consistently captured at a level of 50% of the thigh measured on the line of the anterior superior iliac spine (ASIS) to the superior part of the patella (Figure 15A). Cross-sectional area and echo intensity of the rectus femoris will be measured on each of the participants' right leg from these images (Figure 15B).

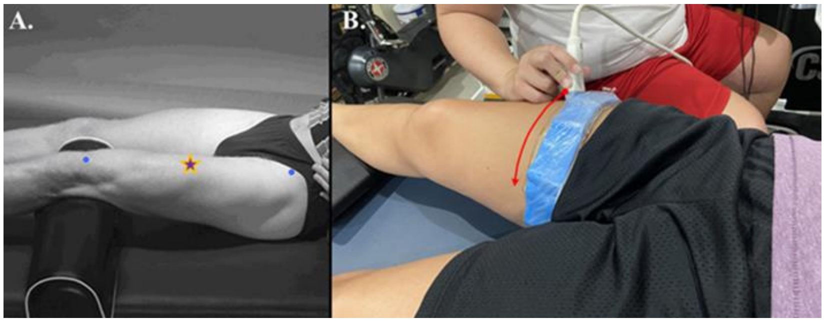


Figure 3.1. A) Identification of level of interest for the assessment of the rectus femoris muscle (★) B) demonstration of panoramic imaging of the rectus femoris at the level identified in 15A.

B-mode longitudinal images of the rectus femoris will be obtained at this same 50% level of the thigh centered in the belly of the rectus femoris which will be determined by palpation during a knee extension contraction. The longitudinal images will allow for the assessment of muscle thickness, fascicle length, angle of pennation and subcutaneous adipose thickness. The patellar tendon structural characteristics will be assessed with the participant in similar position with a bolster positioned under their knee to allow for 10-15° of knee flexion. Longitudinal images will be captured of the entire patellar tendon ensuring that both the distal pole of the patella and the osteotendinous junction at the tibial tuberosity can be seen in the images. These images will be used to identify tendon length. Transverse images will be taken at the mid-tendon region which will be identified by measuring the distance between the inferior pole of the patella and the proximal osteotendinous junction at the tibial tuberosity (Figure 16). Both the inferior pole of the patella and the osteotendinous junction will be identified by palpation. The transverse patellar tendon images will allow for the assessment of tendon thickness, cross-sectional area, and echo-intensity. A generous amount of water-based lubricant will be applied for all areas to be imaged to enhance image quality.

Figure 3.2. Reference points for patellar tendon assessment identifying the distal pole of the patella (●), the proximal OTJ on the tibial tuberosity (●), and the mid-tendinous ROI (★).

Image Analysis

All US images will be analyzed utilizing a third-party image analysis software (ImageJ; Version 1.47v, National Institutes of Health, Bethesda, MD, USA). Prior to analysis, each image will be scaled individually from an area in pixels at centimeters with the straight-line function using a known distance of 2cm. The superficial and deep fascia on muscular ultrasound images will be identified and stationary lines will be superimposed to extend the trajectory of both anatomical structures. All measurements will be in centimeters, save for echo intensity which will be designated by arbitrary units.

Longitudinal images of muscle will be analyzed for rectus femoris thickness (distance between the superficial and deep fascia), pennation angle (angular relationship between the deep fascia and a single muscle fascicle), and fascicle length (measurement of a single muscle fascicle identified between the fascial layers) (Figure 17).

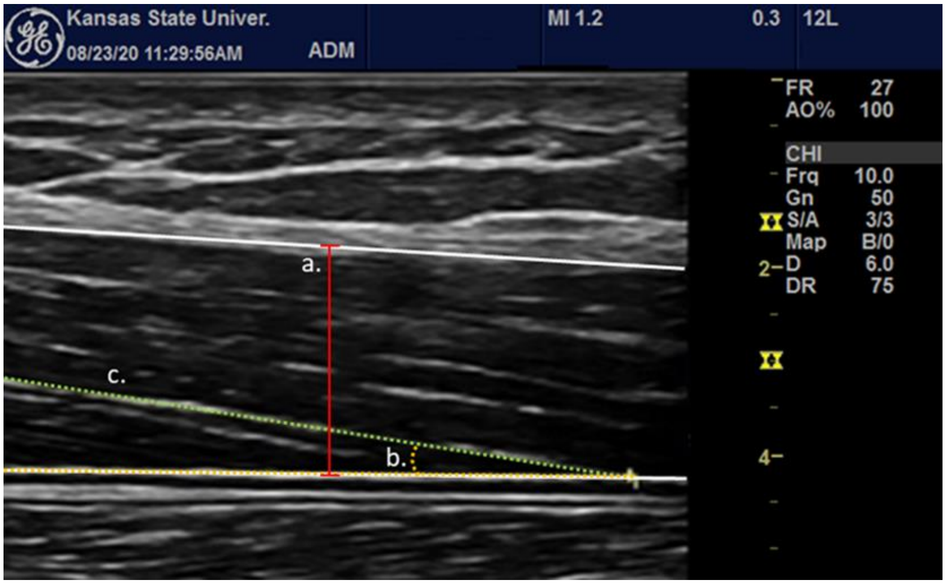


Figure 3.3. Longitudinal US image identifying muscle thickness (a.), pennation angle (b.), and fascicle length (c).

The panoramic muscular images will be analyzed to determine rectus femoris cross-sectional area and echo intensity. A region of interest will be identified by tracing the fascial barriers of the rectus femoris muscle and calculating the area and gray scale of that region of interest. The gray scale obtained via ultrasonography of the RF is indicative of muscle quality where the darker regions are muscular tissue and the lighter areas are non-muscle potentially made up of intermuscular fat or fluid. Care will be taken to ensure that only muscle tissue is included within the region of interest (Figure 18).

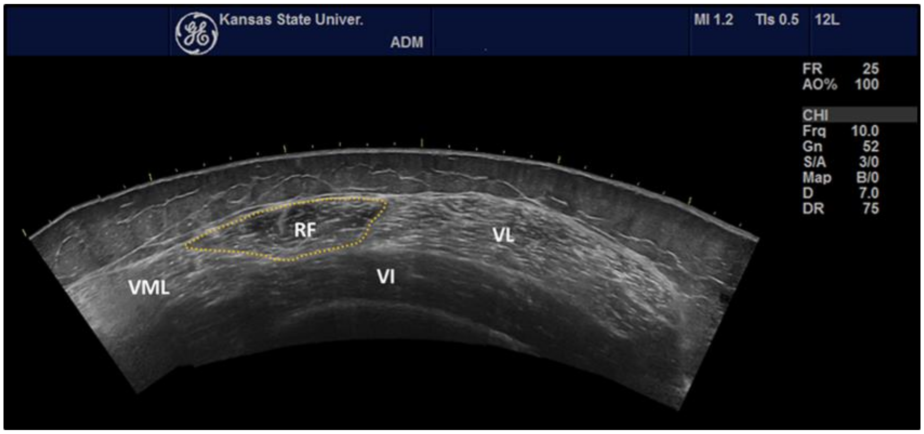


Figure 3.4. Panoramic US of the quadriceps muscle group. Rectus femoris (RF) region of interest identified by yellow boarder.

Patellar tendon transverse image analysis will include the measurement of tendon thickness, cross-sectional area, and echo intensity. Superimposed straight lines will be placed at the level of the anterior and posterior peritendon, and thickness will be measured as the distance between these lines. Similar to the muscle cross-sectional area assessment, tendon cross-sectional area and echo intensity will be measured by identifying a region of interest that will trace the paratendinous layer and a calculation of this region of interest will be taken for area and gray scale (Figure 19).

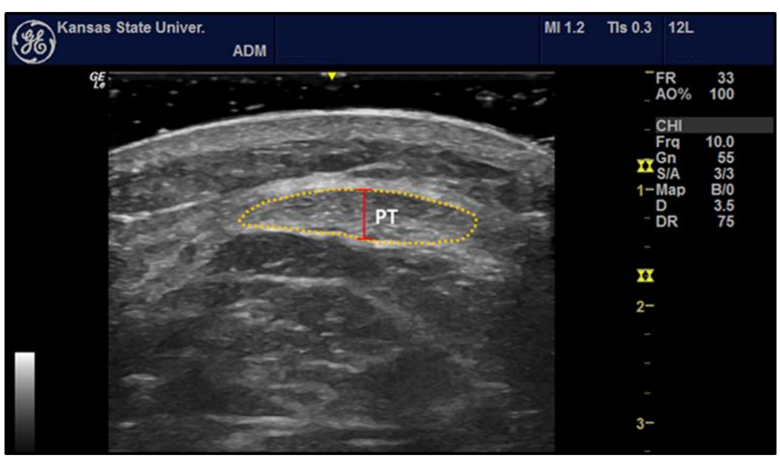


Figure 3.5. Transverse US of the patellar tendon (PT) within the yellow boarder, PT thickness is identified by red line.

The longitudinal images of the patellar tendon will be used to identify tendon length. For this, a measurement will be taken from the inferior pole of the patella to the osteotendinous junction at the tibial tuberosity (Figure 20). Care will be taken to only capture tendinous tissue and avoid capturing bony tissue.

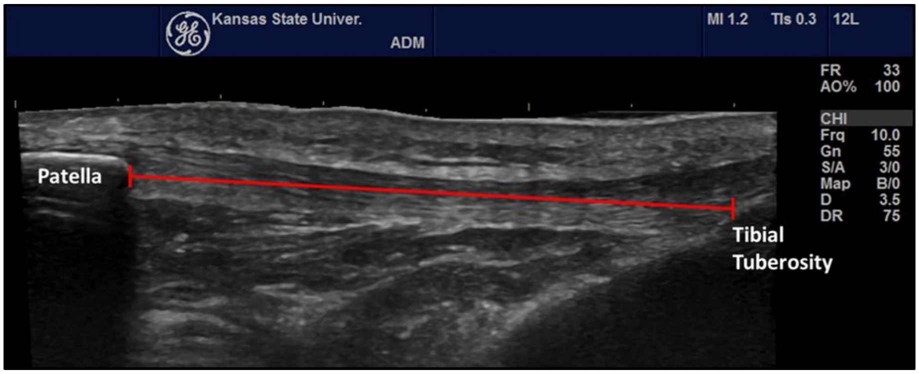


Figure 3.6. Longitudinal US of patellar tendon with tendon length identified in red. Both the distal pole of the patella and the proximal OTJ at the tibial tuberosity are visible to identify entire PT.

Myotonometry

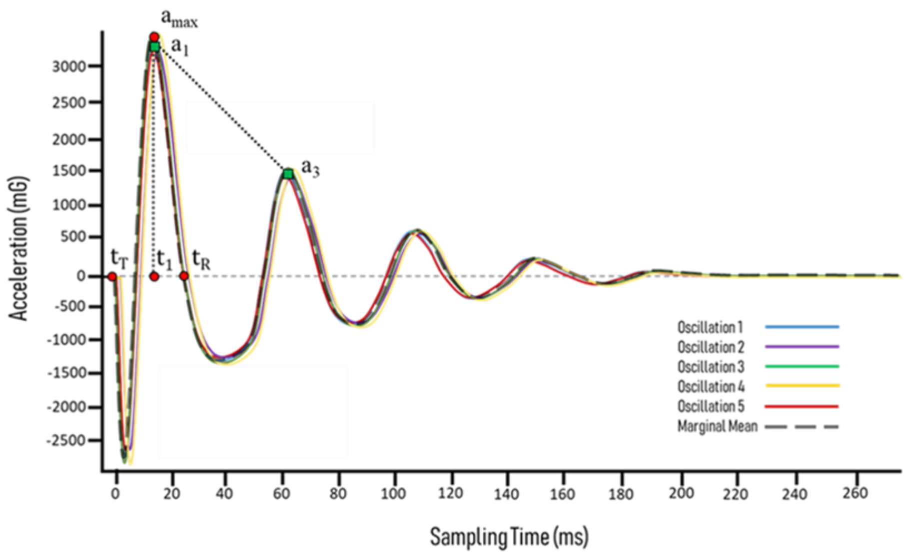
Muscle and tendon mechanical properties will be non-invasively assessed utilizing the myotonometric digital palpation device, MyotonPRO (Myoton, AS, Tillan, Estonia). This hand-held device applies light pulses and analyzes the returning oscillations to evaluate stiffness, creep, time to relax, elasticity of soft tissues. For this investigation the MyotonPRO will be used as a baseline assessment, inter-set assessments, and post-lengthening protocol assessments of the rectus femoris with the participants in a seated position with their knee held at 90° of flexion in order to obtain measurements for the rectus femoris and the patellar tendon. Between each set of the ECC protocol, the MyotonPRO will also be applied to the rectus femoris and patellar tendon to evaluate the time course of tendinous adaptations during acute repeated eccentric contractions.

Immediately following the completion of the eccentric protocol, myotonometry assessments will be repeated at 0, 15, and 30 minutes post. The subsequent 48-hour time point will also include these assessments.

The probe of the MyotonPRO will be held perpendicular to the skin surface at the levels for rectus femoris and patellar tendon ultrasonography as identified previously. This probe is then pushed against the test area to reach the required depression depth as set by the manufacturer. This will be indicated with a green light on the device (Figure 21).

Figure 3.7. MyotonPRO applied to mid-tendinous ROI identified in Figure 16. Application for rectus femoris will occur at previously described target position (reference Figure 15A).

Once this light appears, five short impulses at a tap interval of 0.8 seconds will be automatically implemented by the device to induce a mechanical oscillation in the soft tissue. The MyotonPRO device provides tissue tone by way of oscillation frequency (Hz), stiffness (N/m), elasticity as the proportional inverse to the decrement, mechanical stress relaxation time (ms), and creep as a ratio of deformation and relaxation time. The average of all the successfully delivered tap variables will be used for further statistical analysis (Figure 22).



$$S = \frac{a_{max} \cdot m_{probe}}{\Delta l} \qquad D = \ln\left(\frac{a_1}{a_3}\right) \qquad C = \frac{R}{t_1 - t_T} \qquad R = t_R - t_1$$

Figure 3.8. Visual representation of MyotonPRO oscillation analysis. Calculations for Stiffness (S), Elasticity (D), Creep (C), and Mechanical Stress Relaxation Time (R) are provided.

Isometric Strength Assessment

Isometric strength familiarization and assessment will be conducted for the quadriceps complex using an isokinetic dynamometer (Biodex Medical Systems Inc., Shirley, NY, USA). Participants will sit in an up-right position in the cushioned dynamometer chair with proper restraints at the chest, waist, thigh and ankle to avoid compensation and excessive movement during assessments (Figure 23). The input axis of the dynamometer is aligned with the axis of rotation of the knee.

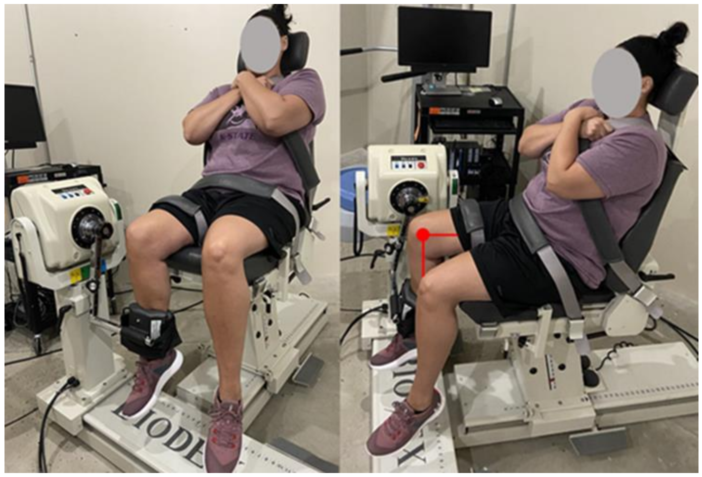


Figure 3.9. Demonstration of dynamometer positioning for MVCs and inter-set MyotonPRO assessment. 90° of knee flexion is represented by the superimposed goniometer in red.

During the first and third visit to the laboratory participants will perform a brief warm-up consisting of three submaximal isokinetic leg extensions at $60°\cdot s^{-1}$ at approximately 75% of the participants' perceived maximal effort. Participants will then be asked to complete 2-3 MVC at 90° knee flexion with 1-minute rest between each attempt for leg extension. During all MVC measurements the participants will be verbally instructed with "push", "as hard and fast as possible" for the duration of each contraction, 3-4 seconds. During the second visit, participants will be asked to perform two MVCs for the knee extensors immediately following every 10^{th} repetition of the eccentric contraction protocol and at 15- and 30-minutes post-eccentric exercise protocol.

Eccentric Contraction Protocol

The eccentric contraction protocol will be performed within a dynamometer. Participants will be asked to try to maximally contraction their knee extensors in order to maintain knee extension while working against the moving dynamometer lever arm that will be inducing a lengthening contraction of the knee extensor muscle group at a speed of $60°\cdot s^{-1}$. These lengthening contractions will be followed by an immediate concentric knee extension contraction at a speed of $150°\cdot s^{-1}$ in order to return the lever arm to the starting position. Participants will complete a total of 100 eccentric contractions arranged in a 10×10 set/repetition scheme. Participants will be afforded a quite rest between each set, immediately following the performance of knee extension and flexion MVCs. Participants will have a total of 60 seconds of rest in which myotonometry assessment of the RF and PT will be captured. At the completion of the eccentric contraction protocol, MVCs and myotonometry assessments will be repeated immediately after and at 15- and 30-minutes post protocol.

Electromyography

In conjunction with MVC performance, surface electromyography (EMG; MP160WSW; Biopac Systems, Inc., Santa Barbara, California, USA) will assess Root Mean Square (RMS) of the quadriceps muscle group (i.e. rectus femoris). Prior to electrode placements, the skin at the specified locations of the rectus femoris will be shaved, abraded, and cleaned with isopropyl alcohol. This will be completed in order to reduce inter-electrode impedance and increase the signal- to-noise ratio. Pre-gelled bipolar surface electrodes (EL502, Biopac Systems Inc. Goleta, CA, USA) with an inter-electrode distance of 25 mm will be placed over the rectus femoris and the biceps femoris. Electrode placement for the rectus femoris will be at 50% of the thigh measured on the line of the anterior superior iliac spine (ASIS) to the superior part of the patella[223] (Figure 15A). A second electrode will be placed on the tibial tuberosity as a grounded reference.

The torque (Nm) and EMG (μV) signals will be sampled simultaneously at 2kHz with a Biopac data acquisition system, stored on a personal computer, and processed off-line with custom-written software (LabView 8.5, National Instruments, Austin, TX, USA). The scaled torque signal will be filtered using a fourth-order, zero-phase shift, low-pass Butterworth filter with a 10Hz cutoff frequency. The passive baseline torque value will consider the limb weight and subtract that from the signal so a new baseline value will be set at 0 Nm. All subsequent analyses will be performed on the scaled, filtered, and gravity-corrected torque signal. Isometric MVC will be determined as the highest 0.5s epoch during the entire 3-4 seconds MVC plateau. The raw EMG signals (μV) will be digitally bandpass filtered at 20-400 Hz using a zero-phase fourth-order Butterworth filter and subsequently rectified.

The MVC peak torque (Nm) will be calculated as the highest mean 500-ms epoch during the entire 3- to 4-s MVC plateau. The RTD ($N \cdot m \cdot s^{-1}$) will be as the linear slope of the torque-time curve (Δtorque/Δtime) at time intervals of 0-50 (early RTD) and 0-200 (late RTD) milliseconds (Figure 24). These time intervals were chosen for analysis because they represent rapid torque characteristics in the early (0-50ms) and later (0-200ms) phases of muscle contractions and have been previously shown to effectively differentiate among strength-related performances [64]. For each MVC, the onset of contraction will be determined as the point when the torque signal reached a threshold of 4 N·m. Of the MVCs performed throughout the study, the MVC with the higher peak torque will be selected for analysis.

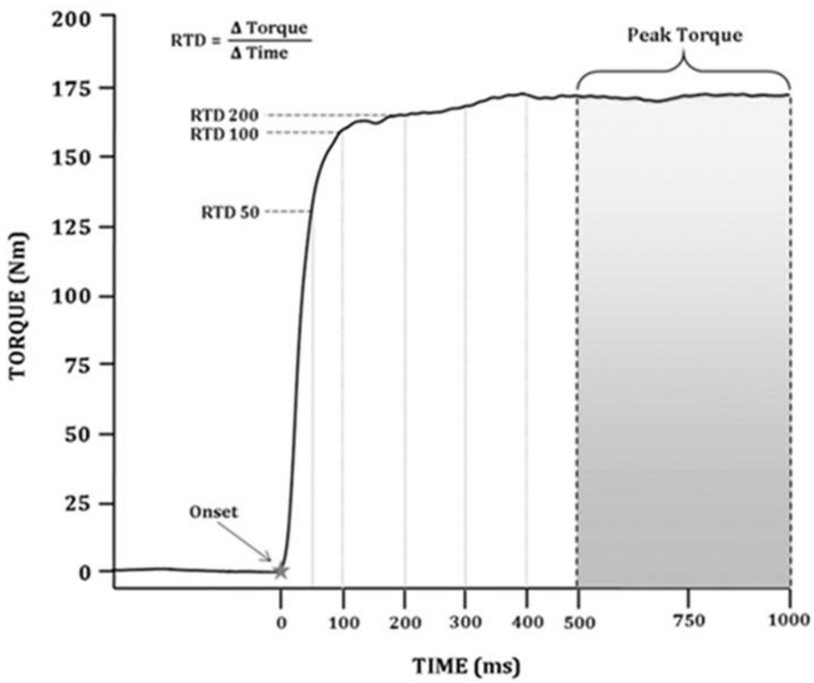


Figure 3.10. Example of the Torque-Time Curve obtained identifying various time points of Rate of Torque Development (RTD) as well as Peak Torque (PT).

The EMD will be identified as the time (ms) difference between the EMG and torque onsets. The EMG onset will be determined manually, using custom-written software will provide an interactive graph from which a high-resolution window will be subsequently scaled to establish an optimal view for cursor placement and consequently onset detection. A horizontal line will be displaced on the graph that represent 3 standard deviations above baseline and provide a visual guide to aid in determining the EMG onset (Figure 25). Torque onset determination will be automated and initiated when the torque signal reaches 7.5 for leg extensors.

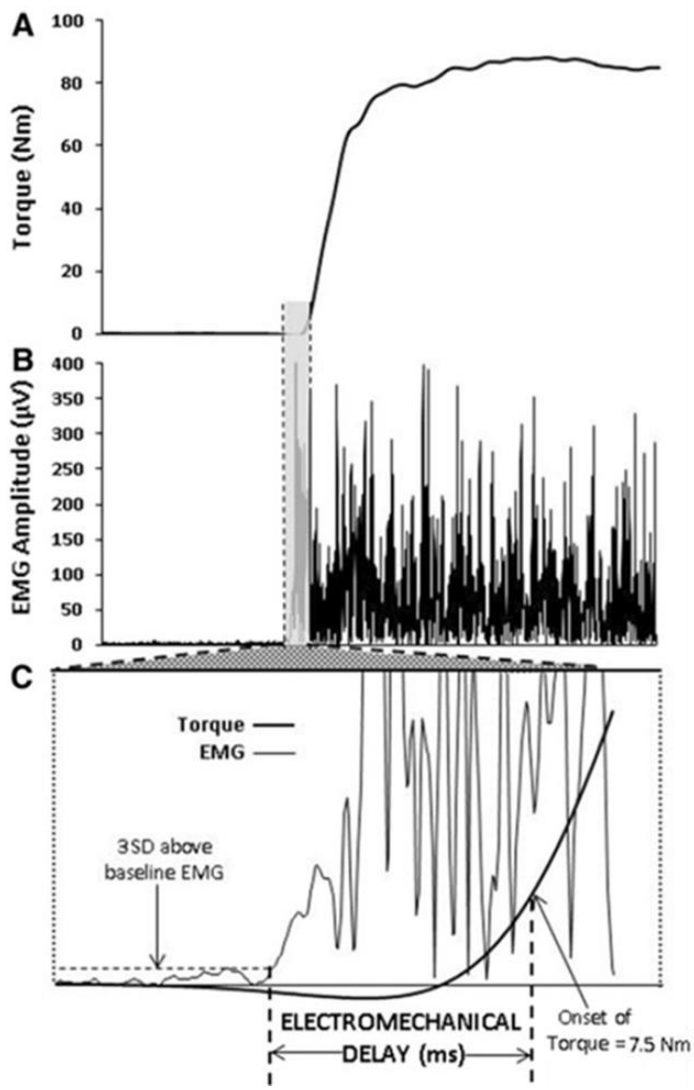


Figure 3.11. A) Diagram showing a filtered torque and B) rectified EMG tracing from which C) EMD was determined as the time difference (ms) between EMG and torque onset.

3.2.2. Statistical Analyses

A two-way mixed factorial ANOVA will be used to analyze separate muscle-tendon complex variables of structure and mechanics with neuromuscular variables by age across time [age x (young vs. old) x time (pre vs. post-1st set vs. post-2nd set vs. etc…)]. When appropriate, to identify single factor analysis, separate one-way repeated measures ANOVAs and independent samples *t* tests with Bonferroni corrections on either the simple main effects or main effects collapsed across the opposing variable. Pearson-correlation coefficients will be calculated to examine the relationships between muscular performance, architecture variables with tendon structure and mechanics, time, and age. IBS SPSS (SPSS Inc., Chicago, IL) will be used for all statistical analyses and an alpha level of $P \leq 0.05$ will be used to determine statistical significance.

3.3. Pilot Data

Pilot testing was undertaken to explore the influence of a short, acute eccentric dominant exercise on RF and PT passive mechanical properties of stiffness and elasticity. A convenience sample of 10 healthy, college age individuals (4 males, 6 females) participated in a drop jump exercise. Volunteers started on a platform approximately 91 cm high, from an athletic stance position on this platform volunteers were asked to then jump off the platform onto the stable ground with weight evenly distributed with both feet contacting the ground at the same time. Additional guidance was provided for each participant to land as quietly as they could and decelerate upon landing to approximately 90° knee flexion with limited torso flexion. Volunteers performed 2×10 with no more than 3-s between each jump and 30-s between each set. Pre- and post-drop jump exercise, RF and PT stiffness and elasticity were gathered using the MyotonPRO. Myotonometry was performed on the right limb of all volunteers with the knee and hip positioned at 90°. Independent *t*-test demonstrated that pre-exercise stiffness was significantly different between the muscle (RF) and the tendon (PT) tissues where mean PT stiffness was greater than RF stiffness ($p = 0.001$). Separate 2-way repeated measures ANOVA were used to analyzed tissue*time [tissue (RF; PT)*time (pre-exercise; post-exercise)], which identified an interaction for time*tissue. Further paired samples t-test exhibited that RF stiffness values prior to the drop jump exercise were lower than those collected following the drop jump exercise (p = 0.027). The increased muscular stiffness observed during the pilot investigation is in line with similar findings of the influence on acute exercise on immediate muscular stiffness alterations[224,225]. Additionally, greater stiffness of the PT compared to the RF is expected given the densely packed collagenous tissue and is supported in previous literature with stiffness measured via shear wave elastography and myotonometry[162,226].

The pilot investigation was conducted to explore the sensitivity of the myotonometric device as well as explore the influence of eccentrically dominant exercise on the passive mechanical properties of the muscle-tendon complex. While the results were not groundbreaking, results were beneficial in that they reflected the known behavior of muscular and tendinous tissue alterations following acute exercise while utilizing the novel myotonometer. The exercise implemented during the pilot investigation was also much shorter (2×10) than the proposed eccentric protocol of the culminating project (constant speed 10×10). It would be anticipated that a more controlled, higher intensity exercise intervention like that of the culminating project would elicit different soft tissue responses. Additionally, the length and highly eccentric nature of the culminating project intervention has a greater potential to alter tendon structural and mechanical properties that often require longer duration and higher intense muscular activity to produce tendinous changes[124,132,145].

Chapter 4 - General Discussion

The overarching objective of this document was to investigate the cooperative relationship between the muscle-tendon complex. This chapter will provide an encompassing discussion, outlining the major findings from previously conducted studies described in the previous chapters. Additionally, a brief discussion on how the progression of the studies detailed previously help to support the study design and methodology of the culminating project.

Study 1 (2.1) was developed to identify the reliability and correlation between Brightness mode (B-mode) and panoramic diagnostic ultrasound techniques. While panoramic imaging can provide a more robust image of a single muscle or muscle group, ultrasound devices equipped with the function that allows for panoramic imaging may be too much of a financial burden compared to B-mode enabled models. Additionally, capturing clear panoramic images from highly contoured areas of the body of from patients/participants of small stature (i.e. females) can be challenging and images are often of compromised quality. For this investigation, both imaging techniques were deployed to capture structural properties of the medial gastrocnemius in female participants. Imaging capturing in this study was based off previous literature for B-mode and panoramic imaging methods. However, during image analysis, a unique 1×1cm region of interest was used in addition to traditional image analysis methods. From this study, it was found that both imaging techniques demonstrated good to excellent reliability for all muscle variables (ICC 0.699-0.999; SEM% 0.839-7.949%). Additionally, there was no significant difference for muscle thickness and echo intensity between the two imaging techniques (p = 0.174-0.828). Positive correlations existed between all of the echo intensity variables (EI_{Long}, EI_{Pan}, and EI_{ROI}) and muscle thickness obtained from B-mode ultrasound was positively correlated with that variable obtained from panoramic images (r = 0.892, R^2 = 0.796, p = <0.001). The findings from this study suggest that both imaging techniques are reliable when assessing the curved medial gastrocnemius muscle in females. Furthermore, results demonstrate that for some muscle structure variables the two imaging techniques may be interchangeable techniques if one or the other of the techniques is not immediately available or financially feasible.

Study 2 (2.2) was designed to identify gender-dependent characteristics of the free Achilles tendon. The free Achilles tendon region is most susceptible area of the tendon for tendinopathies to develop and ruptures to occur. Tendinous ruptures most often plague recreationally active males through the third to fourth decade of life. Tendon thickness, cross-sectional area and echo intensity were assessed via transverse B-mode ultrasound of the free Achilles tendon while free tendon length was gained from extended field of view longitudinal ultrasound. In comparison of demographic and body composition means, body mass was the only significant difference between males and females. The primary finding from this investigation demonstrated that echo intensity is gender-dependent, with males in this study exhibiting lower echo intensity values (darker) than females. Additionally, it was found that while males did not significantly differ from females for lower leg circumference, this anthropometric property was significantly correlated with free tendon thickness and free tendon length in males but not females. Muscle girth measurements have often been used as indirect measures of muscle strength. Also, greater body mass (i.e. weight and body mass index) have been associated with greater isometric strength and enlargement of tendinous tissue thickness. To this end, these findings along with previous literature may demonstrate that greater load (body mass) and triceps surae muscular stress is applied to the free Achilles tendon in healthy, young adult males resulting in lower echo intensity values as compared to females.

Study 3 was constructed to investigate relationships among passive mechanical properties and structural properties of the triceps surae muscle-tendon complex with postural stability utilizing the novel MyotonPRO, diagnostic ultrasound imaging, and a Biodex balance system, respectfully. Previous literature has shown tendinous alterations are regionally specific based off the line of tensile strain of one of all of the muscles that make up the triceps surae group. The tendinous region of interest within this investigation was selected for its clinical relevance in tendinopathies and due to its aponeurotic linkage with all three muscles of the triceps surae. Additionally, the medial gastrocnemius was targeted because it has been identified to produce the greatest muscular force out of the triceps surae group and plays a primary role and in balance and stability tasks. Findings from this study demonstrated that logarithmic decrement (inversely related to elasticity) of the Achilles tendon was positively associated with medial gastrocnemius size (cross-sectional area and thickness), tendon thickness was negatively associated with muscle quality (echo intensity), and that muscle thickness was significantly linked to postural stability in the anterior-posterior plane of stability. This study seems to have been the first to combine measurements gained from the MyotonPRO, diagnostic ultrasound in the explorations of muscle-tendon complex relationships in addition to investigating these variables potential connection to postural stability. Muscle size and quality have been associated with muscular strength and specific tension, where increases in muscle size (assessed via ultrasound, magnetic resonance, and computed tomography) and muscle quality (evaluation of echo intensity from ultrasound and magnetic resonance imaging) predict increases in muscle force or torque. Additionally, increases in muscular strength can lead to alterations in tendon mechanics (decreased elasticity) that may improve neuromuscular efficiency and force transmission through the muscle-tendon complex. Regarding single-leg postural stability, as mentioned above muscle size is often an indirect measure of muscle strength, thus increases in muscle thickness would assumedly increase intrinsic and specific tension which may provide improved ankle kinematic control during dynamic single-leg postural stability tasking. The cumulative findings appear to support previous findings on the role of muscle structure and quality inducing mechanical alterations of tendinous tissue in addition to the importance of the medial gastrocnemius on postural stability performance.

The culminating project (Chapter 3) was assembled to address areas of muscle-tendon complex research that have yet to be fully explored due, in part, to limitations in technology. The complexity and expense of previous investigative methods for assessing muscle-tendon mechanical properties and function with acute eccentric exercise interventions have obstructed researchers from gaining important information on immediate alterations that may impede muscle-tendon efficiency to store and transfer elastic energy. The previous studies detailed above demonstrates research and laboratory reliability with methods that allow for simplified assessments of the muscle-tendon complex structure and mechanical characteristics that will support the completion of the culminating project. Additionally, assessments from the studies detailed previously have demonstrated the assessment of isolated muscle and isolated tendon characteristics which will assist in gaining tissue specific information along with conjoined musculotendinous information prior to, during, and after exercise. Ultimately, the previous work captured within this dissertation have greatly benefitted the design of the culminating project which will seek to explore isolated tissue mechanical response in conjunction with neuromuscular performance prior to, throughout, and after an acute eccentric exercise to obtain information on the time-course of muscle-tendon alterations.

Chapter 5 - Conclusion

The objective of this dissertation was to present completed work within the realm of non-invasive, *in vivo* muscle-tendon complex research and demonstrate continued growth within this area. This dissertation has illustrated reliability of ultrasonographic assessments of lower body musculature and tendinous tissues, region specific muscle-tendon complex variance between genders, the relationship between isolated muscle structure and mechanics with isolated tendon structure and mechanics, and how isolated tissue characteristics relate to postural stability intervention performance. The culminating project will attempt to provide additionally information regarding mechanics of isolated tissue properties with neuromuscular performance throughout a high strain, eccentric dominant acute exercise and how isolated tissue properties and neuromuscular performance may differ between young adults and older adults.

From the results of this dissertation and the completion of the culminating project, some lines of future research can be developed to further explore muscle-tendon complex properties and adaptations. At this time, our knowledge and understanding of tendon mechanics and adaptation from a non-invasive, *in vivo* model is highly dependent on the inclusion of muscular contractions to calculate tendon specific properties. Additionally, there is a wealth of knowledge specific to muscular performance that neglects to assess or acknowledge the influence of tendinous mechanical properties on muscle performance. The muscle-tendon complex is a sum of its parts. Continued exploration of isolated tissue mechanics and performance will support enhanced knowledge of the function and adaptation of the muscle-tendon complex as a whole in a variety of population, intervention, and pathologies. Additionally, further exploration of isolated and cumulative muscle and tendon structure, mechanics, and neuromuscular properties would greatly improve clinical knowledge and may potential provide methods for assessing pathology, treatment, and rehabilitation progression.

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Appendix A - Definition of Terms

Anterior-posterior Index (API): represents the variance of foot platform displacement in degrees in the anterior and posterior planes of postural stability; Biodex Balance System variable

Aponeurosis: sheet of fibrous tissue that extends from muscle that anchors a muscle or connects a muscle with another structure in the body

Concentric contraction: muscle action in which the muscle shortens because the contractile force is greater than the resistive force

Cross-sectional area (CSA): area of a tissue assessed from transverse or cross section view/plane

Electromyography (EMG): recording of the electrical activity of muscle tissue obtained either form the surface of the skin or inserted electrode; EMG represents surface of skin recording, herein

Elasticity: ability of tissue to resume normal/resting shape after a stress/force is applied

Elastic strain energy: form of potential energy that is stored within elastic tissues upon deformation

Electromechanical delay (EMD): time delay between onsets of muscle activation and muscle force production; reflects both electro-chemical processes and mechanical processes; considered a rapid strength characteristic

Eccentric contraction: also referred to as lengthening contraction; muscle action that occurs when the total length of the muscle increases as tension is produced; initial tissue lengthening occurs within the tendinous tissue

Echo intensity (EI): mean pixel intensity of a specific region of interest from an ultrasound or magnetic resonance device measured on a gray-scale histogram value measured in arbitrary units on a scale of 0-255 (black = 0, white = 255)

Free tendon: region of tendinous tissue that consists of only collagenous tendon fibers with no muscular aponeurosis or muscle fascicle attachment

Hysteresis: viscoelastic property of tissue that represents energy dissipation of transmission; difference between the strain energy required to generate a given stress in a material, and the material's elastic energy at that stress; higher hysteresis is associated with greater energy dissipation

Isokinetic: muscular contraction that develops and occurs at all joint angles throughout a range of motion; speed or velocity is held constant because there is accommodating resistance at a controlled speed of movement

Isometric: muscle action in which muscle length does not change because the contractile force is equal to the resistive force

Maximal voluntary contraction (MVC): muscular strength assessment in which participants attempt to concentrically contract as hard as possible with the limb in a fixed position

Medial-lateral Index (MLI): represents the variance of foot platform displacement in degrees in the medial and lateral planes of postural stability; Biodex Balance system variable

Muscular strength: ability of muscle to exert maximal force on a single occasion

Muscle-tendon complex: general term to refer to the muscle and associated tendon as a combined structure; not a specific region (see MTJ)

Musculotendinous junction (MTJ): region where muscular fascicles or aponeurosis converge with collagen fibers and peritendon of the associated tendon

Myotonometry: soft tissue assessment using a device that provides external oscillations and records subsequent tissue response; provides variables of tissue tension, biomechanics, and viscoelastic properties; interchangeable when referencing MyotonPRO herein

MyotonPro: hand-held, non-invasive palpation device used to measure tissue mechanics such as tone, decrement, stiffness, mechanical stress to relaxation time, and creep

Osteotendinous junction (OTJ): tendinous tissue insertion into bone

Overall Stability Index (OSI): represents the variance of foot platform displacement in degrees in all motions during postural stability; culminating score incorporating values of anterior-posterior and medial-lateral planar motion; Biodex Balance System variable

Postural stability: dynamic; ability to maintain an upright position while positioned on an unstable surface or experiencing external perturbations

Quadriceps muscles: group of muscles located in the anterior/front of the femur/thigh; comprised of four singular muscles: vastus lateralis, rectus femoris, vastus intermedius, and vastus medialis

Rate of torque development (RTD): duration in time to attach maximum torque or force; rapid strength characteristic

Early: occurring from 0-50ms of torque-time curve; associated with initial motor unit recruitment and firing rates also reflective of intrinsic muscle properties

Late: occurring from 100-200ms of the torque-time curve; associated with maximal strength and muscle size and considers other structural factors including tendon stiffness and muscle pennation angle

Recreationally active: defined by the Physical Activity Guidelines for Americans as moderately active; consisting of 2.5-3 hours per week of moderate-intensity activity including various combinations of aerobic and anaerobic exercise with strength training

Region of Interest (ROI): subset of an image or region of the body. With imaging, a ROI is often created to assess a targeted/specific portion of the image and is made by superimposing boundaries around the targeted area of the image and analysis is collected from within the boundaries.

Specific tension: maximum force developed per unit of cross-sectional area frequently used to estimate force

Stiffness: measured as the elastic modulus, the resistance to deformation; expressed as the magnitude of a stress/force divided by the strain (deformation) induced by stress/force

Strain: specific to tissue deformation (compression, elongation, displacement); not associated to orthopedic injury herein

Strength: the maximal force that a muscle or muscle group can generate at a specified velocity

Stress: force/tension applied to a tissue that typically results in linear tissue deformation

Tendon: specialized tissue comprised primarily of collagen protein fibers that exhibits viscoelastic characteristics that attach muscle to bone and facilitate locomotion

Tissue thickness: structural variable of a tissue when measured from the superficial to the deep aspect of the tissue

Torque: measurement of force that accounts for rotation about an axis

Triceps surae: group of three muscles located on the posterior/back side of the low leg/calf; muscles of this group include: lateral gastrocnemius, medial gastrocnemius, and the soleus muscle

Ultrasound (diagnostic; US): also referred to as ultrasonography; real-time imaging method that uses high-frequency sound waves to produce images of structures within the body

> *B-mode*: Brightness mode (2D mode), linear array of transducer simultaneously scans a plane through the body that can be viewed as a 2-dimensional image
>
> *Panoramic:* also referred to as extended field of view; real-time imaging method that provides a panoramic image broadening the scope of the linear array transducer allowing for automated sequential alignment of individual images

Velocity: rate of change of limb position with respect to force production over time; speed at which force is produced

Appendix B - Review of Literature

This review of literature is organized in a study-by-study manner. The article summaries are provided in chronological order, and a section summary is provided at the conclusion of each section.

B.1 Musculotendinous Stiffness & Elasticity

Cavagna et al., 1964

During a two experiment series which involved adding up all the increases in kinetic and potential energy and dividing them by the metabolic energy cost of running, the authors were able to measure the energy fluctuations in human runners. This calculation gave an efficiency that was much higher than muscle efficiencies calculated from previous experiments. Cavagna and his colleagues concluded that kinetic and potential energy lost at one stage of a running stride was stored as elastic strain energy and returned later in a recoil. The authors concluded that the elastic energy must be stored in the muscles.

Griffiths, 1991

Through experimentation using felines, Griffiths was able to highlight the significance of tendon compliance through examination of muscle fiber and muscle spindle during active stretching of the medial gastrocnemius. During the contraction of muscle, the tendons are stretched by an amount that is determined by their compliance, which eventually reaches a constant level. Tendon stretch exceeds that applied to the muscle, and the muscle fibers and spindles shorten during muscle stretch except at high rates of stretch. When the muscle is being stretched at low speeds the muscle fibers can actually shorten because of the compliance of the tendons. By way of the viscoelastic composition of tendons, they are able to act as a mechanical buffer to protect muscle fibers from damage during lengthening contractions.

Narici et al., 1996

This investigation implemented a six month long strength training intervention to explore human quadriceps group adaptation by the assessment of muscle fiber cross-sectional area, torque, and neural activation. Through the participation of seven young adult males, the research team applied a training protocol that consisted of six series of eight unilateral leg extensions at 80% of the individual one repetition maximum. Following training, an uneven distribution of hypertrophy was found amongst and within the components of the quadriceps muscle group. Also, there was an observed increase in muscle torque, however this occurred in the absence of change in neural activation as assessed through indwelling EMG. Researchers also identified a significant decrease in time to peak torque after training, which they suggested may indicate an increase in musculotendinous or series-elastic stiffness.

Kubo et al., 1999

The aim of this study was to identify the elastic properties of tendon structures of the knee extensors *in vivo* in order to investigate their role on jump performance with and without countermovement. Utilizing ultrasonography, investigators were able to track the lengthening of the musculotendinous junction and in conjunction with knee extensor force production calculated the elastic property of the tendon in 31 young adult males. Researchers found that tendon stiffness was inversely correlated with the difference in jump height between the vertical jumps performed with and without a countermovement. This finding suggested that tendon stiffness may have a favorable effect on stretch-shortening cycle exercises, potentially due to adequate storage and recoil of the elastic energy.

Bobbert, 2001

The aim of this study was to determine the dependence of human squat jump performance on the compliance of series-elastic elements of the triceps surae muscle consisting of the gastrocnemius and soleus through computer simulation. Utilizing data from an experienced male jumper, a two dimensional forward dynamic model of the human musculoskeletal system was developed. The simulation showed that the compliance of the series-elastic elements had considerable effect on the maximum height achieved during a squat jump. These results support the importance of the elastic components for energy storage and distribution. The author postulates that the lengthening of the elastic tendon during the downward squat allows the series-elastic system to achieve a higher energy storage, and by virtue of the elastic recoil can then transfer more energy allowing for higher power output at the ankles during the last part of the push-off phase of the jump.

McNeill Alexander, 2002

This paper shows how tendon elasticity affects muscle function, especially during normal locomotion. As identified via Young's Modulus of elasticity (a gradient of a graph of stress against strain), tendinous elasticity is low at low stresses. The author reports that tendons are not efficient at energy dissipation, returning via elastic recoil approximately 93% of the work previously done stretching it. This demonstrated that metabolic energy can be saved in locomotion if tendons stretch and then recoil. Tendons can also recoil elastically much faster than muscles can shorten, enabling getter force output than the muscle can provide on its own. Through review, it was concluded that tendon elasticity makes position control more difficult and force control easier.

Roberts, 2002

Within this publication, Roberts provides a review of the function of tendons elastic characteristics that should be considered when examining the contractile characteristics of muscle. The authors identifies that more often than not, the mechanical roles of tendon and muscle contractile elements are often considered independently of each other however they are tightly integrated. Tendon stretch and recoil not only reduce muscular work, but also allows muscle fibers to operate nearly isometrically, during which the muscle fibers can develop high forces. The author also postulates that the elastic energy storage and recovery in tendons may provide a key mechanism to enable individual muscles to alter their mechanical function, from isometric force-producers to actively shortening power-producers. The evidence presented within this paper suggests that during running accelerations work is transferred directly from muscle to tendon as the tendon is stretched early in the step powered by muscle shortening. This energy stored in the tendon is later released to help power the increase in energy of the body in motion. Finally, Roberts explains that the tendon elastic energy storage and recovery extends the functional range of muscles by uncoupling the pattern of muscle fiber shortening from the pattern of movement of the body.

Reeves & Narici, 2003

Utilizing ultrasonography, the authors explored the behavior of human muscle fascicles during dynamic contractions. Across a range of lengthening velocities controlled by a dynamometer, there was negligible change in the length of muscle fascicles measured from rest to a given point of joint excursion. It was also found that during eccentric muscle actions, the fascicles contracted quasi-isometrically, independent of angular velocity. The application of a rapid stretch applied to a fully active muscle can initially be taken up almost entirely by stretching of the series-elastic tendon. The authors believed that this behavior was reflective of the degree of stretch through the series-elastic components. These findings led to the conclusion that the series-elastic component acts as a mechanical buffer during active lengthening.

O'Brien, 2007

The purpose of this publication was to provide a comprehensive review of the structure and metabolism of human tendons. The densely packed collagen fibers and high water content of tendons provide the tendon with viscoelastic properties. This collagen composition influences the tensile strength and the tissue's ability to resist high compressive forces. This structure and composition of tendons allow for a unique mechanical behavior that is dependent on the rate of mechanical strain. The author identifies that the relationship between stress and strain for a tendon is not constant but depends on the time of displacement or load. This viscoelastic tissue is more deformable at low strain rates, but less deformable at high strain rates. Therefore, tendons at low strain rates tend to absorb more mechanical energy, but are less effective in carrying mechanical loads. However, tendons become stiffer and more effective in transmitting large muscular loads to bone at high strain rates.

Roberts & Azizi, 2010

The purpose of this study was to examine the mechanical function of tendons during lengthening contractions, when muscles absorb energy. Force, length, and power were measured in the gastrocnemius muscle of wild turkeys. Sonomicrometry was used to measure muscle fascicle length separate from musculotendinous junction length change modulated by servomotor in the presence of electrical stimulation to induce contraction. It was observed that during musculotendinous junction lengthening, the muscle fascicles underwent shortening. Energy input to the musculotendious junction during the fastest lengthening speed was much greater than that of the muscle fascicles. The authors identified that this discrepancy indicated that energy was first absorbed by the elastic tendon, then released to do work on muscle fascicles after the lengthening phase of the contraction. This temporary storage of energy by the elastic elements also resulted in a significant attenuation of power input to the muscle fascicles. It was concluded that the results obtained in this investigation demonstrated that tendons may act as mechanical buffers by limiting peak muscle forces, lengthening rates, and power inputs during energy-absorbing contractions.

Konow et al., 2012

This investigation sought to test the idea that tendons reduce the rate of energy absorption by skeletal muscle during energy-dissipating activities, or lengthening contractions. Utilizing wild turkeys because of the ossified portion of the distal gastrocnemius tendon, muscle force and lengthening was assessed during controlled landings that required rapid energy dissipation. Researchers found that there was a rapid musculotendinous junction lengthening immediately following impact that involved little or no muscle fascicle lengthening. Following the early contact period, muscle fascicles were observed to lengthen and absorbed elastic energy. This late lengthening occurred after most of the ‘ankle’ joint flexion had already occurred demonstrating the transition from flexion to extension was mainly driven by tendon recoil. It was concluded that temporary tendon energy storage led to a significant reduction in muscle fascicle lengthening velocity and the rate of energy absorption, providing evidence that tendons function as power attenuators that probably protect muscles against damage from rapid and forceful lengthening during energy dissipation.

Roberts & Konow, 2013

This review provides evidence and commentary on the role of tendon as a buffer for energy dissipation. The authors explain that muscles dissipate energy when they actively lengthen, and energy dissipation is required for any activity involving deceleration of the body or limbs. The muscles ability to dissipate energy undoubtedly affects a wide range of locomotor activities, and it is during these dissipative activities that injuries tend to occur. Several studies are presented that have addressed the role of tendons in energy-dissipating activities, and there is a general consensus that tendons act as a mechanical buffer to protect muscles from possible damage associated with rapid active stretching. The authors concluded that tendons protect muscle fibers from the damage associated with eccentric contractions by delaying and reducing energy absorption by active muscle fibers. They also propose that while the temporary storage of energy in tendons does not significantly reduce muscle lengthening, it reduced that chance of damage by allowing for muscle contractions to occur slower, less powerfully, and at lower forces.

Arellano et al., 2019

The purpose of this study was to explore the aponeuroses *in vivo* and it's energy storing capacity during contractions that altered the tissue's width. The aponeuroses are elastic-sheet-like tendons that cover the surface of muscle bellies, and these can be clearly distinguished from the extramuscular 'free' tendon. Using wild turkeys that were taken through controlled landings and jumps, it was observed that changes in aponeurosis width were inconsistent across each task. However, by organizing the data into tasks that exhibited width increases and those that exhibited width decreases, the researchers were able to identify aponeuroses shape-changing behavior that effectively modulated the stiffness and thus the elastic energy storing capacity of this tissue. Increased stiffness was identified when this tissue underwent increases in width allowing for a greater percentage of elastic energy storage. The results of this investigation provide evidence of the variable energy storing capability of the aponeuroses.

Chen et al., 2019

The aim of this study was to analyze the within-day inter-operator and between-day intra-operator reliability of the MyotonPRO for the assessment of tone and stiffness specific to the quadriceps muscle group and the patellar tendon at different knee angles. Stabilizing participants' knees at 0°, 30°, 60°, and 90° of knee flexion, the MyotonPRO device probe applied a series of oscillations to the targeted tissues. The results indicated that the MyotonPRO is reliable for detecting the tone and stiffness of the quadriceps femoris as well as the patellar tendon regardless of rater and time. However, joint positioning does influence the measurement of tone and stiffness due to the variation in tissue tension at different degrees of motion.

In summary, musculotendinous stiffness has been assessed through various imaging modalities, including ultrasonography and myotonometry. Greater stiffness is commonly observed in thicker, shorter tendons. Alterations in stiffness are associated with tissue elasticity and influences muscular contraction ability. More specifically, tendon compliance may be critical in altering the timing of muscle fascicle shortening. However, optimal musculotendinous stiffness has been recognized to vary depending on the role of the muscle-tendon complex, task intensity, and duration/consistency of loading.

B.2 Muscle-tendon Complex Adaptation to Eccentric Exercise

Cleak & Eston, 1992

The aim of this study was to investigate the magnitude and time course of performance decrements following high-intensity eccentric contractions. Following the performance of 70 maximal eccentric elbow flexion contractions muscle soreness, swelling, stiffness, and strength were assessed immediately after and then again every 24 hours for a total of 11 days. Peak decrements were observed between 24 hours post and 96 hours post. All variables except for isometric strength returned to baseline by day 11. These result indicate that while the more sensory variables of soreness, swelling and stiffness fully recovered, the continued strength loss may increase risk of injury following exercise that includes high-intensity eccentric contractions.

Warren et al., 1993

Researchers behind this publication sought to identify the mechanism of contractile failure associated with eccentric contractions performed by the soleus muscle within mice. Subjecting the isolated muscles to one of three contraction protocols that included 20 eccentric contractions, 10 eccentric contraction and 20 isometric contractions, Warren and his colleagues measured performance of a passive stretch, twitch and tetanus, and force production induced through a caffeine buffer. Results from this investigation indicated that force deficits observed in the muscles that performed 20 eccentric contractions was from a failure of the excitation process at some step prior to calcium release by the sarcoplasmic reticulum. Further examination of the sarcolemma of this same group suggested that membrane resting conductance was normal, supporting the idea that the ability of the injured fibers to conduct action potentials was most likely not impaired.

<u>Cook & McDonagh, 1995</u>

This publication provides findings specific to the function of human muscle-tendon complex through electrically stimulated contractions. While the first dorsal interosseous muscle was under stimulation, constant velocity stretches were applied inducing a lengthening contraction. The stretch produced an increase in muscular force of up to 80% during the stretch, however the magnitude of this increase was influenced by both the amplitude and velocity of the stretch. These findings demonstrate that force production during eccentric contraction is significantly greater than that of concentric and isometric force production. In concluding this study, the authors highlight that the presence of an elastic tendon may in fact being behavior differently than muscle fibers during lengthening contractions. Finally, the authors remark that during lengthening contractions the role of motor unit activation and elastic tendon lengthening requires further investigation in order to identify whether duration, velocity, or tension might lead to injury commonly observed with such contractions.

Kawakami et al., 2002

The purpose of this study was to identify the interaction between muscle fibers and tendinous tissues during different tasks and with counter movements. The authors hypothesized that the tendinous tissues, not muscle fibers, behave as elastic structures that are capable of energy storage and release, thus enhancing the joint performance during counter movement tasks. It was found that during counter movement actions (plantar flexion preceded by dorsiflexion of the ankle), muscle fascicle lengthened initially with little EMG activity during the dorsiflexion phase and remained constant while the whole muscle-tendon unit lengthened before decreasing in the final plantarflexion phase. During a task with no counter movement (plantar flexion only) muscle fascicle length decreased throughout the movement. From these results, it was concluded that during counter movement tasks, muscle fibers optimally work essentially isometrically, leaving the task of storing and releasing elastic energy for enhancing exercise performance to the tendon.

Arampatzis et al., 2007

The aim of this investigation was to determine whether the magnitude of the mechanical load induced as cyclic strain applied to the Achilles tendon may have a threshold that may prompt adaption effects on tendon mechanical and morphological properties. The cyclic loading intervention lasted a total of 14 weeks, and consisted of five sets of isometric plantarflexion contractions. Participants performed either a low-magnitude strain cyclic loading exercise or a high-magnitude strain exercise. It was found that only the high-magnitude strain exercises resulted in decrease strain at a given tendon force, an increase in tendon stiffness and tendon elastic modulus, and tendon hypertrophy of the Achilles tendon that was region specific. These results indicate that in order to achieve tendon biomechanical and structural adaptations, loading must include chronic high-magnitude strain.

Duchateau & Enoka, 2008

This article is a topic review on task dependent neural control during concentric and eccentric contractions. The presented evidence explains that when tasks involve submaximal contractions, the amount of motor unit activity differs during the shortening and lengthening phases of contractions. The greater force capacity of muscle during lengthening contractions, there are fewer motor units are recruited and discharge rate is lower during lengthening contractions compared with shortening contractions. While there is a known depression of voluntary activation in several muscles during maximal lengthening contractions, the mechanism(s) for this inhibition have yet to be identified. Finally, the authors state that there is a critical need for investigations that seek to determine the differences in performance of shortening and lengthening contractions in terms of the specific adjustments used by the nervous system to control the activity of the involved motor neurons and to what extent due sensory reflex organs play in activation modulation.

Guilhem et al., 2010

The aim of this review was to explore the properties of an eccentric contraction and compare neuromuscular and muscle-tendon system adaptations induced by isotonic and isokinetic eccentric trainings. During eccentric contractions, the shock-absorber, sping-like properties of the elastic tendon contribute to external torque production. These forced lengthening contractions are associated with high tension that cause both a deterioration of the cytoskeleton and a local inflammation response known as Delayed Onset Muscular Soreness. While there are neural processes that work to inhibit activation to preserve force producing tissues, investigations into the influence and timing of decay in the elastic tendon buffer capacity may provide more insight into not only the muscle-tendon complex but also sensory reflex activation.

Fouré et al., 2013

The aim of this study was to determine the specific effects of 14 weeks of eccentric training on muscle and tendon mechanical properties assessed in active and passive conditions *in vivo*. Along with simultaneous ultrasonography the series-elastic stiffness under active and passive plantar flexion, Achilles tendon stiffness and dissipative properties during isometric and passive contractions were measured pre- and post-training in 24 subjects. The active part of the series-elastic component, those that undergo lengthening during the eccentric contraction, demonstrated a significant decrease stiffness becoming more compliant. However, there was a significant increase in the Achilles tendon stiffness, thus becoming less compliant. There were no significant alterations that occurred between pre- and post-training in the triceps surae muscles and Achilles tendon architectural properties. In totality, these results demonstrate that the major effect on eccentric training produces a decrease in the active series-elastic component stiffness which leads to an increase in energy that could be stored during the lengthening contraction. This, coupled with the increase in Achilles tendon stiffness, allowing for more efficient transmission of energy; support the use of eccentric training for the modulation of mechanical properties of the muscle-tendon complex.

Gonzalez-Izal et al., 2014

The purpose of this study was to compare the progression of muscle force and the different EMG parameters that are traditionally used to assess muscle fatigue and muscle conduction velocity during a fatiguing exercise consisting of repetitive eccentric or concentric actions. Young adults performed four sets of 20 maximal contractions of either concentric knee extension or eccentric knee extension. It was observed that following the concentric protocol participants experienced greater immediate muscle force loss, blood lactate concentrations and greater change in EMG parameters. Both protocols similarly reduced muscle conduction velocity. Through ultrasonography, it was found that the eccentric protocol increased echo intensity of the quadriceps muscles indicating greater muscle damage. These results suggest that the effect of muscle damage from the eccentric exercise on the transmission of action potentials may be similar to the effects of hydrogen accumulation produced by concentric exercises. However, neuromuscular performance deficits following concentric exercise may have a greater influence on force and torque production than eccentric exercise.

Yin et al., 2014

This study sought to measure and compare patellar tendon stiffness and microcirculation in athletes and non-athletes following eccentric exercises. Participants completed four sets of 40 repetitions of eccentric knee extension. Through the use of ultrasonography it was found that following the eccentric protocol, the athletes who performed four sets of 80 and the non-athletes who performed four sets of 40 both experienced a reduction in patellar tendon stiffness. The athletes who only performed the four sets of 40 protocol had no changes in tendon stiffness. The authors concluded that while the reduction of tendon stiffness occurred in conjunction with increases in microcirculation, a cause-and-effect relationship cannot be determined from these results.

Chaudhry et al., 2015

The aim of this study was to investigate how tendon mechanical properties and tendon perturbations compared between eccentric and concentric exercise cycles. Utilizing a heel raise and lower exercise, researchers targeted the Achilles tendon in eleven participants. The exercise was performed so that each participant completed two repetitions of eccentric on one leg and two repetitions of concentric on the contralateral leg, and was repeated three times. Researchers determined that there was no alteration in tendon stiffness, however tendon perturbations, or movements, were significantly higher during eccentric contractions compared to concentric. Furthermore, these perturbations during the eccentric exercise were negatively correlated with tendon stiffness. These results may support that it is the tendinous tissue perturbations, and not specifically changes in stiffness, that lead to the positive observed clinical effects of the use of eccentric exercises for the treatment of Achilles tendinopathy.

Guilhem et al., 2016

The purpose of this investigation was to determine the relationships between muscle-tendon complex mechanics during maximal eccentric contractions and the extent of subsequent functional impairments induced by muscle damage. The musculotendinous junction, fascicles, and tendinous tissues were continually measured in the medial gastrocnemius using ultrasonography during 10 sets of 30 maximal eccentric plantarflexion contractions performed by nine young adults. The musculotendinous junction, fascicles, and tendinous tissues were all stretched beyond baseline values during and following the eccentric protocol. It was also found that fascicle stretch length, lengthening amplitude and negative fascicle work beyond the slack length were significantly related to decreases in force 48 hours after the eccentric protocol. These results indicate that contraction strain greatly influence the magnitude of muscle damage and that Achilles tendon compliance decreases the amount of strain. This tendinous alteration may attenuate muscle fascicle lengthening and subsequently muscle damage.

Geremia et al., 2018

This study sought to document the magnitude and the time course of neuromechanical and morphological adaptations in response to a triceps surae eccentric training program. The training program consisted of three 4 week mesocycles of three sets of 10 maximal repetitions of eccentric plantarflexion for a total of 12 weeks. This training program led to a greater increase in eccentric torque compared to isometric and concentric as well as increased eccentric EMG activity. It was also found that torque and muscle thickness increased until the 8th training week, while eccentric and isometric activation increased until the 4th training week. Finally, this intervention also produced a shift in the angle of peak torque in eccentric torque production that shifted towards longer muscle lengths. It was concluded that the changes in maximum eccentric force production were due to adaptations in muscle activation and muscle mass. Also, the shift in the optimal length for force production towards longer lengths are advantageous for increasing total joint range of motion and potentially lower predispositions for muscle strain injuries.

In summary, the muscle-tendon complex respond differently to eccentric training and acute eccentric exercise. Increased tendon stiffness is often the result of eccentric training, and is one reason as to why eccentric training is included in the rehabilitation of tendinopathies. However, acute bouts of eccentric exercise can lead to muscle damage and decreased tendon stiffness. The velocity and induced strain on the muscle-tendon complex during eccentric contractions greatly influences the alterations observed in both tissues. During eccentric contractions, initially the tendinous tissue succumbs to lengthening while the muscle fascicles are nearly isometric. Once the tendon has reached a lengthening threshold, the muscle fascicles then begin to lengthen and subsequently shorten at the very end phase of the contraction. This specific sequence of events is the primary basis for the above mentioned mechanical buffering role of the tendon.

B.3 Impact of Age on Muscle-tendon Complex

Dressler et al., 2002

The aim of this study was to investigate age-related changes in the biomechanical properties of patellar tendons to determine whether these declines are associated with changes in fibril diameter or collagen type. Researchers excised the patellar tendon of 50 rabbits, 17 young and 33 old, include the distal tibial attachment. It was found that maximum tendon stress declined approximately 25% and strain energy density declined approximately 40% in the specimens from the older rabbits compared with the younger specimens. Also, the distribution of collagen fibrils from the older rabbits' tendon were significantly smaller in diameters and type V collagen was observed only in these specimens. It was concluded that with increasing age after skeletal maturity, the presence of type V collagen may help regulate the composition of tendon however, the decrease in diameter of the collagen fibrils may adversely affect patellar tendon strength.

Karamanidis & Arampatzis, 2006

The purpose of this study was to examine the effects of aging and endurance running on the mechanical and morphological properties of different muscle-tendon complexes *in vivo*. Thirty older adults and 19 young adults were divided into two sub-groups of non-active and endurance runners, and all performed maximal voluntary contractions of plantarflexion and knee extension on a dynamometer. Ultrasonography was applied to examine muscle and tendon structure noninvasively. The aging group exhibited a reduction in stiffness of the patellar tendon and quadriceps aponeurosis, but this was not identified in the Achilles tendon and aponeurosis. In both older adults and young adults, musculotendinous junctions showed no differences between those who were endurance runners and those who were inactive. In conclusion, it was suggested that tendon changes related to aging do not occur proportionally throughout the body and increased chronic loading of the musculotendinous junction may not be sufficient to produce mechanical adaptation.

Onambele et al., 2006

The aim of this study was determine whether compromised postural balance in older subjects is associated with changes in calf muscle-tendon structural and mechanical properties. Young and old participants underwent single-leg and tandem postural stability trials for the evaluation of center of pressure displacement, as well as muscle-tendon complex examination through dynamometry with simultaneous ultrasonography. Both trial duration and center of pressure displacements were negatively impacted by age. Similarly, muscle strength, size, and activation capacity as well as tendon mechanical properties significantly decreased with age.

These results suggest that age-related changes in muscle-tendon complex characteristics may be one cause of the variance in balance task performance outside of habitual bipedal stance.

Karamanidis & Arampatzis, 2007

The purpose of this investigation was to examine the lower musculotendinous junction capacities in older individuals and how that may affect their ability to recover balance with a single-step after a fall. A secondary aim was to determine whether running experience, chronic loading, enhanced and protected the motor skills for recovery. Thirty older adults, 60-69 years old, and 19 younger adults, 21-32 years, performed an induced forward fall and were asked to take a single step forward to correct this fall. It was found that the age-related decreased tendinous stiffness, which was seen to a greater extent in the patellar tendon than the Achilles tendon, decreased the older subjects' ability to recovery from the forward fall. However, older individuals who were classified as endurance runners, had a quicker recovery time and required less steps for recovery following the forward fall compared to the non-active older adults. Researchers identified that while endurance running may not be successful at preventing the age-related decline in tendinous mechanical properties, it may maintain or enhance motor skills useful in correcting forward falls.

Bento et al., 2010

This study sought to determine the relationship between muscle peak torque and rate of torque development of the lower limb joints in older adult females with and without fall history. The participants within the fall group were identified to have experienced a fall within one year of their participation in this study. While peak torque was not significantly different for the either group specific to knee flexor muscles, rate of torque development was greater, faster, in women who had not experienced a fall within the last 12 months. The authors concluded that perhaps training that was specific to improve rate of torque development by incorporating ballistic exercises may improve older adults independent ambulation.

Stenroth et al., 2012

The aim of this study was to exam the concurrent age-related differences in muscle and tendon structures and properties. Achilles tendon morphology and mechanical properties as well as the triceps surae muscle architecture were measured in 100 subjects. Utilizing motion analysis-assisted ultrasonography, researchers were able to determine tendon stiffness, Young's modulus, and hysteresis during isometric ramp contractions. Ultrasonography was also used to measure muscle and tendon architectural features and size. Older participants demonstrated a 17% lower Achilles tendon stiffness and a 32% reduction in Young's modulus compared to the younger participants. Age also seemed to influence tendon cross-sectional area, as older participants exhibited a 16% larger cross-sectional area. Total triceps surae muscle size was smaller and medial gastrocnemius muscle fascicle lengths were shorter as well in the older participants. Researchers suggested that older individuals may compensate for lower tendon material properties by increasing tendon cross-sectional area, and that lower tendon stiffness might be beneficial for movement economy in low-intensity locomotion for older adults.

Thorpe et al., 2013

Utilizing an equine model, this study examined the alterations in whole tendon, fascicle and interfascicular mechanical properties in a high-strain energy storing tendon and a low-strain positional tendon with increasing age. While investigations were carried with horses, the equine superficial digital flexor is comparable to the human Achilles tendon and the equine common digital extensor is similar to the human anterior tibialis tendon. That data analyzed indicated there were no alterations in the mechanical properties of the whole superficial digital flexor with aging, however there was significantly less sliding at the fascicular interface. This increase in stiffness of the interfascicular matrix may result in fascicles being loaded at an earlier point in the stress-strain curve, increasing risk of damage.

Csapo et al., 2014

The purpose of this investigation was to determine whether the age-related decrease in tendon stiffness would lead to greater muscle fascicle strains during an isometric contraction. Dynamic velocity-encoded phase-contrast magnetic resonance imaging of the medial gastrocnemius muscle and the Achilles tendon were captured in six young and six older adults during a submaximal isometric plantarflexion contraction. Despite identifying a lower Achilles tendon stiffness in the older adults, contraction-induced changes in the medial gastrocnemius muscle fascicle lengths were similar in both young and older adults at equal levels of absolute force. These results suggested that factors other than Achilles stiffness might serve as compensatory adaptations to limit the degree of muscle fascicle strains upon contractions.

Ruan et al., 2015

The aim of this study was to investigate Achilles tendon elasticity in health volunteers utilizing acoustic radiation force impulse to identify shear wave velocities. This study included 56 health individuals ranging in age from 25-65 years old. Shear wave velocities increased with increasing age during relaxation and contraction, indicating a decrease in tendon elasticity. However, this study was limited by the small mid-tendon region of interest. This may be problematic because it is commonly supported that the poles of energy storing tendons are the regions that 1. Experience the greatest adaptation to loading and unloading and 2. Tendinous injuries commonly occur at or near the poles of the tendon, this includes ruptures.

Intziegianni et al., 2016

The purpose of this study investigated the influence of age and pathology on Achilles tendon properties during single-leg vertical jump. Thirty volunteers, 10 children, 10 asymptomatic adults, and 10 tendinopathic patients, performed single-leg vertical jumps on a force-plate with an ultrasonographic probe positioned at the musculotendinous junction. The mean age of the children was 13 years old, the adults was 37 years old, and the tendinopathic patients was 40 years old. The children exhibited the highest level of Achilles tendon elongation, compliance, and strain. There was no significant difference between Achilles tendon length, force, or strain between the adults and the tendinopathic patients. However, Achilles tendon elongation and compliance were higher in the patients compared to the asymptomatic adults but this did not reach a significant level. These authors suggest that higher elongation, compliance and strain are indicative of healthy tendon and that a higher compliance might be considered a protective factor against load-related injuries.

Holzer et al., 2018

The study investigated the relationship between ankle plantarflexor muscle strength and Achilles tendon biomechanical properties in older female adults. Thirty female participants between the ages of 60 and 75 years old underwent MRI imaging of their low leg in order to provide measurement of the Achilles tendon cross-sectional area. Achilles tendon force, stiffness, and Young's modulus were assessed through ramped isometric contractions on a dynamometer with simultaneous ultrasonography of the musculotendinous junction. The results of this study indicated that Achilles tendon force is positively correlated with Achilles tendon stiffness and Young's modulus; however this is only true for the dominant leg, no significant correlations between these variables were found in the non-dominant leg. The authors conclude that increases in muscle strength in older adults may lead to changes in tendon stiffness primarily through alterations in material composition rather than size.

Slane et al., 2018

The purpose of this study was to evaluate middle-aged Achilles tendon displacement patterns *in vivo* using ultrasonography. Health middle-aged adults with a mean age of 49 years old performed eccentric trials of dorsiflexion and plantarflexion. Under passive loading, the average tendon displacement was significantly higher in the middle-aged adults compared to a younger adult group. However, during eccentric loading there was no difference between the age groups for tendon displacement. The difference between passive and eccentric loading conditions was significantly greater for tendon displacement in the middle-aged adults. These results suggest that aging contributes to specific changes in tendon compliance and deformation. These changes may have implications for increase in injury incidence observed in middle-aged adults.

Quinlan et al., 2018

The aim of this project was to establish the muscle and tendon contributions to differences in rate of torque development between young and older adult men. Muscle and tendon structure and biomechanical properties were examined by magnetic resonance imaging and ultrasonography. Rate of torque development was obtained through the analysis of EMG root mean square values during isometric maximal voluntary contractions and electrically stimulated supramaximal contractions. Absolute rate of torque development was lower in older males, and when normalized to muscle cross-sectional area remained lower. Maximal muscle twitch, time to peak torque, and activation capacity were significantly affected in the older men, as well. Similarly, tendon stiffness was impacted by age as stiffness was found to be lower in older men and was also related to the normalized rate of torque development. Researchers concluded that these results provide evidence that a lower rate of torque develop seen in older adults is caused by slower muscle contraction speeds, slower time to peak torque, decreased muscle cross-sectional area, and decreased tendon stiffness. It was suggested that these findings reinforce the importance of both muscle and tendon characteristics when considering rate of torque development

In summary, it has commonly been observed that the musculotendinous tissues become more compliant with age. There is also evidence that indicates age-related declines in neuromuscular variables such as peak torque, EMD, and RTD. While often times not investigated simultaneously, it is has been concluded that both increased compliance of tendinous tissue as well as age specific decreases in muscle activation are factors increase the risk of falling and potentially disrupt independent living for older adults.

Appendix C - Culminating Project IRB Approval

KANSAS STATE UNIVERSITY | University Research Compliance Office

TO: Ryan Thiele Proposal Number IRB-10586
Dean of Health & Human Sci
Manhattan, KS 66506

FROM: Rick Scheidt, Chair
Committee on Research Involving Human Subjects

DATE: 03/12/2021

RE: Approval of Proposal Entitled, "Time-course of tendon and muscle mechanical properties during eccentric exercise in young and old men."

The Committee on Research Involving Human Subjects has reviewed your proposal and has granted full approval. This proposal is **approved for <u>three years</u> from the date of this correspondence.**

APPROVAL DATE: 03/12/2021

EXPIRATION DATE:03/11/2024

In giving its approval, the Committee has determined that:

No more than minimal risk to subjects

203 Fairchild Hall, Manhattan, KS 66502 | (785) 532-3224 | fax: (785) 532-3278

This approval applies only to the proposal currently on file as written. Any change or modification affecting human subjects must be approved by the IRB prior to implementation. All approved proposals are subject to continuing review, which may include the examination of records connected with the project. Announced post-approval monitoring may be performed during the course of this approval period by URCO staff. Injuries, unanticipated problems or adverse events involving risk to subjects or to others must be reported immediately to the Chair of the IRB and / or the URCO.

Electronically signed by Rick Scheidt on 03/12/2021 12:27 PM ET