THE INFLUENCE OF AGE ON MUSCLE AND TENDON LENGTH CHANGES DURING VISCOELASTIC CREEP.

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ABSTRACT

MICHAEL J. SCHARVILLE: The influence of age on muscle and tendon length changes during viscoelastic creep.
(Under the direction of Eric Ryan)

The purpose of this study was to examine the influence of age on muscle-tendon behavior during a bout of constant-torque (CT) stretching. Eighteen young and 16 older healthy men performed a 60-s CT stretch at their pre-determined torque threshold. Changes in ankle joint position and muscle and tendon lengths from pre- to post-stretch were examined using an electrogoniometer and ultrasonography, respectively. Ankle joint position increased for both groups, however the younger men (2.01°) experienced a non-significant (P=0.072) greater increase when compared to the older men (1.41°) and a simultaneous greater increase in muscle-tendon unit length (P=0.043). Furthermore, muscle and tendon lengths increased for both groups; however the muscle increased more (P<0.001) in the young (Δmuscle length – 77%; Δtendon length – 23%) and the tendon increased more (P<0.001) in the old (Δmuscle length – 36%; Δtendon length – 64%). These findings may be due to the age-related increases in tendon compliance and muscle stiffness.

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LIST OF ABBREVIATIONS

AT	Achilles tendon
CT	Constant-torque
EMG	Electromyography
MG	Medial Gastrocnemius
MTJ	Myotendinous junction
MTU	Muscle-tendon unit
MVC	Maximal voluntary contraction
SOL	Soleus
TA	Tibialis Anterior
UNC-CH	University of North Carolina at Chapel Hill
US	Ultrasound
US-tape	Ultrasound tape measure

CHAPTER I

INTRODUCTION

Skeletal muscles and tendons respond viscoelastically, exhibiting a combination of viscous and elastic behaviors in response to tensile loads. Each behavior exists along a viscoelastic continuum and is independently based on the rate and load applied to the tissue (56). Elastic resistance to elongation is instantaneous and proportional to the load placed on the tissue (52). However, viscous resistance is time dependent and relative to the rate of elongation (56). Two commonly measured properties of viscoelasticity are stress-relaxation and creep. Viscoelastic stress-relaxation is the decrease in resistance (force or torque) when the muscle-tendon unit (MTU) is held at a constant-angle or length. Viscoelastic creep is the amount of deformation, or passive lengthening of the MTU, as a result of a constant load placed upon the MTU (56).

Many of the original studies investigating the viscoelastic responses during passive stretching have utilized static or constant-angle stretching techniques where the joint angle is held constant and the MTU exhibits stress-relaxation (31-34). For example, McHugh and colleagues (34) were the first to examine the viscoelastic stress-relaxation *in vivo* during constant-angle stretching of the hamstring muscles. These authors examined the stress-relaxation response during a stretch at the point discomfort resulting in noticeable electromyography (EMG) activity and a stretch where the muscles were passive (<5% muscle activity of active stretch). The relative stress-relaxation response was similar between both stretches (-14.4 ± 2.2% active, -13.0 ± 2.3% passive) indicating that the viscoelastic response was primarily mechanical in nature. More recently, studies have suggested that constant-torque (CT) stretching may be more beneficial than constant-angle maneuvers as it elicits more work on the MTU, thus reducing passive stiffness and increasing joint range of motion to a greater extent (17,50,64,65). As its name implies, CT stretching is when a participant is stretched with a powered dynamometer at a constant passive torque. CT stretching routines result in viscoelastic creep or an increase in joint position. Viscoelastic creep has been examined indirectly through repetitive constant-angle stretches starting at the same initial torque (52,53,56), and during single (49) and repetitive (48) bouts of CT stretching. For example, Taylor et al. (56) developed an *in vitro* model to examine the viscoelastic creep response during repetitive constant length stretches of rabbit MTUs and reported that the MTU increased by 3.46 ± 1.08% of its initial length following the 10 stretching cycles, with 80% of the changes in length occurring during the first four stretches. Furthermore, Ryan et al. (49) determined that a single bout of CT stretching results in a reliable *in vivo* viscoelastic creep response of the plantarflexor muscles with most of the increase in joint position occurring in the first 15-20 seconds.

Previous studies have examined MTU length changes throughout a range of motion and have indicated that both the muscle and tendon play a role in the passive lengthening of the MTU. However, there are contrasting findings that have suggested the increase in MTU length is primarily due to increases in tendon length (16,25,26), while other studies have indicated that these increases are primarily due to increases in muscle length (1,5,7,21,37). For example, Herbert and colleagues (16) examined the relative contribution of the muscle and tendon with the muscle fully shortened (knee flexed, ankle plantarflexed) and the muscle fully lengthened (knee extended, ankle dorsiflexed). They determined that the increase in MTU length was largely due to increases in tendon length with only a 27% increase in medial gastrocnemius (MG) muscle fascicle length. In contrast, Abellaneda et al. (1) determined that the increase in muscle fascicle length accounted for approximately 70% (16.8 ± 5.2 of the 24.4 ± 1.8 mm) of the total MTU length changes during a dorsiflexion range of motion assessment. Other recent studies have also demonstrated that that the muscle is the primary structure being lengthened (1,5,7,21,37) during increases in dorsiflexion range of motion. However, the tissues that account for the stretching-induced viscoelastic creep response are unclear. Morse and colleagues (37) were the first to examine MTU changes following a series of passive stretches and reported that the reduced resistance to stretch within the same common range of motion was due to a 0.24 cm increase in muscle length. Interestingly, there was no change in fascicle length from pre- to post-stretch indicating that the stretching-induced changes were likely influenced by the intramuscular connective tissues (i.e. perimysium). In addition, other structures have been attributed to the viscoelastic response during passive stretching which include lengthening of the sarcomere cytoskeleton (titin and desmin) (9,36,57), rearrangement of the perimysial collagen fibers (46), and relaxation of the proteoglycan matrix tissue (14), and deformation of the aponeuroses (37).

Physiological changes that occur with aging have been well documented, however the extent to which these changes influence the viscoelastic properties of the MTU remains unclear. Older individuals exhibit decreases in muscle size (11,40,42) and architecture (24,41) with an increased proportion of stiffer slow twitch fibers (3,28,39) and an infiltration of adipose and connective tissue (2,29,47). All of these factors may contribute to the increases in passive stiffness seen among older adults which in theory may alter MTU compliance and alter the viscoelastic creep response during common stretching exercises (9,10). The role of passive stiffness on the viscoelastic creep response has recently been investigated by Sobolewski et al.

(53) in a sample of young individuals dichotomized into low and high passive stiffness groups. The authors indirectly examined creep across four 30-sec constant-angle stretches starting at the same passive torque value. The results indicated that the rate of change in viscoelastic creep was lower for the high stiffness group when compared to the low passive stiffness group. The authors (53) suggested that the stiffer participants displayed less creep because they exhibited greater elastic resistance to stretch across subsequent stretches. However, further investigation by Sobolewski et al. (52) using the same stretching protocol indicated that older men (67 ± 3 years) had a similar viscoelastic creep response when compared to younger men, despite differences in passive stiffness. As demonstrated by Dierick and colleagues (6), it is possible that aging did not influence viscoelastic creep because there is a simultaneous age-related increase in elastic stiffness and decrease in viscous stiffness. However, future studies are needed that directly examine viscoelastic creep in both young and old adults during CT stretching protocols.

The extent to which the muscle and tendon increase in length during a passive CT stretch and the resultant viscoelastic creep response remains unclear. Based on the work by previous authors (22,37), it is possible that the muscle accounts for the majority of the increase in MTU during CT stretching. However, this response may be altered in older adults. For example, Mian and colleagues (35) examined the age-related differences in muscle and tendon contribution during the stance phase of the gait cycle. The authors determined that although there were no changes in MTU length between ages, the older adults experienced greater increases in tendon length when compared to muscle. Narici et al. (40) determined that older tendons were approximately 15% more compliant than younger tendons with disparities conceivably due to an increase in elastin content, reduction in collagen fibril crimp angle, and/or reduction in extracellular water content. These age-related changes have recently been supported in the literature (40,54); however, further investigation into the structural alterations during CT stretching is warranted. Therefore, the purpose of the present study is to investigate the influence of age on muscle and tendon contribution during viscoelastic creep.

Research Questions

1. Is the viscoelastic creep similar between young and older men?
2. Are the relative changes in MG and AT length during viscoelastic creep different between young and older individuals?

Hypotheses

1. Older adults will exhibit a reduced absolute viscoelastic creep response.
2. The tendon will account for a greater percentage of the increase in MTU length during CT stretching in older adults when compared to young adults.

Delimitations

1. Forty recreationally active men will be recruited to participate in the study. Recreationally active will be defined as those who exercise between 1-5 hours per week.
2. Twenty participants will be between the ages of 65 and 74 and 20 participants will be between the ages of 18-25.
3. The study requires two visits to the Neuromuscular Research Laboratory on separate days (at least 48 hours apart) at the same time of day (±2 hours).

Limitations

1. The participants are not truly random as they will be recruited from UNC-Chapel Hill and the surrounding area.

2. The stretching protocol may not reflect everyday stretching because it will occur in one leg and it will be done in an isokinetic dynamometer that most people do not have access to.

Theoretical Assumptions

1. Participants will provide accurate medical and physical activity history.
2. Participants will be honest in the subjective torque threshold bout.
3. Participants will adhere to the guidelines of no strenuous lower limb exercises 24 hours prior to testing.

Operational Definitions

1. *Constant-Torque Stretch (CT stretch):* A powered dynamometer will stretch the plantarflexor muscles into dorsiflexion at a fixed pre-determined passive torque value that will remain constant during the entire stretch.
2. *Muscle-tendon unit (MTU):* Gross anatomical structure composed of a proximal MG portion and a distal AT portion.
3. *Passive Stretch:* Presence of minimal, or negligible electromyography activity during a stretch.
4. *Viscoelastic creep:* Time-dependent property in which there is an increase in position or length with the MTU held at a constant torque.

CHAPTER II:

REVIEW OF LITERATURE

This review will start by introducing viscoelastic properties of the MTU with the primary focus on viscoelastic creep. Previous research shows that viscoelastic creep can be measured indirectly through repetitive stretches at the same starting tension or measured directly when the MTU is stretched at a constant-torque. The constant-torque stretch may increase the joint position and lengthen the MTU during a single bout. However, the specific tissue alterations (muscle and tendon) associated with this passive lengthening have yet to be quantified. Several studies will be discussed that have been able to identify muscle and tendon contribution when the MTU is stretched through a range of motion. To my knowledge, few studies have examined the viscoelastic responses in older adults. Therefore, a review of the physiological changes that occur with the aging MTU will be used to help support the hypothesis. In conclusion, a few studies will be highlighted to illustrate how to estimate MTU length during rest and stretching.

Viscoelastic Properties of the Muscle-Tendon Unit

Taylor, Dalton, Seaber, and Garrett (1990)

The purpose of this investigation was to examine the acute effects of passive stretching on the viscoelastic properties of the muscle-tendon unit (MTU). Forty extensor digitorum longus (EDL) and 20 tibialis anterior (TA) rabbit MTUs were used in this study. This study consisted of

3 parts: (I) viscoelastic response of eight EDL MTUs during 10 repeated cyclic stretches to a pre-determined length (10% beyond its resting length), (II) viscoelastic response of 12 EDL MTUs during ten 30-s static stretches to a pre-determined tension (78.4 N), and (III) the viscoelastic response of 20 EDL and 20 TA MTUs performing cyclic stretching to a pre-determined length at varying rates (0.01, 0.1, 1, and 10 cm/sec) and while the MTUs were innervated and denervated. For part I, peak tension decreased 16.6% from the 1st to 10th cycle, however, only the first four stretches were significantly different ($P<0.05$) from the remaining stretches. Part II illustrated that the stress relaxation curves for the first two stretches were significantly different ($P<0.05$) from the remaining stretches. In addition, the MTU increased by $3.46 \pm 1.08\%$ of its initial length following the 10 stretching cycles, with 80% of the length changes occurring during the first four stretches. The results for part III indicated that peak tensile force (N) and energy absorbed (N·cm) by the MTU increased ($P<0.0001$) with increasing stretch rates. However, the MTU responded similarly (peak force and energy absorbed) during both innervated and denervated conditions. These results suggested that the rabbit MTU exhibits viscoelastic properties during stretching techniques commonly used in clinical and athletic settings. Specifically, the decline in peak tension and increase in MTU length under the same tension demonstrate stress relaxation and muscle creep properties, respectively. This study also illustrated that peak tensile force and absorbed energy were dependent on the rate of stretch and that slower stretches allow for a greater degree of stress relaxation to occur. Finally, this study alluded to the fact that only four stretches may be necessary and lead to most of the MTU elongation.

McHugh, Magnusson, Gleim, Nicholas (1992)

The purpose of this study was to determine whether viscoelastic stress relaxation could occur in human skeletal muscle, independent of a stretch-induced electromyography (EMG) response. A total of 15 subjects (nine men and six women) with a mean age of 28.7 ± 1.2 years participated in the study. The hamstring muscle group was stretched to a fixed angle via a passive straight leg raise at a constant velocity while each subject laid supine with their contralateral limb strapped to the table. Each subject underwent two tests in which hip flexion range of motion (ROM), the stretch-induced EMG response of hamstring muscle group, and the resistance to stretch (N) were measured. An electrogoniometer was placed over the hip joint to account for the hip ROM during the stretch. Surface EMG electrodes were placed midway between the gluteal fold and the knee joint, over the semitendinosus muscle belly measuring muscle activity throughout the stretch. Resistance to stretch or tensile force was measured using a load cell fastened to a chain fixed around the subject's ankle. The testing protocol consisted of two stretches at a constant velocity. During the first trial, the researcher stretched the muscle at a rate of 1.63 ± 0.46 degrees/second to the maximum tolerated ROM and then held that angle constant for 45 seconds. The hip joint angle was 90 ± 6° and all 15 subjects experienced a stretch induced EMG response (542.6 ± 107.1μV•s). Also, with the first trial there was a significant decrease in force from 65.8 ± 5.3 to 54.4 ± 4.6 N (P<0.0001) demonstrating stress relaxation. The second trial was completed by 10 of the subjects and consisted of a straight leg raise (2.04 ± 0.19 degrees/second) brought to 5 degrees below the ROM at which the onset of EMG activity occurred. At that position (53 ± 6°), the stretch was held for 45 seconds. The second stretch illustrated negligible EMG activity (18.0 ± 4.7 μV•s); however, there was still a significant decline in force from 28.3 ± 4.4 to 24.1 ± 3.2 N (P<0.05) indicating that stress relaxation can occur independent of EMG activity. Although there was a greater absolute decrement in force from test 1 to test 2, the relative decreases in force throughout the stretch for the 10 subjects who completed both tests was not significantly different between the tests (14.4 ± 2.2% test 1, 13.0 ± 2.3% test 2). Test 2 was able to demonstrate that viscoelastic stress relaxation in human skeletal muscle is a mechanical response and can occur with the muscle in a passive state.

Yeh, Tsai, & Chen (2005)

The purpose of this investigation was to compare the effectiveness of a constant-torque prolonged muscle stretching (PMS) treatment on the inhibition of ankle hypertonia (high tension or abnormally rigid muscle) with that of constant-angle PMS treatment. A total of 30 spastic hemiplegia subjects (20 men, 10 women) with a mean age of 54 ± 9 years participated in this study. Each subject completed a constant-angle and constant-torque PMS treatment of the calf on two different occasions separated by one week and about the same time of day. The PMS treatment included 30 minutes of continuous stretching with the order of stretches being randomized. Each subject underwent a series of pre- and post-treatment assessments that included frequency-varied sinusoidal movements and a passive maximum range of motion (ROM) test. Three sinusoidal stretching motions (±3°) were performed at 10 different frequencies (3-12 Hz) in a random sequence and used to compare the two different PMS treatments effect on the viscous and elastic components. Prior to the stretching treatment, each subject completed a maximum passive dorsiflexion ROM assessment using a motor-driven stretching device at a rate of 5°/s. For the constant-torque PMS, 80% of the passive torque that was generated at the maximum ROM was used to hold the stretch at a constant-torque for 30 minutes. In contrast, the constant-angle PMS dorsiflexed the ankle to the maximum ROM and held the angle constant for 30 minutes. During each treatment, a torque sensor was used to measure reactive torque, and angular changes were recorded using a potentiometer mounted on the ankle joint. Results from the study indicated that both viscoelastic stress relaxation and creep occurred in constant-angle and constant-torque stretches, respectively. The constant-angle PMS resulted in decreases in torque from -18.71 to -9.32 Nm. The constant-torque PMS elicited an increase in dorsiflexion position from 14.91 to 18.89°. After a single treatment session, ROM increased from an average value of 8.77 ± 4.84° to 12.57 ± 4.90° (P<0.01) for the constant-angle treatment, and the ROM increased from an average value of 8.73 ± 2.85° to 13.5 ± 3.28° (P<0.01) for the constant-torque PMS. However, there was no difference (P≥0.05) in the ROM between the constant-angle and constant-torque PMS groups. Lastly, using a second-order biomechanical torque model, elastic and viscous components were quantified for each stretch. Results indicated that the constant-torque PMS was more effective than constant-angle PMS in reducing both the viscous and elastic components after a single treatment session (P<0.05). This study suggests that constant-torque PMS is more effective for inhibiting spasticity, increasing ROM, and altering skeletal muscle viscoelastic properties.

Ryan, Herda, Costa, Walter, Hoge, Stout, Cramer (2010)

The purposes of this study were to characterize viscoelastic creep *in vivo* in the human skeletal muscle-tendon unit (MTU) and examine the consistency of these responses during a 30-second stretch. Twelve healthy men (age 22 ± 3 years) volunteered for the study and completed three separate trials about the same time of day (±2 hours). A familiarization trial was used to introduce participants to the dynamometer as well as to determine the maximum tolerable passive torque threshold (torque at which the participant felt comfortable holding the stretch and the lower limb muscles were passive). The resulting torque threshold was used for the viscoelastic creep assessments during both experimental trials. For each experimental trial, the participants completed one 30-second constant-torque stretch in the seated position of the dynamometer. During each stretch, the lever arm of the dynamometer passively dorsiflexed the foot (5°/s) from -20° of dorsiflexion to the maximum tolerable passive torque threshold and held the torque constant for 30 seconds. Surface electromyography electrodes were placed on the medial gastrocnemius (MG), soleus (SOL), and tibialis anterior (TA) according to SENIAM guidelines. A maximal voluntary contraction (MVC) of the plantarflexors was completed following the constant-torque stretch. To ensure that the creep assessments were passive, MG and SOL values were normalized to the electromyography amplitude values recorded from the MVC. The raw electromyography amplitudes from the TA during the stretch and the MVC were used for analysis. Six separate one-way repeated measures analyses of variance were applied to examine the changes in position, torque, and the normalized and raw electromyography amplitude values were collected every 5seconds during the 30-second stretch. Test-retest reliability statistics were analyzed at 5-second increments for position. Six separate intraclass correlation coefficients (ICCs) model 2,1 per the recommendations of Shrout and Fleiss (1979) and the standard errors of the measurement (SEM) were calculated to analyze the test-retest reliability. Results indicated a significant and continuous increase in position ($P<0.001$) over 30 seconds during the constant-torque stretch. Relative consistency was high with ICC values >0.994 and absolute consistency was low with SEM values being <1.54% (expressed as percentage of mean). However, most of the increase in position occurred during the first 15-20 seconds (73-85%). These findings demonstrate that viscoelastic creep of the human skeletal muscle-tendon unit can be reliably examined *in vivo*. Further, the viscoelastic creep response appears to be independent of muscle activation as there was no main effect for any of the electromyography amplitudes.

Ryan, Herda, Costa, Walter, Cramer (2012)

The purpose of this investigation was to characterize the viscoelastic creep responses during repeated constant-torque stretching exercises using stretch durations commonly performed in athletic and clinical settings. Twelve men (age 22.4 ± 3.2 years) volunteered for the study. Each participant visited the lab on two occasions performing a familiarization and an experimental session. The familiarization session and setup was similar to the procedures described by Ryan et al. (49) (see above). During the experimental session, four 30-second constant-torque stretches at the pre-determined maximum tolerable torque threshold were completed. A 20-second rest period between stretches was given were the ankle was returned to -20° of dorsiflexion. The velocity at which the foot was dorsiflexed during the stretch and plantarflexed during the rest period was constant at 5°/s. An isometric maximal voluntary contraction (MVC) was completed after the fourth stretch. Surface electromyography electrodes were placed on the medial gastrocnemius, soleus, and tibialis anterior according to the SENIAM guidelines. Position, torque, and electromyography amplitude values were monitored every five seconds. The normalized EMG amplitudes for the MG and SOL and the raw TA amplitudes values during each stretch did not change ($P \geq 0.05$) and were negligible with a mean response of 1.6% of the MVC values indicating that each stretch was passive. The absolute changes in position were plotted on a logarithmic time scale and fit with a linear regression line to examine the rate of increase and the overall increase in position for the entire stretch. Results from the study indicated a significant main effect for time. The 0-5-second time period showed the greatest relative change in position in comparison to all other time periods ($P<0.001$). The 5-10-second time period had a greater relative change in position in comparison to the 10-15, 15-20, 20-25, and 25-30-second time periods ($P<0.001$). Also, the 10-15 and 15-20-second time periods were greater than the 25-30-second time periods ($P<0.001$). Over the course of the four stretches, the creep response was similar with no significant differences ($P\geq0.05$) between the slope values when plotted on the logarithmic time scale. However, the absolute ankle position and the y-intercept values increased following both stretch 1 and 2 ($P<0.05$) while stretch 3 and 4 started and ended at the same joint angle. This study illustrates the dynamics of viscoelastic creep and specifically that the rate (relative) and amount (absolute) of creep are similar over the four 30-second stretches with the majority of creep (increase in joint angle) occurring in the first 15-20 seconds, similar to findings of many previous studies examining stress relaxation.

Sobolewski, Ryan, Thompson (2013)

The purpose of this study was to determine if the acute viscoelastic stress relaxation and creep responses to a practical bout of passive stretching were influenced by differences in maximum range of motion (MROM) and passive stiffness using a new *in vivo* technique similar to that described by Taylor et al. (56). Thirty-seven men (age 24 ± 3 years) volunteered for the study and visited the lab on two separate occasions (2-7 days for subsequent session). The first visit was a familiarization session in which each subject practiced the stretches and determined a maximal tolerable passive torque threshold as previously described by Ryan et al. (49) (see above). During the experimental session, a MROM, an isometric maximal voluntary contraction (MVC), and four 30-second stretches were completed. A MROM assessment was conducted using the dynamometer to passively dorsiflex the foot from 20° of plantarflexion to the participants maximally tolerated end ROM (onset of pain) and then released back down to the starting position (velocity constant at 5°/s). Passive stiffness values were calculated from the MROM assessment as the slope of the angle-torque curve at 10° of dorsiflexion. Surface electromyography electrodes were placed over the medial gastrocnemius (MG) and the soleus (SOL) and used to ensure each stretch was completely passive. To ensure that each stretch was passive, an MVC of the plantarflexors was completed to normalize the electromyography amplitude. A total of four stretches were completed where the dynamometer lever arm dorsiflexed the calf muscle-tendon unit (5°/s) to the torque threshold with the corresponding angle held constant for 30-seconds. A 30-second break was used in between each of the four stretches. Viscoelastic stress relaxation was examined via the torque responses collected every five seconds. The absolute changes in torque were plotted on a logarithmic time scale and fit with a linear regression line to examine the rate of decrease during the four stretches. The relative changes in torque were also calculated for each 30-second stretch. Viscoelastic creep was measured indirectly based on the position changes at the start of each stretch. Each starting position was plotted on a logarithmic time scale and fitted with a linear regression line to examine the rate of increase in position. Following the stretches, the individuals were ranked and then dichotomized into high and low passive stiffness and large and small MROM groups. Both MROM and passive stiffness groups were significantly different from one another (P<0.001). The results indicated that viscoelastic stress relaxation was greater (P≥0.05) in the first stretch than any other stretch when ranked by both MROM and passive stiffness. However, MROM and passive stiffness did not influence any of the stress relaxation variables (P≥0.05). MROM had no influence on viscoelastic creep; however, the rate and relative change in creep was influenced by differences in passive stiffness. The rate of change in creep was decreased among passively stiffer individuals ($P<0.05$). In conclusion, this study demonstrates that MROM had no influence on viscoelastic stress relaxation or creep; however, the rate and relative change in creep was influenced by differences in passive stiffness but had not effects on stress relaxation. It was also suggested that the passively stiffer group may exhibit less creep because they exhibit greater elastic resistance to stretch.

Contributions of Muscle and Tendon

Kubo, Kanehisa, Kawakami, Fukunaga (2001)

The purpose of this study was to investigate the effects of static stretching on the viscoelastic properties of human tendon structures *in vivo*. Seven recreationally active, healthy men (age 25.3 ± 1.4 years) participated in the study. Each subject rested prone with the knee fully extended and the foot placed on the footplate of an isokinetic dynamometer. The subject performed two ramped isometric maximal voluntary contractions (MVC) with a three-minute rest period between trials before and after the 10 minute static stretch. To measure tendon elongation, an ultrasound (US) was placed over the midbelly of the medial gastrocnemius. A marker was placed between the skin and the US probe to confirm the probe did not move during the MVC. The displacement of the aponeurosis is considered to indicate the lengthening of the deep aponeurosis and the distal tendon. During the MVC, the aponeurosis moved proximally and was defined as the length change of tendon and aponeurosis during contraction. The displacement value at MVC was converted to strain by the following equation: Strain (%) = $L \bullet TL^{-1} \bullet 100$ where TL is the length of the tendon structure and L is the displacement of the aponeurosis. To calculate stiffness, the measured torque (TQ) during the MVC was converted to muscle force (Fm) via the following equation: $Fm = k \bullet TQ \bullet MA^{-1}$ where k is the relative contribution of the physiological cross-sectional area of the medial gastrocnemius and MA is the moment arm length at neutral ankle joint (0°). The Fm and L values above 50% of MVC were fitted to a linear regression equation with stiffness being derived from the slope of the regression line. Hysteresis was also calculated as the area under the Fm-L curves (ascending and descending phases). Static stretching was administered by the footplate moving from a neutral position (0°) dorsiflexion to 35° of dorsiflexion at a constant velocity of 5°/s and held constant at that position for 10 minutes. Passive torque was detected during the stretch using the dynamometer. Surface electromyography (EMG) electrodes measured activity from the medial gastrocnemius, lateral gastrocnemius, soleus, and tibialis anterior to ensure each stretch was passive. Prior to the stretch, strain, stiffness, and hysteresis calculated from the Fm-L curves 8.1 ± 1.6%, 22.9 ± 5.8 N/mm, and 20.6 ± 8.8%, respectively. Results from the stretching bout indicated viscoelastic stress relaxation occurred during the static stretch as the passive torque peaked initially at 36.1 ± 7.0 Nm with a mean rate of decline at 23.6 ± 8.5% ($P<0.05$). The MVC post stretch was not significantly different in terms of force ($P\geq0.05$), but the tendon length values at near MVC were significantly greater after stretching ($P<0.05$). The static stretch technique resulted in the tendon structures being more compliant by decreasing stiffness from 22.9 ± 5.8 to 20.6 ± 4.6 N/mm. Hysteresis also decreased significantly from 20.6 ± 8.8 to 13.5 ± 7.6% ($P<0.05$) suggesting that the viscosity within the tendon structures decreased making the tendon more prone to lengthen. To conclude, static stretching decreases the viscosity of the tendon structures as well as increases the elasticity ($P<0.05$) providing a background for reducing passive resistance and improving joint range of motion after stretching.

Herbert, Moseley, Butler, Gandevia (2002)

The purpose of this investigation was to determine how much of the total increase in muscle-tendon unit (MTU) length was attributed to the muscle fascicles and how much was taken up by the elongation of the tendons. A total of five experiments were performed on 24 healthy volunteers between the ages of 24 and 44 years. For the first experiment (n = 9) the change in length of the medial gastrocnemius (MG) muscle fascicles and tendon were analyzed using ultrasonography (US). Each individual sat semi-reclined and strapped to a footplate with surface electromyography electrodes placed on the lateral gastrocnemius. A linear array US probe was placed over the midbelly of the medial gastrocnemius (MG) to determine the length of individual muscle fascicles (measured off-line). Separate measurements of the MTU and muscle fascicles were collected when the knee was flexed and the ankle plantarflexed and then with the knee extended and the ankle dorsiflexed. The rationale was to see the change in MTU and muscle fascicle length from a fully shortened position to a fully lengthened position. The second experiment (n = 6) examined the change in length of the tibialis anterior (TA) muscle fascicles and tendon. For these measurements, the TA was stretched via plantarflexion with the knee positioned between 15 and 30° of flexion. The US probe was placed on the midbelly of the TA and the fascicle was captured. The third experiment (n = 3) examined the effect of a prior contraction on MG fascicle length. Prior to each measurement, an isometric contraction at the test angle was performed for approximately three seconds. Similarly, experiment four (n = 3) looked at the effect of a prior contraction on TA fascicle length. An isometric contraction was performed at the test angle (full plantarflexion) prior to each measurement. Lastly, the fifth experiment (n = 9) tested whether the relationship between changes in TA muscle fascicle and muscle-tendon lengths were different at rest and during contraction. Each subject was positioned with the knee flexed between 60 and 90° with the foot strapped to an isokinetic dynamometer. Surface electromyography electrodes were placed over the TA to measure muscle activity throughout. Three different fascicle length measurements were randomly assigned that included the subject completely relaxed, or during contractions at 5 or 100% of the maximal isometric torque (at a neutral ankle joint). For all analyses, the relative compliance of the muscle fascicles and tendon were determined from the estimation of MTU length and changes in joint position typically seen from cadavers (Grieve et al., 1978; Spoor et al., 1990; Visser et al., 1990). Linear regression was used to determine the slope of the relationship between muscle fascicle length and the change in MTU length. Results from the first two experiments indicated that the relationship between muscle fascicle length and the change in MTU length for the MG is approximately linear. The mean slope of the linear regression line for experiment one and two were 0.27 ± 0.09 and 0.55 ± 0.13, respectively. These findings demonstrate that 27% of the total change in MTU length occurred in the MG muscle fascicles; thus, the remaining 73% was presumed to occur in primarily in the tendon. Experiment two revealed that 55% of the total change in MTU length was attributed to the TA muscle fascicles and 45% of the change occurring from the tendon. Results from experiment three and four indicated that there was no effect of a prior contraction on the contribution of muscle and tendon length changes (slopes of 0.21 and 0.61, respectively) ($P>0.05$). In experiment five, the results indicated that as the TA contracts, a greater contribution comes from the muscle fascicles ($P=0.06$). The mean slope at rest, 5% MVC, and 100% MVC were 0.44 ± 0.19, 0.66 ± 0.26, and 0.60 ± 0.22, respectively. In conclusion, these five experiments suggest that when moved through a range of motion or a contraction, the tendon is more susceptible to changes in length in comparison to the muscle fascicles.

Morse, Degens, Seynnes, Maganaris, Jones (2008)

The purpose of this investigation was to examine the changes in the muscle-tendon unit (MTU) during passive lengthening of the medial gastrocnemius (MG) and to determine whether the muscle or tendon contributes to the increased range of motion (ROM) following multiple bouts of repeated stretching. Eight recreationally active males (age 20.5 ± 0.9 years) volunteered for the study. Each participant was positioned prone with the knee fully extended and the foot strapped to an isokinetic dynamometer. An electrogoniometer was placed on the ankle joint to account for heel displacement that potentially occurs during dorsiflexion. Two unipolar surface electromyography electrodes were placed on the MG to record muscle activity during each stretch. Those values were normalized to a plantarflexor maximal voluntary contraction to ensure each stretch was passive. Passive maximum range of motion (ROM) of the ankle joint was determined with the ankle dorsiflexed at 1°/sec from -10° of dorsiflexion to the point of discomfort. The corresponding angle at the end ROM was used to complete three rounds of the stretching ROM assessments. The stretching ROM assessments (same velocity and angle as maximum ROM assessment) were completed before and after the stretching intervention. The stretch intervention consisted of five stretches in which the ankle was passively dorsiflexed at 5°/sec to the end ROM and held for one minute. On a separate visit, a series of 10 rapid stretches (60°/sec) that moved the foot from 0 to 10° of dorsiflexion were carried in place of the stretching intervention. Thus, the stretching ROM assessments were completed prior to and immediately after the rapid stretches to see if there was a change in MTU stiffness. The rapid stretches simulated a ballistic-type maneuver and were tested to see if MTU stiffness could be attributed to a resetting of the thixtropic properties of the muscle. B-mode ultrasonography (US) with a linear array probe was used to identify the myotendinous junction (MTJ) of the MG during the stretches. Displacement of the MTJ was measured relative to an acoustic marker located between the skin and the probe. The total length of the MTU was determined by a tape measure laid over the skin and an US to specify the insertion of the proximal and distal attachment of the MTU. The change in MTU length at each joint angle was estimated using the cadaveric regression model (12). The amount of shortening of the tendon distal to the MTJ, and extension of the muscle proximal to the MTJ, were determined from the distal displacement of the MTJ. The movement of the skin markers was analyzed throughout the stretch at 0, 5, 10, 15, 20, and 25° of dorsiflexion. During the third ROM assessment, the elongation of muscle, tendon, and fascicles were recorded pre- and post-stretching. Prior to the stretching intervention, MTU increased in length by 2.19 ± 0.14 cm throughout the dorsiflexion ROM. The muscle accounted for 47% of the MTU elongation, while the tendon accounted for 53%. Following the stretching protocol, ROM increased from 28.1 ± 2.3 to 32.7 ± 2.4° ($P<0.05$) along with an increase in MTU from 2.19 ± 0.14 to 2.52 ± 0.14 cm ($P<0.05$) at the end ROM. Also, there was an increase in MTJ elongation from 1.04 ± 0.08 to 1.38 ± 0.07 ($P<0.05$) with no significant changes in tendon elongation or passive torque between the two points at the end ROM. Results from the third stretching ROM assessment indicated significant increases ($P<0.05$) in MTJ elongation (from 0.92 ± 0.06 to 1.16 ± 0.05 cm) and decreases ($P<0.05$) in tendon elongation (from 1.06 ± 0.04 to 0.82 ± 0.05 cm) with no change in MTU elongation and fascicle elongation from 0-25° of dorsiflexion ($P\geq0.05$). Muscle stiffness decreased from 38.8 ± 8.4 to 17.2 ± 3.5 $Nm•cm^{-1}$ ($P<0.05$) with no change in tendon stiffness pre- and post-stretching. However, results from the rapid stretching trial did not show a significant decrease in MTU stiffness ($P>0.05$) suggesting that the thixotropic properties of the muscle are unlikely the mechanism for reducing MTU stiffness. Overall, the results indicated that prior to stretching, the MTU elongation is a result of both muscle and tendon lengthening equally; however, after the stretching intervention, MTU and ROM increase with subsequent decreases in MTU stiffness. Further, muscle lengthening after the stretching intervention is not explained by a change in fascicle length; therefore, the increased compliance (decreased muscle stiffness) is likely due to alterations in connective tissue properties and primarily the perimysium.

Abellaneda, Guissard, Duchateau (2008)

The purpose of this study was to investigate whether changes in medial gastrocnemius (MG) architecture differ between individuals who produce different passive torques during muscle stretching of the plantarflexors and whether the relative contribution of muscle and tendon structures to the increase in total muscle-tendon unit (MTU) lengthening varies among subjects. A total of 16 subjects (12 men and 4 women) with a mean age of 24.1 ± 2.7 years participated in this study. Each subject rested prone with the right foot strapped to a footplate and a potentiometer placed on the ankle joint to account for angle changes. The ankle was rotated from -10° dorsiflexion to 30° dorsiflexion in 10° steps at an angular velocity of 2.5°/s and held at a constant-angle for 20 seconds (s). Torque was measured during the stretch by a strain-gauge transducer. Surface electromyography (EMG) of MG and soleus were used to monitor muscle activity during passive ankle dorsiflexion. An isometric maximal voluntary contraction (MVC) of the plantarflexors was performed following the stretches with the ankle in a neutral position (0° dorsiflexion) to obtain a maximum EMG value defined as a 2-s epoch at torque plateau. Passive torque and EMG activity were measured during the last 5 s at each 20-s hold with the slope of the passive torque-angle curve from +20° to +30° to estimate passive stiffness. B-mode ultrasonography was used to analyze the MG architecture during the stretch. A linear-array probe was fixed over the midbelly of the MG to measure fascicle length and pennation angle. The longitudinal displacement of the MG and soleus insertions on the deep aponeurosis during dorsiflexion represented the lengthening of aponeurosis and free tendon distal to that point. MTU length was measured using a tape measure from the medial femoral condyle to the superior edge of the calcaneum. The change in MTU length was estimated using the regression equation by Grieve et al. (12). To estimate the extent of tendon lengthening, the fascicle length was subtracted, at each ankle angle, from the respective change of MTU length. The muscle fascicle was defined as the fiber bundle between the two aponeuroses. Pennation angle was determined as the angle at which the fascicle inserted into the deep aponeurosis. Fascicle length was estimated using trigonometry when the fascicle extended off the static image and calculated as: fascicle length = lf 1 (measured fascicle length) + lf 2 (estimated fascicle length) = lf 1 + (h/sin μ), where h is height. Results from the study indicated that passive torque increased exponentially with relatively little muscle activity (<3%). Fascicle length increased from 57.6 ± 8.9 mm at -10° to 80.5 ± 10.1 mm at +30° (P<0.001) with pennation angle decreases from 21.2 ± 4.3° at -10° to 14.5° ± 3.0° at 30° (P<0.001). The MTU lengthened from 471 ± 35.4 to 496 ± 371 mm when the foot was dorsiflexed from 0° to 30°. The average lengthening of the MG fascicles and aponeurosis was 17.5 ± 6.4 mm, which accounted for 71.8 ± 27.3% of the total increase in MTU length meaning that the tendon elongation contributed to 6.9 ± 6.5 mm or 28.2 ± 27.3% of increase in MTU length. Upon a clear indication of a bimodal distribution for passive torque, two groups were formed with the passively stiff group designated as those over

30 Nm at 30° of dorsiflexion. The passively stiff group (39.6 ± 13.4 Nm) in comparison to less stiff group (23.7 ± 5.7 Nm) reported a significant decrease in fascicle length and greater EMG activity in MG ($P<0.05$) with the tendon length trending towards greater values. In conclusion, this study demonstrates an increase in passive torque during passive stretching of the plantarflexors is accompanied by more of an increase in muscle than tendon; however, the relative compliance of each tissue influences the MTU lengthening differently.

Blazevich, Cannavan, Waugh, Fath, Miller, Day (2012)

The purpose of this study was to describe the relative contributions of fascicle lengthening and tendon lengthening relative to muscle-tendon unit (MTU) lengthening during ankle rotations, and to determine if muscle and tendon lengthening during a stretch was related to maximum range of motion (ROM). Twenty-one healthy men (age 19.6 ± 0.8 years) volunteered for this study and visited the laboratory two times at the same time of day. The first visit was a familiarization session, which allowed each participant to practice the maximum ROM assessment. During the experimental trial, each subject was seated with the knee fully extended in an isokinetic dynamometer with the foot and heel securely strapped to the footplate. B-mode ultrasonography (US) with a linear array probe was used to analyze the movement of the myotendinous junction (MTJ) during the maximum ROM assessments. Surface electrodes were placed over the medial gastrocnemius (MG), soleus, and tibialis anterior according to the SENIAM guidelines to monitor the muscle activity of each stretch. Two maximum ROM assessments were completed with the dynamometer rotating the ankle joint from 30° plantar flexion to 30° dorsiflexion at 2°/s and stopped when the participant pushed a button as they reached their maximal stretch limit. After a five-minute break, a second series of stretches involved fixing a US probe positioned midbelly over the MG to identify the muscle fascicles. The dynamometer rotated the ankle joint from 30° plantar flexion to 30° dorsiflexion at 2°/s, but was held constant for 5 seconds at each 10° interval where a static image from the US probe was collected. Upon completion of the stretches, two isometric maximal voluntary contractions of the plantarflexors and dorsiflexors were completed to normalize EMG amplitude and ensure each stretch was passive. MTU length was measured at rest using a tape measure from the medial femoral condyle to the superior insertion point of the Achilles tendon on the calcaneus. MTU length changes were estimated using the cadaveric equation by Grieve et al. (12). Muscle length was defined as the part that lies proximal to the MTU, and Achilles tendon length was derived from the difference between MTU and muscle length (distal portion of the MTU from MTJ). MG fascicle length was calculated as the distance of a fascicle extending from the superficial to deep aponeurosis. MG fascicle rotation was determined as the change in pennation angle (angle at which the fascicle inserts into the deep aponeurosis) during the assessments. The results of this study demonstrated a bimodal distribution in maximum ROM. Thus, flexible (53.4 ± 3.9°) and inflexible (35.8± 6.3°) (P<0.001) groups were created based on maximum ROM. MG and soleus peak EMG amplitudes were 8.5 ± 5.8% and 5.5 ± 5.2%, respectively with minimal activation from the TA (<0.01 mV). To determine EMG onset, the data was first manipulated to remove the two subjects who did not have any EMG activity. The second method involved assigning a nominal value to each subject based on the subject's maximal ROM. The average joint angles at which EMG onset occurred was 31.1 ± 9.8° and 29.9 ± 9.2° (first and second method, respectively) (P≥0.05). A significant relationship was observed between the angle of EMG onset and maximum ROM (P<0.05), which indicated that a later onset of EMG was associated with the achievement of a greater dorsiflexion angle. Resting length measurements of the muscle, tendon, and MTU did not differ between groups ($P \geq 0.05$). Peak resistive torque was greater for the flexible group (115.2 ± 29.3 Nm) in comparison to the inflexible group (77.5 ± 19.3 Nm) ($P<0.001$). Throughout the ROM, the MTU elongation was lengthened more by the muscle (30 mm) with a lesser contribution form the tendon (22.mm). The contribution of muscle fascicle length (20%) was greater than that of rotation (8%) suggesting that within the muscle, the fascicles are primarily responsible for the length changes. However, toward end ROM (20° dorsiflexion to end ROM), much of the elongation of the MTU resulted from a tendon stretch rather than the muscle stretch. There were no differences in muscle and tendon rate of change between the flexible and inflexible subjects during the stretch. However, flexible subjects did exhibit a significantly greater tendon ($P<0.001$) and muscle elongation ($P<0.05$) at stretch termination than the inflexible subjects. Fascicle length and rotation were measured every 10° from -20 to 30° of dorsiflexion. There was no change in fascicle length through the ROM, but the estimated percent lengthening at the maximum ROM was greater in flexible (69.5 ± 32.2%) than inflexible subjects (46.7 ± 20.3%) ($P<0.05$). There was a difference between groups in fascicle rotation (represented as rate of angle change) from 10 to 30° of dorsiflexion for the flexible and inflexible groups at 9.7% and 5.9%, respectively ($P<0.05$). Similar differences were also noted for fascicle rotation at maximum ROM ($P<0.05$). These findings suggest that the muscle is more compliant than the tendon during a passive stretch. The change in MTU length is due more to changes in the muscle with the contribution of fascicle rotation being negligible in comparison to fascicle rotation. Toward the end ROM, most of the MTU elongation was a result of tendon lengthening. Furthermore, fascicle rotation during the stretch was greater than fascicle lengthening for the flexible group suggesting that the intramuscular connective tissue is possible the main contributor to the elongation of the muscle in flexible subjects.

Influence of Age on The Muscle-Tendon Unit

Lexell, Taylor, Sjöström (1988)

The purpose of this study was to examine the influence of aging on muscle area, total number, size, proportion, and distribution of fiber types. Forty-three males' (15 – 83 years) vastus lateralis cross sections (15 μm) were examined and separated into five groups by age (20, 30, 50, 70, and 80 years). Staining of the sections for myofibrillar adenosine triphosphate were performed to help visualize type I and type II fibers and then mounted on a medium. The total number of fibers (using every 48^{th} square mm cross section) was determined by multiplying the mean number of fibers per square mm by the number of squares counted and by a factor of 48. The mean cross sectional area was determined using 31 of the 43 sections with careful consideration to include both types of fibers in superficial, deep, and central parts of the muscle cross section. Results indicate a quadratic relationship between both muscle area and age and total number of fibers and age ($P<0.001$) with the fitted curve peaking at 23.7 and 24.2 years, respectively. Furthermore, there is an increasing rate of reduction in muscle area and total fiber number with the average reduction of about 40% from 20 to 80 years. However, around age 50, the slope becomes steeper indicating there is an increased rate of reduction past that age. Additional results indicated that there was no relationship between type I fiber size and age, but there was a significant reduction in type II fibers with increasing age ($P<0.01$). Mean fiber size from both type I and II fibers indicated a significant reduction with increasing age ($P<0.05$) and an average reduction of 26% from ages 20- 80 years. Lastly, the author was able to determine the amount of muscle area comprised of muscle fibers. By regressing the product of the fiber sizes and total number of fibers on muscle area and allowing for a changing relationship with age, the author concluded that the younger individuals muscle area comprised of approximately 70% muscle fibers with the older individuals at approximately 50%. The decrease in muscle fiber per muscle area could be due not only to a loss of fibers but also an increase in connective tissue and intramuscular fat. In conclusion, it is clear that with aging there are both significantly fewer and smaller fibers in the old muscles. A reduction in fibers can be attributed to the loss of type II fibers being denervated and reinnervated to type I fibers. However, with increasing age beyond 50 years, the neurogenic process becomes diminished and some fibers are permanently denervated and lost. The loss of fibers is replaced by fat and connective tissue and subsequently alters the proportion of fibers per muscle.

Narici, Maganaris, Reeves, Capodaglio (2003)

The purpose of this investigation was to demonstrate that sarcopenia not only involves a decrease in muscle mass but also entails changes in muscle architecture specifically within the human medial gastrocnemius (MG). A total of 30 physically active men participated in this study which included 14 young (aged 27-42 years) and 16 older (aged 70-81 years) participants. Careful consideration was done to establish that the elderly were matched with the younger group based on physical activity to rule out disuse as a possible explanation of architectural changes. MG anatomic cross sectional area (ACSA) and volume were measured by computerized tomography (CT) while the subject rested supine. Pennation angle and fascicle length were collected by real-time ultrasonography (US) with each participant resting prone. Each image was obtained with the probe fixed on the midbelly of the MG on the dominant leg. Physiological cross sectional area (PCSA) was also estimated using the ratio of muscle volume to fascicle length. The results indicated that all the MG's architectural parameters were significantly reduced in the elderly compared with the younger adults. The differences between the elderly and younger groups for ACSA were 14.0 ± 3.6 and 17.4 ± 2.8 cm^2 (19.1% difference) ($P<0.005$), for volume 208.7 ± 48.5 and 279.3 ± 59.3 cm^3 (25.3% difference) ($P<0.001$), for fascicle length 4.29 ± 0.67 and 4.78 ± 0.55 cm (10.2% difference) ($P<0.01$), for pennation angle 23.6 ± 3.0 and 27.2 ± 4.3° (13.2% difference) ($P<0.01$), and for PCSA 50.1 ± 12.6 and 59.1 ± 14.4 cm^2 (15.2% difference) ($P<0.05$). This study demonstrates that muscle architecture is significantly altered in elderly individuals. Since the groups were matched based on physical activity, the changes in architecture can be accounted for the differences in age. The decrease in muscle volume, ACSA, fascicle length, pennation angle, and PCSA strongly suggests that sarcopenia involves not only of a loss of fibers in parallel, but in series as well. The decreased muscle volume and fascicle length that accounts for the decreased PCSA could illustrate a loss of muscle function and likely reduce muscular strength. Another possible reason the reduction of muscular strength is that there could be a decrease in tendon stiffness. This would alter the force-generating potential putting less strain on the fascicles and over time cause a decrease in pennation angle.

Gajdosik, Vander Linden, Williams (1999)

The purpose of this study was to examine the influence of age on length and passive elastic stiffness (PES) characteristics of the calf muscle-tendon unit (MTU) when stretched through the full, available dorsiflexion range of motion (ROM). Twenty-four younger women (aged 29.7 ± 6.2 years), 24 middle-aged women (aged 50.2 ± 6.1 years), and 33 older women (aged 72.9 ± 7.3 years) participated in this study. Surface electromyography (EMG) electrodes were placed over the muscle bellies of the medial gastrocnemius, soleus, and tibialis anterior and each subject was encouraged to maintain a silent EMG response throughout the stretch. All the subjects assumed a supine position with the right foot strapped to an isokinetic dynamometer. To familiarize each subject with the setup, 10 repetitions of 10 seconds of static stretches of the calf MTU were initiated. The maximum dorsiflexion angle was determined by manually moving the ankle-foot apparatus into dorsiflexion and end maximum ROM was defined as a marked presence of EMG activity from the calf muscles or the angle achieved just prior to pain or discomfort. After the maximal dorsiflexion angle was found, the ankle-foot apparatus was moved from this angle through 60° into plantar flexion. Three trials were performed in which the foot was dorsiflexed at 5°/sec through a 60° ROM (stopping at pre-determined end ROM angle minus 1°). The maximal passive resistive torque was measured and passive angle-torque curves were constructed for the complete dorsiflexion ROM. The maximal passive dorsiflexion angle, maximal passive resistive torque (PRT), angular change for the full stretch ROM, and average PES for the full stretch ROM were examined for group differences and their relationships with age. Results indicated that maximal passive dorsiflexion angle, passive angular change, and maximal PRT decreased with increasing age groups ($P<0.05$). Post Hoc analyses indicated that older women had a smaller maximal passive dorsiflexion angle (15.39 ± 5.78°) in comparison to the middle aged (22.75 ± 4.38°) and younger women (25.83 ± 5.5°) ($P<0.001$). Maximal PRT for the older women (12.61 ± 5.69 Nm) was also less then the maximal PRT for both middle aged and younger women (17.95 ± 5.43 and 21.68 ± 5.33 Nm, respectively). The average PES for the last half of the complete stretch ROM decreased with increasing age. The average PES was less for older women than for younger women (P = 0.019), but no differences were found with either group in comparison with the middle-aged women. With aging, the decrease in the PES could be a result from loss of motor units and decrease in number and size of both type I and type II muscle fibers. The reduced PES with aging could have also been attributed to the increased amount adipose tissue and other connective tissue within the muscle. In conclusion, older women demonstrated that maximal ROM, maximal PRT, and average PES were all decreased suggesting the MTU was less extensible.

Dierick, Detreffmbleur, Trintignac, Masquelier (2011)

The purpose of this study was to assess the passive elastic and viscous stiffness components of ankle joint in young, middle-aged, and old adult female fibromyalgia subjects and compare the results with age-matched healthy control subjects. A total of 52 healthy, sedentary (<2 hours per week of physical activity) female subjects (age 20-72 years) were enrolled in the study. Each subject was placed supine with both knees extended and one foot in the footplate of an electromechanical device (allowed for flexion-extension at ankle joint). The footplate was able to oscillate at varying frequencies (4-12 Hz) and produced displacement of the ankle (±2.5°) from the neutral position (90° between foot and ankle). A potentiometer and four strain gauges were mounted to the rotational axis to measure angular displacement and torque, respectively. Surface electromyography (EMG) electrodes were placed mid-belly of the medial gastrocnemius and tibialis anterior and subjects were encouraged to keep electrical activity at baseline (relaxed muscle). Three separate treatments were given to each ankle in which a series of 27 oscillation trials, consisting of nine different frequencies (4-12 Hz) were recorded. The amplitude and phase-shift of the torque signal relative to the displacement signal were computed. The amplitude of the torque responses that is in phase with the ankle displacement is called the elastic torque and the one that is 90° out of phase with the displacement is called the viscous torque.

Each amplitude of the elastic and viscous torque were expressed in terms of stiffness Nm/rad (raw torque/ankle displacement). Viscoelastic stiffness was determined over the range of different oscillation frequencies and each mean value of elastic stiffness and viscous stiffness were computed. Results from this study indicated that elastic and viscous stiffness were significantly different for the three age groups ($P<0.001$). Older women had a significantly higher value for elastic stiffness (60.48 ± 8.81 Nm/rad) in comparison to the younger women (17.68 ± 8.81 Nm/rad) and middle-aged women (34.66 ± 4.53 Nm/rad) ($P<0.001$). Viscous stiffness was significantly higher in younger women (10.65 ± 0.97 Nm/rad s) in comparison to middle-aged (6.41 ± 0.50 Nm/rad s) and older women (5.28 ± 0.97 Nm/rad s) ($P<0.001$). To conclude, the results indicated that passive stiffness increases with age meaning there is a greater absolute torque at a given joint angle. The increased elastic resistance could be due to changes in intramuscular content (increased collagen) that inhibits range of motion. However, the decreased viscous resistance with aging could be due to the content of the myoplasm and the desmin intermediate filament.

Sobolewski, Ryan, Thompson (2013)

The purpose of this study was to determine the influence of age on the acute viscoelastic responses to a practical stretching intervention. Twenty-two young (24 ± 3 years) and 14 old (67 ± 3 years) males participated in this study and visited the laboratory on two separate occasions. Each participant performed a standard dorsiflexion ROM assessment to assess passive stiffness (50) and an isometric MVC of the plantarflexors to ensure that each stretch is passive. The experimental setup was identical to the previous study from Sobolewski et al. (53). A total of four 30-s constant angle stretches with a 30-s rest period was completed. Viscoelastic stress relaxation and creep were assessed as the absolute (across all four stretches) and relative (from stretch to stretch) changes in the decrease in force and increase in position, respectively. The rate of viscoelastic creep was examined indirectly through the rate of change in position at the start of each of the four stretches (53). The results indicated that the older men (0.155 ± 0.068 $Nm \cdot deg^{-1} \cdot cm^{-1}$) exhibited a greater passive stiffness (*P*=0.044) at a common joint angle in comparison to the younger men (0.114 ± 0.048 $Nm \cdot deg^{-1} \cdot cm^{-1}$). Additionally, there were no change in the absolute viscoelastic stress relaxation responses between groups ($P \geq 0.05$); however, there were differences in the relative change in stress relaxation between the young and old for stretch one (*P*=0.01) only. The younger men demonstrated a greater relative stress relaxation response for the first stretch when compared to the third (*P*=0.018) and fourth (*P*=0.002) stretch. The older adults, however exhibited a similar stress relaxation response across all four stretches (*P*=0.917). Furthermore, there were no differences in the absolute or relative changes ($P \geq 0.05$) in the viscoelastic creep response between young and old men. These findings demonstrate that although there was an increase in passive stiffness among the older group, the viscoelastic creep response was unaffected. However, the viscoelastic stress relaxation response across repeated stretches is altered in older adults.

Mian, Thom, Ardigò, Minetti, and Narici (2007)

The purpose of this study was to determine if there are age-related differences in gastrocnemius fascicle-tendon interactions during a fundamental locomotor walking task. Eight young (27.3 ± 3.8 years) and 8 elderly (76.6 ± 4.3 years) men and women participated in this study and were match-paired based on leg, shank, and gastrocnemius muscle-tendon unit. Measurements of muscle-tendon architecture, joint kinematics, and electromyography (surface electrodes placed on lateral gastrocnemius and tibialis anterior) were made during treadmill walking at 1.11 m/s. Data was captured for 20 seconds and from that, the first two consecutive strides were analyzed per participant. B-mode ultrasonography (US) was used to assess the parameters of muscle architecture (fascicle length and pennation angle) with the US probe fixed to the midbelly of the lateral gastrocnemius. When the length of a fascicle extended off the image, the length was estimated by linear continuation. Pennation angle was also measured as the angle at which a fascicle inserts into the deep aponeurosis. This angle was used to calculate muscle length as the product of fascicle length and the cosine of pennation angle. Length changes of the lateral gastrocnemius muscle-tendon unit were determined using the equation from Grieve et al. (12) and the change in tendinous tissue was accounted for by subtracting the fascicle length from the muscle-tendon unit length. The heel contact phase served as the reference length and did not differ between groups. However, there were significant changes during the gait cycle (from heel contact to peak) in muscle fascicle and tendon length. Although the muscle-tendon unit length did not differ during the cycle, the contribution of fascicle (young: 3 ± 2; old -1 ± 5 mm) and tendon (young: 5 ± 3; old: 9 ± 4 mm) were significantly different between groups ($P<0.05$). Therefore, these results suggest that the increase in MTU during the stance phase of gait cycle is similar in each group. However, the increased contribution from the tendon in the elderly subjects could be due to an increase in tendon compliance.

Methodology for Quantifying Muscle-Tendon Unit Length

Grieve (1978)

The purpose of this study was to provide a technique whereby the length of the human gastrocnemius muscle can be estimated from angular measurements of the lower limb. A total of eight non-pathological cadaveric limbs were used from subjects deceased over the age of 60. As the knee joint was stabilized, the ankle joint was manipulated in 10° increments with length measurements recorded to the nearest millimeter. Length changes were expressed as a percentage change from the reference angle (90° between leg and foot). A scatter of the raw data was used to derive the best-fit polynomials. In addition, lines of best fit were used to relate the change in muscle length from the reference joint angle. The equation conceived from this study to estimate MTU length with the reference knee (0°) and ankle (90°) joint angles was: $\Delta L = -22.815 + 0.30141(90 + \theta A) + 0.00061(90 + \theta A)2$ where ΔL is the change in MTU length due to the change in dorsiflexion angle (A). The predictive equations that this study identifies for muscle length using joint angle changes helps to provide more insight into the mechanics of muscle action.

Barber, Barrett, and Lichtwark (2011)

The purpose of this study was to determine the validity and reliability of muscle length measures obtained using a novel ultrasound (US) and tape measure (US-tape) method in a typically developed (TD) and individuals with cerebral palsy (CP). Muscle length measures of the medial gastrocnemius (MG) using the US-tape approach were compared to measures obtained using a 3-dimensional US (3DUS) approach. A total of 25 individuals (15 TD and 9

CP) participated in the study. Both the US-tape and 3DUS were performed with the participant lying prone and dorsiflexed at an ankle angle of 30, 60, and 100% of total range of motion (ROM). B-mode US was used with a tape measure fixed to a linear transducer to specify landmarks of interest. The landmarks included the most superficial point of the medial femoral condyle (MFC), the most distal point of the myotendinous junction (MTJ), and the calcaneus. The tape distance from the MFC to the calcaneus illustrated the muscle-tendon unit (MTU) distance and the tape distance from the MTJ to the calcaneus illustrated the Achilles tendon (AT) distance. Post-processing of each image takes into account the US image depth and distance which can be used in conjunction with the tape measure distance to calculate both MTU and AT length. Pythagoras' theorem calculates length as: MTU and AT length = $\sqrt{}$ (tape distance + US image distance)^2 + (US depth)^2. Muscle length was then calculated as the difference between the MTU and the AT. Freehand 3DUS was used to generate a 3D image of the MG using the same specified landmarks as the US-tape. The measurement of muscle length was obtained from the 3D model using the Stradwin software measurement tools. There was a good agreement between the two methods with the US-tape method overestimating MG muscle length by 0.2 mm (<0.1%) in the TD group and by 0.3 mm (0.1%) in the CP group across all three ankle joint angles. The limits of agreement between the two methods over the three joint angles were 15 mm (6%) for the TD group and 13 mm (6%) for the CP group. The intraclass correlation coefficient (3,1) values over the three joint angels were all >0.999 indicating that the methods are reliable. In conclusion, these results suggest that the US-tape method is a valid and reliable measure that could be used for future study to assess specific muscle and tendon changes.

CHAPTER III

METHODOLOGY

Participants

Twenty-four recreationally active young males (18-30 years) and 22 recreationally active elderly males (65-74 years) were recruited for this study. Young participants were solicited through classes at the University of North Carolina at Chapel Hill (UNC-CH). Older participants were recruited via informational emails from UNC-CH, and flyer advertisement throughout UNC-CH and the surrounding area.

Upon arrival, all participants were required to complete a health history and exercise status questionnaire and an informed consent document specifying the experimental protocol along with the potential risks and benefits associated with participation in the study. The written informed consent document complies with the University's institutional review board. Individuals were eligible to participate in the study based on the following criteria: 1) no prior knowledge of a neuromuscular disease or condition that would affect their ability to complete testing, 2) no history of joint or muscle problems in the low back, hips, legs, knees, ankle, and foot within the past three months, 3) no history of multiple ankle sprains, 4) exercising no more than one to five hours per week, and 5) any participant that does not exhibit excessive dorsiflexion (>55°). Each participant will be specifically instructed to avoid vigorous exercise and stretching 24 hours prior to testing.

Experimental Design

The participants reported to the UNC-CH Neuromuscular Research Laboratory on two occasions separated by 2-7 days at the same time of day (± 2 hours). There was an initial familiarization session followed by an experimental session. During the familiarization session, each participant had their body mass and stature assessed, practiced the isometric maximal voluntary contractions (MVC), determined their maximal tolerable passive torque threshold, and ensured they can fully relax during the CT stretching protocol. Mass and stature was assessed using a scale and stadiometer (Detecto Scale Company, Webb City, MO, USA). Each participant performed several MVCs of the plantarflexors to ensure that they were comfortable giving a maximal effort. Each participant underwent a series of passive stretches to obtain their maximum tolerable torque threshold. The dynamometer was set to a low passive torque (i.e. 5 Nm) with the torque continually increasing to the point of discomfort, but not pain, as verbally acknowledged by the participant. The resultant maximal tolerable torque threshold was used during the experimental session for the stretching protocol(49).

During the experimental session, each participant performed two MVCs of the plantarflexors (two min of rest between contractions) and a 60-s CT stretch. A five minute break was given between the MVCs and the stretch to prevent any alterations that may occur to the muscle-tendon unit (MTU) from the MVC. During the 60-s CT stretch, MTU length was derived and the contribution from the medial gastrocnemius (MG) and Achilles tendon (AT) will be measured.

Viscoleastic Creep Measurements

The CT stretch protocol was conducted on a calibrated, HUMAC Norm dynamometer (Computer Sports Medicine Inc., Stoughton, MA, USA). Each participant was seated at 135° between the thigh and torso and verified by plastic, handheld Model G300 goniometer (Whitehall Manufacturing, City of Industry, CA, USA). The participant's right leg was fully extended (0° below the horizontal plane) with a Velcro restraining strap over the thigh (90 mm width) for stabilization. The right foot was placed firmly against a custom steel footplate (36 x 17 x 2.5 cm) with the lateral malleolus of the fibula aligning to the axis of rotation of the dynamometer. Two restraining foot straps (25 mm width) over the tarsals and metatarsals were used to ensure the foot did not move during the stretching protocol. During pilot testing, the participant's heel was seen to move away from the footplate during dorsiflexion; thus, a 150 mm twin axis goniometer (BIOPAC Systems, INC., Santa Barbara, CA, USA) was fixed to the skin at two mounting points (distal fibula and the lateral aspect of the midfoot). Angular displacement was derived through the change in strain of the composite wire between the mounting points. Prior to the stretch, the participants were asked to remain as relaxed and motionless as possible. The dynamometer footplate passively dorsiflexed the foot at an angular velocity of 5°/s from 20° of plantarflexion until the maximal tolerable torque threshold was met. The dynamometer held this constant passive torque for 60-s and then released back to -20° of dorsiflexion.

Muscle-Tendon Length Assessments

A Brightness-mode (B-mode) portable ultrasound (US) imaging device (Logiq™ e, General Electric Healthcare, Milwaukee, WI, USA) with a multi-frequency linear-array probe (12L-RS; 5-13 MHz; 38.4 mm FOV) was used to identify structural landmarks within the MTU.

The depth and frequency of the US in the musculoskeletal setting was optimized prior to testing to ensure that the designated landmarks fit in the field of view (20-50 mm; 10.0 MHz). A hypoallergenic, water-soluble transmission gel (Aquasonic 100, Parker Laboratories, INC., Fairfield, NJ, USA) was applied to the skin to enhance the acoustic coupling and reduce the possible near field artifacts using minimal probe pressure.

MTU length at rest was assessed using the US tape measure approach adopted from Barber et al. (4) at neutral ankle joint angle (90° between foot and leg). The MTU consists of the MG and AT. An ink mark was made at the AT insertion on the calcaneus (visualized via US) specifying the distal attachment of the MTU. The proximal attachment of the MG is difficult to visualize via the US; therefore, the most superficial part of the medial femoral condyle served as the proximal attachment of the MG. A Gulick anthropometric tape measure (AliMed, Dedham, MA, USA) was fixed to the US probe. The MTU tape length was recorded as the tape distance from the distal edge of the US probe when the probe was over the femoral condyle to the marked AT insertion (tape being pulled taut) (Fig 1). Post-processing using the straight-line function in Image-J (National Institutes of Health, Bethesda, MD, USA, software version 1.49) accounted for the US depth and distance at the femoral condyle (Fig 2). Pythagoras's theorem was used to calculate the total MTU length based on the tape distance plus the US image distance and the US depth of the femoral condyle. Previous data from our lab has shown this method to be reliable with an intraclass correlation coefficient ($ICC_{2,1}$) value of 0.97 and a standard error of the mean at 1.09%.

Prior to the stretching bout and following the MVCs, participants were passively dorsiflexed to their maximal tolerable torque threshold, at which point the isokinetic dynamometer was briefly paused to limit the amount of stretching on the MTU. During that time

(<10-s), identification and ink marks were made at the myotendinous junction (MTJ) and femoral condyle. The MTJ was defined as the intersection of the AT and MG (30). When both landmarks were identified, the participant was returned to their resting position and a hypoechoic marker was placed on the skin just proximal to the MTJ. An anthropometric tape measure was fixed to the skin at the femoral condyle to allow for a baseline assessment of MG length. There was a five-minute break prior to the stretching bout to account for any changes to the MTU.

During the stretch, the participant's foot was dorsiflexed to their torque threshold, at which time the dynamometer was paused to allow for baseline measurements. While paused, a static US image was recorded at the pre-determined MTJ. Simultaneously, MG length was quantified with the tape measure being pulled taut from the femoral condyle to the previously marked MTJ. Upon completion of the two measurements, the dynamometer resumed back to the torque threshold initiating the 60-s stretch. The previous sequence of events occurred in quick succession to reduce the amount of viscoelastic stress relaxation that occurs prior to the stretch (<10-s). After the 60-s, the dynamometer was paused again and another static US image was recorded at the MTJ. The change in length of the MG was determined based on the displacement of the MTJ relative to the marker (proximal [-] or distal [+]). MTU length change during the stretch was estimated using the cadaveric regression equation provided by Grieve et al. (12). The percentage change in MTU length (ΔL) was calculated as follows:

$\Delta L = -22.815 + 0.30141(90 + \theta A) + 0.00061(90 + \theta A)2$ where ΔL is the change in MTU length due to the change in dorsiflexion angle (A). The relative contributions of the MG and AT were determined using the change in MTU length and displacement of the MTJ.

Maximal Voluntary Isometric Contraction

The participants completed two isometric plantarflexion MVCs in the seated position with their leg fully extended and a neutral ankle joint angle (90° between the leg and foot). Participants were instructed to cross their arms at the chest and to plantarflex as hard and as fast as possible for 3-4 seconds. The setup was the same as the creep assessment, with the addition of a thick, rubber heel cup to stabilize the foot. Two MVCs were completed and the highest torque output of the two trials were used to normalize the EMG amplitude values recorded during the passive stretching.

Electromyography Measures

Each participant had pre-amplified, bipolar surface electrodes (EL254S Biopac Systems, Santa Barbara, CA, USA; gain = 350; interelectrode distance = 20 mm) placed over the medial gastrocnemius (MG), soleus (SOL), and tibialis anterior (TA) to ensure that each stretch was passive. The MG and SOL were visually identified and palpated with the participant seated and actively plantarflexed. The TA was determined in a similar manner except the participant was asked to actively dorsiflex. Prior to electrode placement, the skin at the location of the MG, SOL, and TA muscles was shaved, lightly abraded, and cleansed with rubbing alcohol to remove oil and layers of dead skin. Electrode placement for the MG was on the most prominent bulge of the muscle. The SOL electrode was placed at two-thirds of the line between the medial femoral condyle to the medial malleolus. The TA electrode was placed at one-third of the line between the proximal tip of the fibula to the medial malleolus. Each electrode was placed parallel to muscle fiber orientation in accordance with the recommendations of the SENIAM project (18); however, special attention was made to the placement of the SOL electrode to ensure that the electrode does not cover the MG MTJ. A single, pre-gelled, disposable electrode (Ag Cl Quinton

Prep; Quinton Instruments Co, Bothell, WA, USA) was placed over the tibial tuberosity to serve as a reference electrode.

Signal Processing

The position (°), torque (Nm), and EMG (μV) signals were sampled simultaneously at 2.5 kHz with a Biopac MP150WSW data acquisition and AcqKnowledge software (Biopac Systems Inc., Goleta, CA, USA) during the 60-s CT stretch. All signals were stored on a personal computer (Lenovo IBM Thinkpad T420, Morrisville, NC, USA) and processed offline with custom written software (Labview 8.5, National Instruments, Austin, TX, USA). The torque and position signals were smoothed with a zero-phase shift 100 point moving averager and the raw EMG signals were filtered with a bandpass zero-phase fourth-order Butterworth filter (10-500 Hz). Peak isometric MVC torque was determined as the highest 500-ms epoch during the 3-4-s MVC plateau. The same corresponding 500-ms epoch was used to calculate peak EMG amplitude for each muscle and used to normalize the MG and SOL amplitude values during the 60-s stretch based on the procedures of Gajdosik et al. (8).

Statistical Analysis

All descriptive statistics are presented as mean ± standard deviation. Independent samples t-test were used to assess the demographic data including body mass and stature. Normality of the data was confirmed using the Shapiro-Wilk test and homogeneity of variances was verified using Levene's test. Five separate mixed factorial analyses of variance (ANOVAs) (group x time) were used to examine absolute changes in position (viscoelastic creep), absolute length changes (MTU, MG, and AT), and raw EMG amplitude values from the TA (0, 30, and 60-s). A mixed factorial ANOVA (group x muscle x time) was used for the normalized EMG amplitudes for MG and SOL at 0, 30 and 60-s. When a significant interaction was found, follow-up analyses included independent and paired samples t-tests. In addition, four separate analysis of covariance (ANCOVAs) were used to examine the change in ankle joint position, MTU, MG, and AT lengths with the pre-stretch values used as a covariate (59). An effect size (ES) statistic was also incorporated along with the p-value for the change in ankle joint position, and MTU, MG, and AT lengths. ES was calculated as the mean of the young subtracted by mean of the old all over the combined (pooled) standard deviation. A relative contribution of the MG and AT was also calculated as percent change from the entire MTU will be calculated as: $MG = (MGpost - MGpre)/(MTUpost - MTUpre) \times 100$. The alpha level was set at P<0.05, and all analyses were performed using SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA).

CHAPTER IV

RESULTS

Participant enrollment and exclusion data are presented in Figure 3. All raw values are presented in Table 1 and 2. There were no differences between the young and older men for height (P=0.517), but the older men did have a greater body mass (P=0.003).

For ankle joint position during the 60-s CT stretch, there was no interaction (P=0.051) or main effect for group (P=0.380). However, there was a significant main effect for time (P<0.001) indicating that the ankle joint position increased from pre- to post-stretch for both groups. Further, there was no difference in the amount of change in ankle joint position between the young (2.01 ± 0.98°) and older men (1.41 ± 0.72°) (P=0.072;ES=0.66).

There was no interaction for MTU length (P=0.062) or main effect for group (P=0.715). However, there was a significant main effect for time (P<0.001) indicating that MTU length increased from pre- to post-stretch for both groups. There was a significant interaction (P=0.023) between the covariate (pre-stretch MTU length) and the dependent variable (MTU length change) violating the homogeneity of slopes assumption. The Potthoff modified Johnson-Neyman technique (15) was then used and indicated that there was a greater change in MTU length in the young compared to the older men (P=0.043;ES=0.71).

For MG length, there was a significant interaction (P<0.001). There was a significant increase in MG length for both the young (P<0.001) and older men (P<0.001) from pre- to post-stretch. However, there was a greater amount of change in MG length in the young compared to the older men (*P*<0.001;ES=1.40).

For AT length, there was a significant interaction (*P*=0.044). There was a significant increase in AT length for both young (*P*<0.001) and older men (*P*<0.001) from pre- to post-stretch. However, there was a greater amount of change in AT length for the older men compared to the young men (*P*=0.043;ES=0.75).

For torque, there was no interaction (*P*=0.649) and no main effect for time (*P*=0.180) or group (*P*=0.168). Thus, the young and older men stretched at similar torque thresholds (25.22 ± 6.10 and 21.91 ± 7.63 N•m, respectively), which remained constant throughout the stretch.

There was no interaction for normalized MG (*P*=0.759) and SOL (*P*=0.940) EMG amplitudes. There was no main effect for time and group for normalized MG (P=0.066 and P=0.803, respectively) and SOL (P=0.867 and P=0.226, respectively) EMG amplitudes. The mean normalized MG and SOL EMG amplitude values were 1.20 and 1.68% MVC, which has been considered to be passive (11). For the raw EMG amplitude of the TA there was no interaction (*P*=0.815) and no main effect for time (*P*=0.866) or group (*P*=0.163), indicating the participants did not actively contribute to the increase in ankle joint position.

CHAPTER V

DISCUSSION

The primary findings of the present study indicated that the young and old men experienced viscoelastic creep during the 60-s CT stretch which was accompanied by increases in MTU, MG, and AT length for both groups. The young men experienced a non-significant (P=0.072; ES: 0.67) greater increase (2.01° ± 0.98) in ankle joint position from pre- to post-stretch when compared to the older men (1.41° ± 0.72). Similarly, the young men experienced a greater increase in MTU length (P=0.043). However, the muscle increased to a greater extent in the young (ΔMG length – 77%; ΔAT length – 23%) and the tendon increased more in the old (ΔMG length – 36%; ΔAT length – 64%). These findings may highlight the age-related changes in muscle-tendon unit behavior during passive stretching.

Previous studies have examined the viscoelastic creep responses indirectly *in vitro* (56) and *in vivo* (52,53) during repeated constant angle stretching bouts, and directly *in vivo* using CT stretching (48,49,65). Ryan et al. (49) was the first to determine a reliable viscoelastic creep response during a single bout of CT stretching in the presence of negligible EMG activity. Further, Sobolewski et al. (53) determined that an increase in passive stiffness in young adults produced a significantly smaller viscoelastic creep response. Many previous studies have determined that with aging, there is an increase in passive stiffness (9,10) suggesting that there may also be a decrease in the viscoelastic creep response. Dierick et al. (6) reported an increase in elastic stiffness and a decrease in viscous stiffness with age in the plantar flexors; thus, it is possible that an increase in passive stiffness and a corresponding decrease in viscous stiffness could result in a negligible change in viscoelastic creep between age groups. This was supported by Sobolewski et al. (52) who reported similar viscoelastic creep responses between young and older males over four 30-s constant angle stretches ($P \geq 0.05$). However, in the present study, the young men demonstrated a non-significant ($P=0.072$) greater viscoelastic creep response when compared to the older men. Although this increase was not statistically significant, it was a practical difference considering the moderate to large effect size (0.67) (19). The difference between these findings and those reported by Sobolewski et al. (52) may be due to the nature of the acute CT stretching protocol. For example, Sobolewski et al. (52) used repeated 30-s constant angle stretches at same initial torque threshold and assessed the amount of absolute viscoelastic creep from the first stretch to the fourth stretch, whereas the current study examined viscoelastic creep directly during a single 60-s CT stretch.

We are aware of no previous studies that have examined the behavior of the muscle-tendon unit that contributes to the viscoelastic creep response. The results from the present study indicated a simultaneous increase in MTU length and joint range of motion. The muscle and tendon length changes in young adults has been assessed in previous studies primarily with the foot being passively dorsiflexed through a range of motion (1,5,13,21,22,37) and, to a lesser extent, following an acute bout of static stretching (37). A previous study from Herbert et al. (16) found that as the MTU lengthened the relative contribution from the muscle was only 27%, whereas the tendon accounted for the remaining 73% of the change. In contrast, more recent studies (1,5,21) have shown that as the foot was dorsiflexed through a range of motion, the lengthening of the MTU occurred predominantly from the muscle. Specifically, Abellaneda et al. (1) demonstrated that the increase in MTU length was predominantly from the muscle (72%)

with less from the tendon (28%). Similarly, Blazevich et al. (5) measured the muscle and tendon length from 20° of plantrarflexion to 20° of dorsiflexion and found that the muscle accounted for more (58%) of the total increase in MTU length change compared to the tendon (42%). Additionally, Kawakami et al. (21) assessed the contribution from 30° of plantarflexion to 30° of dorsiflexion and found the muscle increased to a greater extent (60%) when compared to the tendon (40%). The contrasting results reported by Herbert et al. (16) could be attributed to the different knee and ankle positions of the testing participants. Specifically, the participants started the range of motion assessment with the knee flexed and ankle plantarflexed, which was thought to make the MTU as short as possible. Taken together, these studies do provide some insight as to the physiological changes that take place while the foot is dorsiflexed, but do not address the direct effects of stretching. Morse et al. (37) performed a pre- and post-range of motion assessment following several bouts of stretching and evaluated the muscle and tendon contribution in each assessment. The authors concluded that both the muscle and tendon equally contributed to the initial range of motion assessment; however, following the five bouts of static stretching, the contribution of muscle and tendon was altered. During the post-stretch assessment, range of motion was significantly increased ($P<0.05$) and the contribution from the tendon remained the same as the pre-stretch value. Thus, the muscle was significantly increased ($P<0.05$) and accounted for the entire increase in range of motion. The present study supports the results from Morse et al. (37) in that the young males' muscle lengthened more relative to the tendon following the 60-s CT stretch. The increase in muscle length following the stretch may be attributed to reductions in passive stiffness that are largely due to increases in muscle compliance (22,37) and little to no change in tendon compliance for the younger men (9,22). Within the MTU, the change in muscle length from passive stretching has been shown to be attributed to the muscle fascicles and/or surrounding connective tissue. Intrafascicular structures that contribute to the viscoelastic properties during passive stretching include the sarcomere cytoskeleton, specifically titin and desmin (9,36,57,62,63). However, Morse et al. (37) estimated muscle fascicle length *in vivo* using ultrasonography and found that there was no change from pre- to post-stretch indicating that the stretching-induced changes were likely influenced by the intramuscular connective tissues (endomysium, perimysium and epimysium) (9). The relatively large amount of perimysium has been considered a major extracellular contributor to passive stiffness (45) and increasing compliance of the muscle may be due to a rearrangement of the perimysial collagen fibers (46). Other physiological changes within intramuscular connective tissue purported to cause an increase in muscle during passive stretching are the relaxation of the proteoglycan matrix tissue (14) and deformation of the aponeuroses (37).

A unique finding from the present study was the altered muscle and tendon contribution amongst the older adults. In the present study, the acute increase in range of motion for the older adults was attributed primarily to the lengthening of the tendon (64%) as opposed to the muscle for the younger adults. These findings are in agreement with those of Mian et al. (35) who measured the muscle and tendon contribution during different phases of the gait cycle between age groups and found that the tendon lengthened more than the muscle to achieve the same MTU length as the young. Possible explanations for this behavior could be an increase in passive stiffness of the muscle (9,10,42) and an increase in tendon compliance in older adults (38,41,43,51,54,55). For example, Gajdosik et al. (10) reported a higher passive resistive torque for older women at common joint angles when the foot was dorsiflexed through a range of motion indicating an increase in passive stiffness for older women when compared to their younger counterparts. Ochala et al. (42) reported higher musculotendionous passive stiffness during sinusoidal stretches in the older men; however, they concluded that with aging, the muscle and tendon might be affected differently. Furthermore, Narici et al. (40) concluded that the AT was less stiff in older men over the age of 60. In the present study, the muscle and tendon both lengthen to contribute to the increased range of motion in older adults. However, due to the increased tendon compliance that occurs with aging, the tendon contributed more to the increase in MTU length.

With aging, there are modifications within the muscle and tendon that specifically influence the compliance of these tissues. Older adults with a higher proportion of type I fibers (28) have an increased collagen content along with different isoforms of the titin protein which yield greater stiffness compared to young adults (3,23,27,39). Additionally, amongst older adults there is a replacement of contractile tissue with adipose and connective tissue (44,47) as well as increases in collagen crosslinking (14) and concentration (3). Thus, increases in both connective tissue (i.e. perimysium) and collagen cross-linking may reduce the muscles ability to lengthen in older adults. Current *in vivo* technology makes it difficult to assess the specific properties that contribute to the age-related increase in tendon compliance. However, *in vitro* studies have examined the age-related changes in tendons (20,51,55,60,61) and have reported increases in tendon cross sectional area and a simultaneously reduction in collagen fibril diameter that results in reduction in tendon quality and lower stiffness values (55). In addition, Ippolito et al. (20) found increases in the volume of the extracellular matrix, thus causing a relative decrease in the number of cells per unit of tissue surface within rabbit AT. The authors suggested there was less organization of the collagen fibril and subsequently less stiffness (20). Similar studies that have examined excised tendons have also reported decreases in collagen, extracellular water, and mucopolysaccharide content with a subsequent increase in elastin content, which is thought to contribute to the age-dependent reduction of stiffness within the tendon (51,55,58,61). Further research is warranted to look at other specific mechanisms within the human AT that contributes to the increased compliance seen to occur with increasing age.

In the present study, it was important that each stretch be passive to ensure that the viscoelastic creep responses (increase in joint range of motion) could be attributed to the mechanical properties of the muscle and/or tendon rather than changes in muscle activation. Gajdosik et al. (11) previously reported that stretches can be defined as passive when surface EMG amplitude values are less than 5% of the EMG amplitude values obtained during a MVC. The mean normalized MG and SOL EMG amplitude values in the current study were 1.20 and 1.68%, respectively and therefore can be determined as passive. However, in order to adhere with the recommendations of Gajdosik et al. (11), five participants from each group were excluded due to their EMG amplitude values exceeding these recommended values.

In conclusion, young and old men experienced viscoelastic creep during the 60-s CT stretch accompanied by increases in MTU, MG, and AT length. The young men experienced greater increases in ankle joint position, MTU length, and MG length and smaller increases in AT length from pre- to post-stretch when compared to the older men. Further research is warranted in human models to identify the specific mechanisms within the muscle and tendon that account for the increased length in both age groups.

APPENDIX A: FIGURES
Appendices are not to be copyrighted

Figure 1.

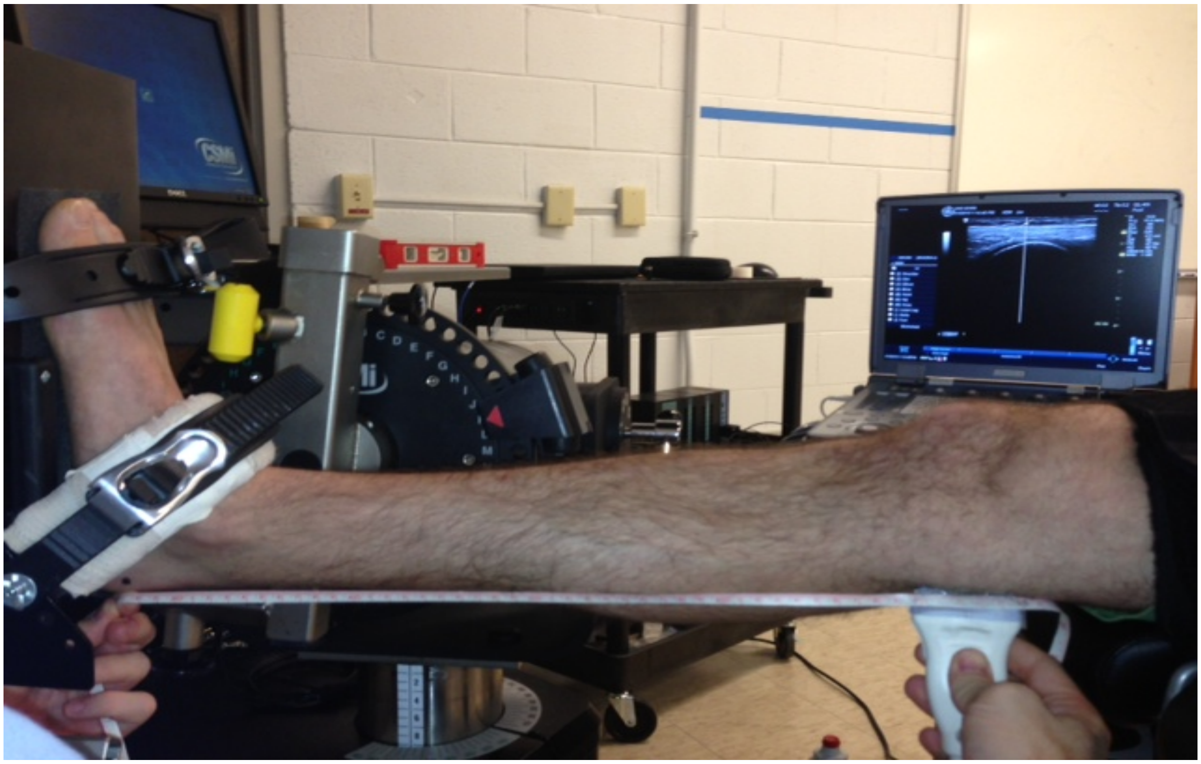


Figure 2.

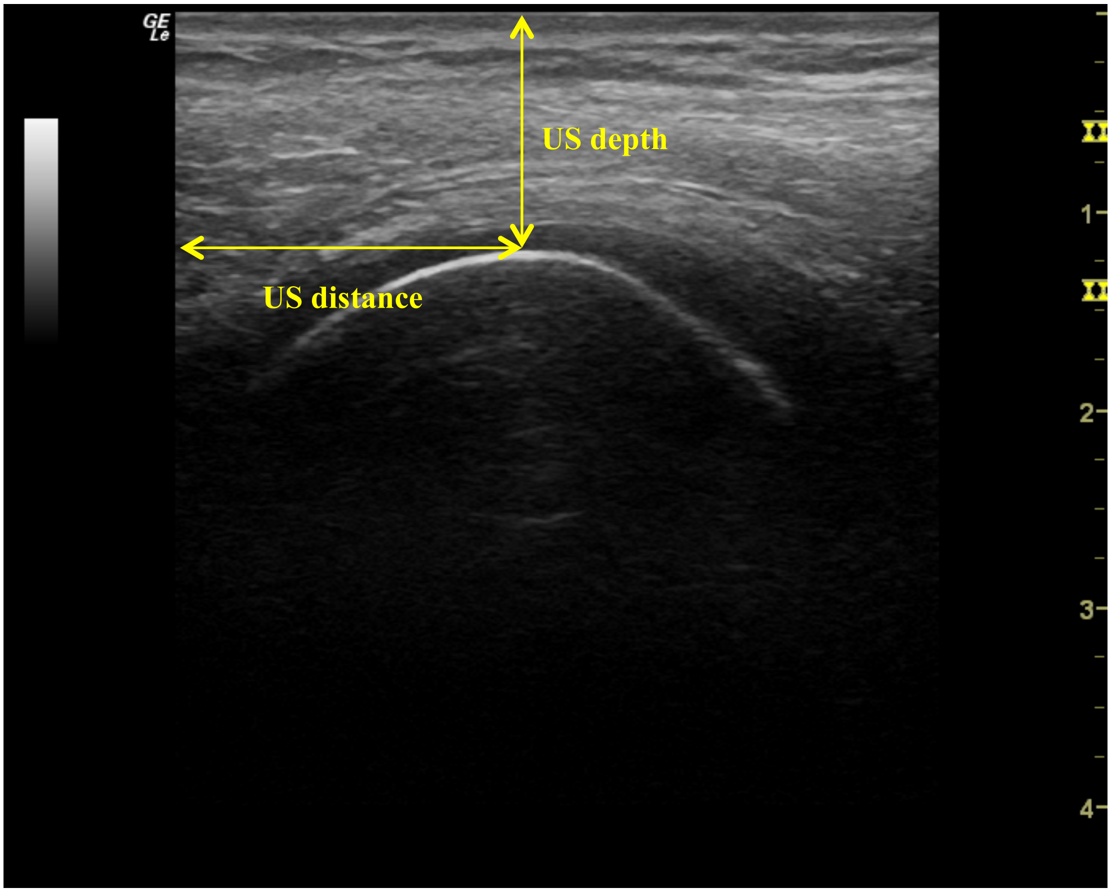
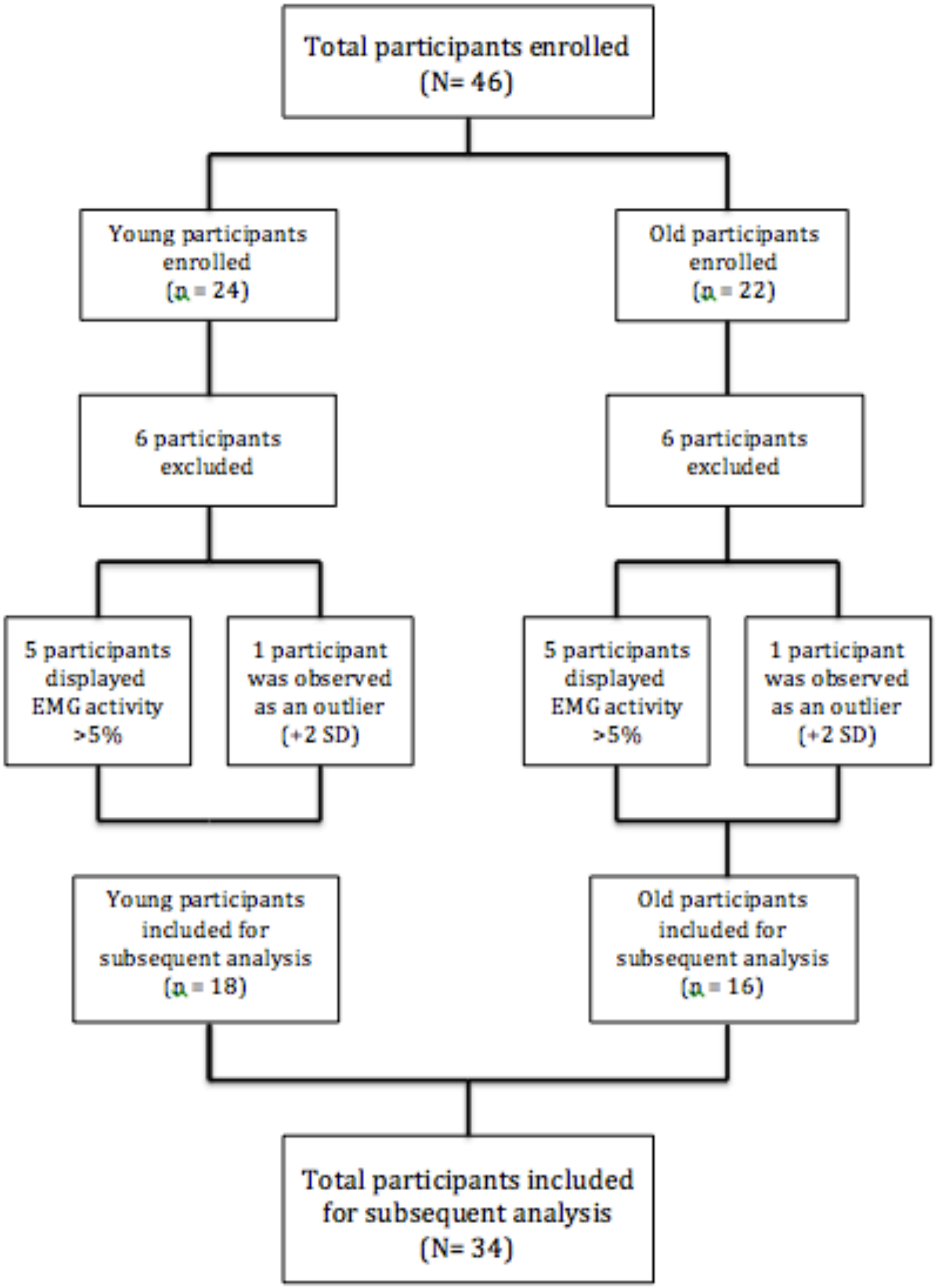


Figure 3.


Total participants enrolled
(N= 46)
Young participants
enrolled
(n = 24)
Old participants
enrolled
(n = 22)
6 participants
excluded
6 participants
excluded
5 participants
displayed
EMG activity
>5%
1 participant
was observed
as an outlier
(+2 SD)
5 participants
displayed
EMG activity
>5%
1 participant
was observed
as an outlier
(+2 SD)
Young participants
included for
subsequent analysis
(n = 18)
Old participants
included for
subsequent analysis
(n = 16)
Total participants included
for subsequent analysis
(N= 34)

APPENDIX B: TABLES

Table 1.

Variable	**Young** (n=18)	**Old** (n= 16)
Age (yrs)	19.78 ± 2.39	68.81 ± 2.90
Height (cm)	174.71 ± 6.56	175.94 ± 3.84
Mass (kg)	71.45 ± 9.23	83.01 ± 11.36*

All values presented as mean ± SD; *significant difference between age groups (P<0.01)

Table 2.

Variable	Group	Pre-stretch	Post-stretch	Change
Ankle joint position (°)	Young	101.06 ± 4.98	103.07 ± 5.45*	2.01 ± 0.98
	Old	99.44 ± 7.30	100.84 ± 7.36*	1.41 ± 0.72
MTU length (cm)	Young	46.05 ± 2.33	46.20 ± 2.30*	0.16 ± 0.07†
	Old	45.80 ± 1.91	45.91 ± 1.93*	0.11 ± 0.06
MG length (cm)	Young	23.43 ± 2.82	23.55 ± 2.82*	0.11 ± 0.04†
	Old	24.74 ± 1.91	24.77 ± 1.91*	0.04 ± 0.02
AT length (cm)	Young	22.61 ± 2.47	22.65 ± 2.46*	0.04 ± 0.04†
	Old	21.06 ± 2.02	21.13 ± 2.04*	0.07 ± 0.04

Values are expressed as mean ± SD. CT, constant torque; MTU, muscle-tendon unit; MG, medial gastrocnemius; AT, Achilles tendon. *Increase from pre- to post-stretch (P<0.05); † Difference between age groups (P<0.05).

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