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Effect of acute tensile loading on gender-specific tendon structural and mechanical properties.

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Effect of Acute Tensile Loading on Gender-Specific Tendon Structural and Mechanical Properties

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ABSTRACT: Stretching is commonly used prior to exercise, as it is thought to reduce the risk of injury, and it is also used in the preconditioning of tendon grafts. As tendon properties have been shown to be different between genders, it is proposed that stretching will differentially affect the structure. Here we examine the effect of acute stretch on the mechanical properties of both male and female medial gastrocnemius tendon. Female [20 years ± 1 (SEM), *n* ¼ 17] and male (22 years ± 1, *n* ¼ 18) subjects underwent a 5-min passive dorsiflexion stretch. Prior to and post stretch medial gastrocnemius tendon stiffness (*K*), length (l) and cross-sectional area (csa) were measured using ultrasonography and dynamometry. Stiffness and Young's modulus (e) were significantly reduced with stretch for both genders ($p < 0.05$). Females showed significantly ($p < 0.05$) greater pre- to poststretch decreases in *K* (22.4 vs. 8.8%) and e (20.5 vs. 8.4%) in comparison to males. The present results show that stretching acutely reduces stiffness of the medial gastrocnemius tendon in females and males, with females showing significantly greater change. The observed disparity between genders may be due in part to variations in tendon moment arm and intrinsic differences in tendon composition. These differential changes in tendon mechanical properties have functional, motor control, and injury risk implications, as well as possible implications for preconditioning of tendon grafts.

Keywords: Stiffness; Young's modulas; Stretching; Sex

Tendon injuries affect millions of people in both athletic and occupational settings,[1] with the achilles and patellar tendons being the two most frequently injured tendons in the human body.[2] The high incidence of achilles tendon injuries is related to the mechanical loading imposed on the tendon during physical activity.[1] Although tendon injury is likely multifactorial, the previously reported greater incidence in tendon injuries among females[3] in comparison to males may be associated with differences in tendon mechanical properties, the stiffness (*K*) and Young's modulus (e) of the patellar tendon[4] and gastrocnemius apponeurosis[5] being significantly lower in females. With reported values of $K = 16.5 \pm 3.4$ N · mm^{-1}, e = 277 ± 25 MPa for females, and $K = 25.9 \pm 7.0$ N · mm^{-1}, e = 356 ± 32 MPa for males for the gastrocnemius apponeurosis.[5] Young's modulus data represent normalized stiffness values, in terms of cross-sectional area (csa) and original length, and indicates that gender differences are not solely due to dimensional factors but that there may be internal structural differences.

Stretching is commonly used prior to exercise, as it is thought to reduce the risk of injury via increasing the range of motion[6] and reducing passive stiffness.[7] However, stretching has been shown to acutely decrease explosive muscle performance,[8,9] and in addition, acutely decrease gastrocnemius tendon stiffness in males,[10] thus increasing tendon mechanical strain for any given load. Stretching or tensile loading protocols are also used in the preconditioning of tendon grafts for ACL reconstructions.[11] Graft preconditioning is suggested to decrease the degree of stress relaxation following graft fixation[12] and thus potentially prevent the development of knee laxity.[13] However, there are conflicting reports about its efficacy,[11–14] and the optimal preconditioning and fixation tension required is undefined.[13] The contrasting reports may well be due to the variations in loading applied to the graft, but also due to the differences between individuals and specifically genders in terms of tendon properties.

Although the effects of passive stretching on reduction of tendon stiffness properties in vivo have been described for males, no such data exists for females. Also, as tendon stiffness for males has been shown to be greater than that for females, it may be possible that structural differences exist between the genders. By its nature, if a set force is applied to a tendon that is more compliant, it will stretch further and so experience a greater strain than a stiffer tendon. This in itself may affect the structural properties of the tendon differentially during acute loading. In light of the above, we hypothesized that acute stretching would have a different effect on the tendon structure between genders. These proposed diverse effects could have implications in both a sporting and clinical setting.

The aims of this study are twofold: (1) to provide novel data on the effect of stretching on female tendon in vivo, and (2) to compare the effect of stretching on the structural and mechanical properties of tendon between genders.

MATERIALS AND METHODS

Participants and Experimental Design

Seventeen females [mean age 20 years ± 1.0 (SEM), height 166.3 cm ± 1.4, and body mass 67.1 kg ± 2.2] and 18 males (mean age 22 years ± 1, height 180.5 cm ± 1.2, and body mass 84.2 kg ± 2.7) participated in the study. The investigation was approved by the local Ethics Committee and all subjects gave their written informed consent to participate. The study conformed to the principles of the World Medical Association's Declaration of Helsinki. Participants visited the laboratory prior to the test session to allow familiarization with the protocols. All measures were preceded by a standardized warmup.

Measurement of Torque

Torque output during isometric plantar flexion was determined using a dynamometer (Kin Com, type 125 AP, Chattanooga, TN). The participants were seated on the dynamometer with the knee fully extended and the hip flexed to 908. The foot was fixed in a neutral anatomical position, where the sole of the foot was at 908 to the tibia. The ankle joint axis was visually aligned with the pivot point of the dynamometer lever arm and the foot was securely fixed to the dynamometer foot plate with Velcro straps. Three maximal isometric plantar flexion efforts were carried out to ensure tendon preconditioning prior to the test. All participants were instructed to gradually develop force from rest to maximum over a 3–4-s time period. The task was repeated three times with a 1-min rest between trials. The torque signals were analogue to digital, converted at a sampling rate of 2 kHz (Testpoint, Keithley instruments, UK) and saved for further analysis.

Measurement of Tendon Elongation

Tendon elongation measurements were taken during the graded isometric plantar flexion test using a 7.5-MHz, 40-mm linear array, B-Mode ultrasound probe (AU5, ESAOTE BIOMEDICA, Italy) with a depth resolution of 49.3 mm. The probe was placed in the sagittal plane over the myotendinous junction of the medial head of the gastrocnemius muscle and fixed in position on the leg. An echo-absorptive marker was placed between the probe and the skin to act as a fixed reference from which measures of elongation could be made. The S-VHS output signal from the ultrasound apparatus was fed to a computer via a video card (RT.X10, Matrox, Canada) and captured at 25 Hz using Adobe Premier Pro (Ver 2.0). The force output from the dynamometer and the ultrasound video were synchronized using a digital trigger (DS7AH, Digitimer, UK). Images from the ultrasound recording were taken at times corresponding 10% force increments from 0 to 100% of maximal voluntary contraction (MVC) and the displacement of the gastrocnemius myotendinous junction relative to the skin marker were digitized using computerized image analysis (Image J, Wayne Rasband National Institute of Health, Bethesda, MD) (see Fig. 1). Previous studies have corrected elongation measurements due to the elongation caused by angular joint rotation.[15] However, Maganaris[16] concluded such approaches should be avoided, as the assumption that isometric plantarflexions and passive ankle rotations in the sagittal plane generate angular displacements around the same axis is invalid. During the plantar flexion effort calcaneal movement is caused by heel lift; however, the effect of this movement on elongation is largely eliminated by the use of a skin marker and fixing the scanning probe.[16] Therefore, this method was used in this study.

Electromyographical (EMG) Measurement

In addition to the plantar flexion efforts, participants performed three dorsiflexion maximal voluntary contractions (MVCs). The dorsiflexions were performed with the subject in the same position as previously described for the plantar flexion efforts. The force produced due to dorsiflexor coactivation during the plantar flexion efforts was approximated assuming a linear relationship between EMG amplitude of the dorsiflexor muscles and force.[17]

Two silver/silver chloride bipolar electrodes (Medicotest UK, type N10A), with a 20-mm interelectrode distance (center to center) were placed midline on the tibialis anterior muscle belly, halfway between the center of the belly and the distal myotendinous junction of the tibialis anterior. Similarly, two electrodes were also placed over the belly of the medial gastrocnemius muscle just distal from the knee and 2 cm medial to the midline. A ground electrode (Medicotest, UK, type Q10A) was placed at the lateral malleolus of the ankle. The participants' skin was carefully prepared prior to electrode attachment (Nuprep, SLE Ltd, South Croydon, UK). The electromyographic signals were high and low pass filtered between 10 and 500 Hz, respectively (Neurolog filters NL 144 and NL 134, Digitimer, UK), preamplified (x1000), (Neurolog remote AC preamplifier NL 824, Digitimer, UK), amplified (x2) (Neurolog isolation amplifier, NL 820, Digitimer, UK), and A/D converted at 2000 Hz (KPCI 3101, Keithley Instruments, UK).

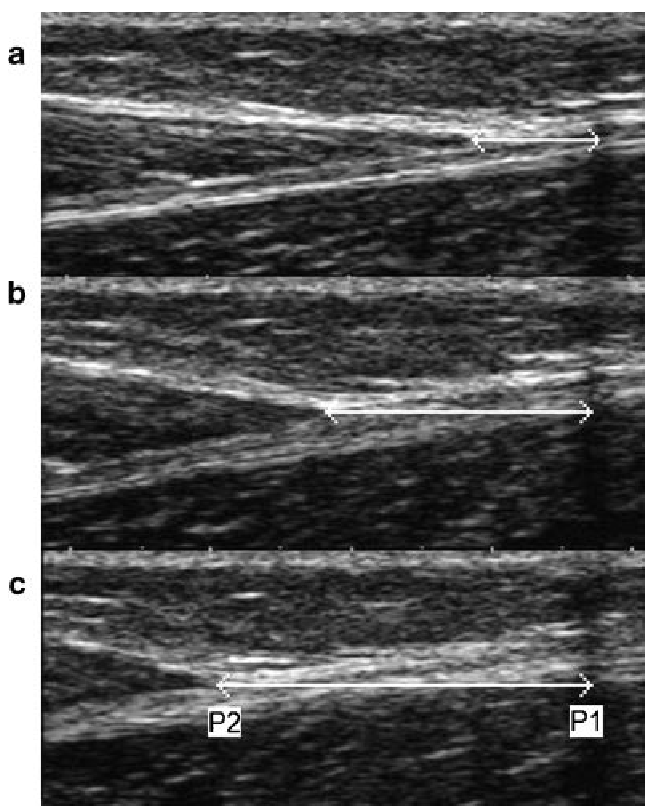

Figure 1. Typical example of medial gastrocnemius tendon elongation during ramped isometric contraction (a—at rest, b—during contraction, and c—maximal contraction). White lines indicating measured distances from echo-absorptive marker (P1) to myotendenous junction (P2).

Measurement of Tendon Dimensions

Tendon Length

At rest, with the foot in neutral position (sole of foot at 908 to the tibia) and lower limb straight, the insertion of the Achilles tendon into the calcaneous was imaged. A mark was made on the skin over the point where the tendon inserted. Similarly, the insertion of the myotendenous junction to the medial gastrocnemius was imaged and a mark made on the skin. The distance between these two marks was measured manually and considered to be the tendon's length. This measurement was conducted three times and an average taken.

Csa

Achilles tendon csa was recorded 1, 2, and 3 cm above the tendon insertion point in the calcaneus.[18] The stills were digitized using computerized image analysis (Image J, Wayne Rasband National Institute of Health). An average of the three stills was taken and corrected based on the assumption that the medial gastrocnemius tendon csa occupies a fraction of the average achilles tendon csa equivalent to the relative physiological csa of the medial gastrocnemius muscle with respect to the entire triceps surae muscle.[19]

Moment Arm Determination

The moment arm length of the gastrocnemius tendon was obtained using the tendon travel method.[20] Briefly, the displacement of the medial gastrocnemius myotendinous junction caused by rotating the ankle from 58 of dorsiflexion to 58 of plantarflexion was recorded using ultrasonography. The tendon moment arm length at the ankle angle of 08 was obtained from the ratio of change in tendon displacement in millimeters to change in angle in radians. This is based on the relationship between tendon excursions, joint displacements, and moment arms, which has previously been demonstrated.[21]

Calculation of Tendon Force

Forces were determined by dividing torque by the tendon moment arm. Cocontraction force was calculated and added to the plantarflexion force. Correction for relative muscle physiological cross sectional area of the gastrocnemius (28%)[19] was applied in the calculation of final tendon force.

Calculation of Tendon Properties

The elongation of the tendon at loads corresponding to 0–100% of the plantarflexion force was measured at 10% intervals. The force elongation relationship was plotted and a second order polynomial fit was applied. Tendon stiffness was defined as the slope of the force displacement relationship at 100% MVC.[22] Young's modulus was calculated as the product of stiffness and the ratio of tendon length to csa. Hysteresis was defined as the area under the ascending curve minus the area under the descending curve expressed as a percentage of the area under the ascending curve.[23]

Tensile Loading Protocol

The participant was positioned in the dynamometer as in the plantar and dorsiflexion efforts (knee straight, hip at 908) and a 5-min passive dorsiflexion stretch applied. During the stretch the EMG activity of the medial gastrocnemious and tibialis anterior was monitored to ensure the participants remained relaxed. The foot plate of the dynamometer (to which the participants foot was attached) was moved into dorsiflexion so that it recorded a passive torque of 35–40 Nm above baseline (as per maximal force in Kubo et al.[10]), this torque level was monitored and maintained throughout the stretch to ensure a standardized level for all participants.

As Achilles tendon moment arms have previously been reported to be shorter in females than males, applying the same passive torque would cause greater forces in the tendons of the females than the males. To account for this difference a second testing session was carried out 1 week after completing the first. This testing session was an exact replica of the first except the passive dorsiflexion stretch was held in a position such that the passive torque was altered to ensure the Achilles tendon was subjected to a force of 730 N above baseline (tendon force based on 40 Nm passive torque and average tendon moment arm). This would control for any differences in moment arm variation between individuals.

Statistics

Descriptive data is presented as means (±SEM). Pre/postgender differences were analyzed using ANCOVA and Student's *t*-tests. Reliability (of force, elongation, csa, length, and EMG) was determined using intraclass correlation coefficients.

Relationships between tendon csa and stiffness/Young's modulus changes were examined using Pearson's correlation coefficients. Alpha levels were set to $p < 0.05$.

RESULTS

No significant differences were seen between the standardized torque and standardized tendon loading method for percent change in stiffness following stretching (-22.4 vs. -16.3% for females and -8.8 vs. -7.3% for males). As such, the data presented here are for the standardized torque methodology, female and male tendon mechanical property data for pre- and post- stretching, are given in Table 1. The within-session intraclass correlation coefficients were 0.95 for elongation, 0.98 for plantarflexion force, 0.99 for csa, 0.97 for tendon length, and 0.98 for RMS EMG. The interday intraclass correlation coefficient was 0.90 for tendon stiffness.

Prestretch Tendon Characteristics

Prior to stretching medial gastrocnemius tendon stiffness was 35% lower in females than males ($P < 0.05$). Initial hysteresis was 33% lower in females in contrast to males ($p < 0.05$). At baseline, female tendon length was shorter (16%) and csa smaller (21%) than their male counterparts ($p < 0.05$). See Table 1 for values.

Table 1. Comparison of Tendon Mechanical Properties between Genders and Pre- and Poststretch ± SEM

Variable	Prestretch	Poststretch	Pre- vs. Post *p*-Value	Prestretch	Poststretch	Pre- vs. Post *p*-Value
Stiffness (Nmm^{-1})	76.7 ± 7.5	68.3 ± 6.9	0.04*	49.6 ± 6.3	39.7 ± 5.4	0.00*
Young's modulus (MPa)	737.4 ± 88.3	648.2 ± 73.2	0.04*	485.6 ± 60.8	394.2 ± 51.4	0.00*
Cross-sectional area (mm^2)	22.5 ± 1.2	22.4 ± 1.1	0.36	17.7 ± 1.0	17.4 ± 0.9	0.12
Length (cm)	19.4 ± 0.5	19.5 ± 0.5	0.055	16.3 ± 0.6	16.3 ± 0.6	0.3
Moment arm (mm)	53.4 ± 2.6	—	—	46.5 ± 5.1	—	—
% Hysteresis	28.1 ± 2.0	24.6 ± 2.6	0.16	18.9 ± 1.7	11.9 ± 1.5	0.01*

*Indicates significant ($p < 0.05$).

Effect of Stretch on Tendon Characteristics

Poststretching, both groups significantly decreased stiffness and Young's modulus. Females showed a decrease in stiffness and Young's modulus of 22.4 and 20.5%, respectively, versus 8.8 and 8.4% for males ($p < 0.05$). These changes can be seen as a decreased gradient in the tendon force–elongation curve and stress–strain curves (see Fig. 2a and b).

Following stretch, decreases in hysteresis of 34.4 and 11.2% were seen for females and males, respectively. Typical hysteresis profiles pre and post stretching are represented in Figure 3a and b.

Tendon length and csa changes following stretching were not significant. In addition, tendon csa was found to be unrelated to degree of change in stiffness ($R = 0.26$) and Young's modulus ($R = 0.28$) ($p > 0.05$).

Females showed a significantly greater decrease in stiffness, hysteresis and Young's modulus following stretch compared to males ($p < 0.05$). When baseline values were entered as covariates in the ANCOVA analysis, it was found that they were not significantly related to the changes in tendon stiffness, Young's modulus, and hysteresis ($p > 0.05$).

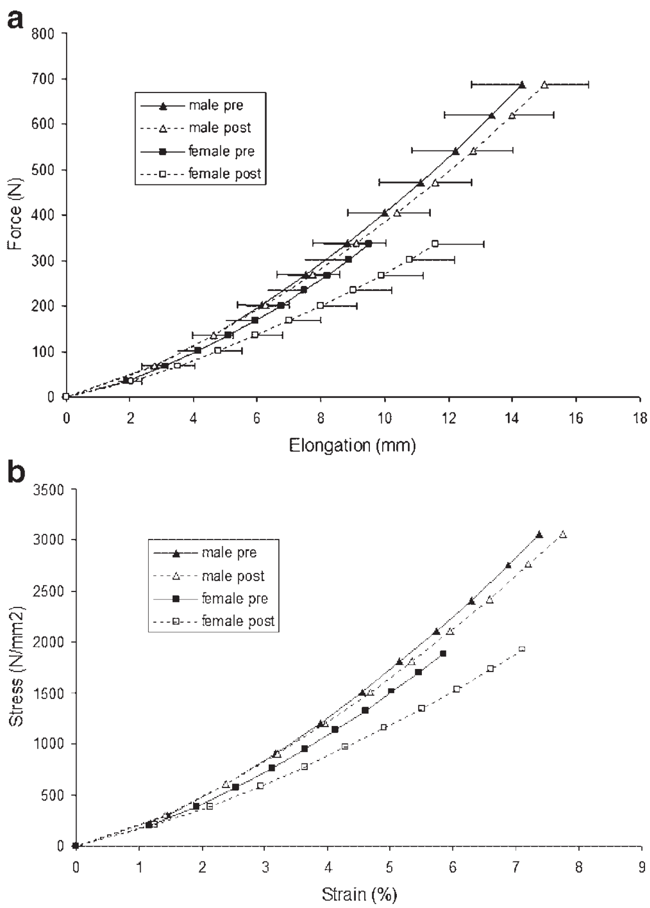

Figure 2. (a) Mean force–elongation curve for females and males pre- and poststretching. (b) Mean stress–strain curve for females and males pre- and poststretching .

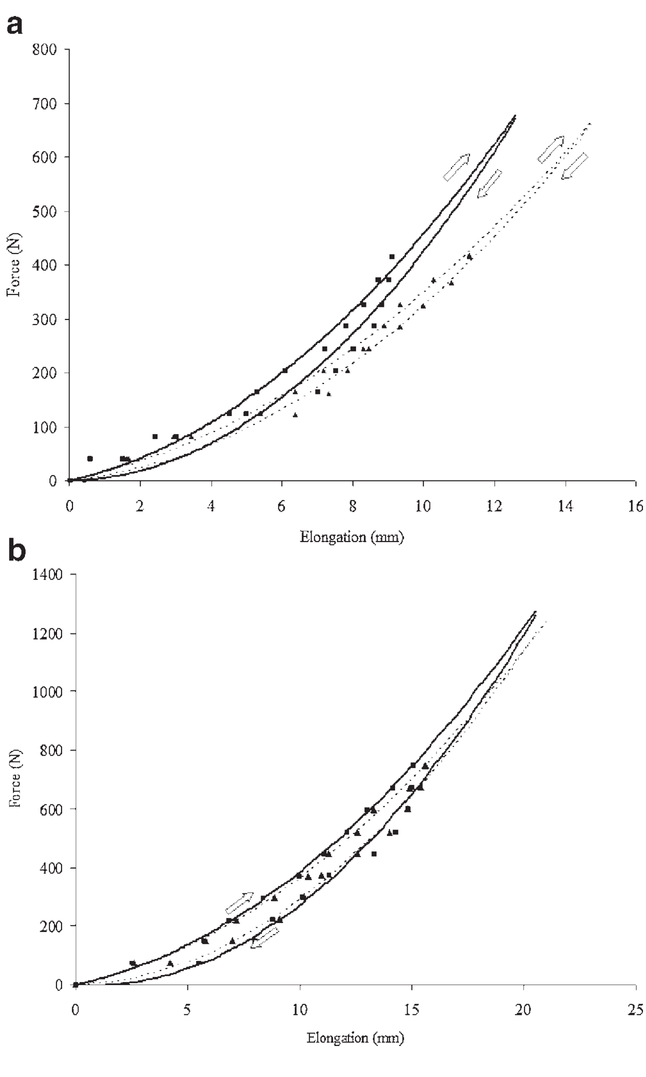

Figure 3. (a) A typical female force elongation profile during both ascending and descending phases showing hysteresis (&, prestretch, ~, poststretch). The difference in the area under the ascending curve to that under the descending curve, up to the maximum recorded data point, represents the hysteresis. (b) A typical male force elongation profile during both ascending and descending phases showing hysteresis (&, prestretch, ~ poststretch).

DISCUSSION

The current study aimed to (1) provide initial data on the effect of stretching on female tendon in vivo, and (2) compare the effect of stretching on the mechanical properties of tendon between genders. Our findings have shown that: (1) in a group of young females, following completion of a 5-min passive dorsifexion stretch, tendon stiffness, Youngs modulus, and hysteresis decreased significantly. (2) There are gender differences with respect to the effect of stretching on tendon with females showing significantly greater decreases in stiffness, Young's modulus, and hysteresis in contrast to males.

It was observed that female tendon stiffness was significantly lower than that for males at baseline; the average tendon stiffness prior to the performance of the passive stretch was 49.6 ± 6.3 N · mm^{-1} for females and 76.7 ± 7.5 N · mm^{-1} for males. These values lie within the range of values obtained in previous studies that have used ultrasonography to measure gastrocnemius tendon and apponeurosis stiffness in vivo.[5,18] Poststretch there was a significant decrease in tendon stiffness in females of 22.4% and males of 8.8% ($p < 0.05$). Previous studies utilizing a similar protocol to that used here only exist for males and measured apponeurosis stiffness; however, the reported changes are very similar to those presented here.[10,24] Following a 10-min passive dorsiflexion stretch held at 358 from neutral, a 10% decrease in stiffness has been shown.[10] Similarly, following the same stretch held for 5 min, a 7.9% decrease in tendon stiffness has been reported.[24] The slightly larger decrease shown by Kubo et al.[10] is most likely due to longer stretch protocol utilized in comparison to this study (10 vs. 5 min). The intensity of the stretch applied in the present study was greater than that stated by Kubo et al.,[24] who reported an average maximal passive torque of 37.8 ± 6.7 Nm. Kubo et al.[24] did not maintain this torque throughout the stretch, thus allowing stretch relaxation to occur, this could account for the slightly greater changes found in the present study as the torque level was maintained throughout the stretch. In addition, caution must be taken when comparing to the results of Kubo[10,24] and colleagues, as they measured apponeurosis stiffness, which has been shown to elongate further than tendon for a given load.[25] Although the time frame of the tendon stiffness change was not investigated in this study, evidence from previous research points to this acute response to static stretching being a temporary effect. Studies measuring tendon and apponeurosis properties the day following completion of a stretch training regimen have shown stretching have no significant effect on tendon and apponeurosis stiffness.[26,27] However, caution must be taken when comparing these results to those found here due to differences in the stretch protocols utilized.

Although there was no significant difference in moment arm length between genders ($p > 0.05$), on average, females were shorter than males (46.5 ± 5.1 mm vs. 53.4 ± 2.6 mm). This shorter moment arm would cause the force experienced in the tendon during the stretch to be greater in the females than the males by on average approximately 13%. A retest was conducted with a stretch intensity standardized for individual's moment arm length, such that the Achilles tendon received a passive force of 730 N above baseline. The percentage stiffness change following the adjusted stretch compared to the original stretch was found not to be significantly different for either gender. However, the stretch adjustment showed a trend toward a decreased stretch effect for the females; that is, decreasing the external stretch intensity decreased the stiffness change. Although non-significant, this trend that suggests that when standardizing the external load gender differences will occur partly due to differences in moment arm length.

During the stretching protocol the same loading was applied to each individual tendon; due to individual and gender differences in tendon dimensions and mechanical properties, this same loading would cause different stresses and strains within the tendon. As female's tendons are initially more compliant and smaller in diameter, the stresses and strains they experience are likely to be larger than the males. These potentially greater stresses and strains may be responsible for the greater changes seen in the females. However, baseline stiffness, Young's modulus and hysteresis values were found to be not significantly related to the degree of change experienced following stretch. This does not align with the rationale that more compliant structures would show a greater change, as they would experience a greater strain for any set level of loading. In addition, tendon csa was found to be unrelated to the degree of stiffness, Young's modulus, and hysteresis change following completion of the static stretch. This indicates that the gender difference in tendon csa was not responsible for the gender-specific changes observed here. These results indicate that as-yet unidentified gender specific factors are responsible for the subsequent load-induced mechanical changes rather than the initial differences in tendon mechanical properties.

It may be that the crosslinking or arrangement of fibers, ratio of collagen fiber type, or water content differs between genders, and this influences the tendon's mechanical properties and acute responses. Hormonal differences between genders could affect tendonous tissue, as estrogen has previously been shown to inhibit collagen synthesis, and thus affects tendon tissue quality.[28] In support of this, it has been shown that tendon collagen fractional synthesis rate is lower in women than men, both at rest and following exercise.[29] A positive correlation has been reported between collagen content and tendon mechanical properties;[30] differences in collagen content between genders could therefore be responsible for the different mechanical properties. However, tendon mass density (which is thought to be indicative of collagen content) has been shown to be not significantly different between males and females in the human patellar tendon.[31]

The mechanisms that cause the decrease in tendon stiffness following stretch are largely unknown. It may be suggested that acute changes in the dimensions of tendons might be involved. However, the results of this study showed that stretching had no significant effect on the length or csa of both female and male tendons, indicating that changes in stiffness following stretch are not due to alterations in structural dimensions. The values for tendon length and csa measured in this study are in line with those previously reported using ultrasonography and MRI.[5,32] When stiffness values were normalized for tendon length and CSA in the calculation of Young's modulus, the significant ($p < 0.05$) decrease in stiffness in both females and males was still apparent, with females still showing a greater change than males ($p < 0.05$).

The in vivo approach of this investigation is associated with some inherent limitations. In the measurements of tendon elongation the myotendinous junction is assumed to displace during the contraction in the scanning plane only; however, as a three-dimensional structure, it is possible that mediolateral displacement may also occur. The ankle neutral joint position is presumed to correspond to 0% tendon strain and 0 N tendon force. Although this has previously been shown to not be strictly correct, the effects on the subsequent calculations of tendon strain have been shown to be negligible.[33] The measurement of the displacement of the myotendionous junction provides an average elongation for the gatrocnemius tendon; this does not allow for the determination of regional differences in tendon proper- ties. In addition, it may be that not all forces seen are transmitted through the tendon, as other structures stiff enough to transmit forces may be involved. The force within the medial gastrocnemius tendon was estimated based on a correction factor for relative muscle physio- logical csa of the medial gastrocnemius within the triceps suare; however, it is not possible to tell whether all sections of the triceps suare are equally activated during the plantarflexion effot. These assumptions may cause a small degree of error in the absolute values of stiffness calculated here; however, these factors will have little impact on the principle aim of this study, which was to compare the changes in tendon properties that occur following an acute stretch and exist in all in vivo noninvasive tendon studies.

The gender difference regarding the effect of stretching on tendon mechanical properties has important implications. When clinicians or trainers are prescribing stretching procedures it is important that they understand they will have different effects when used on different genders, that is, all things being equal, female tendons will show greater changes than the males. Too great a decrease in tendon stiffness will have adverse effects on muscle output, motor control, and injury risk. In addition, this gender specific finding will have implications regarding the pre conditioning procedure used for tendon grafts. Applying the same preconditioning protocols to male and female tendon may produce different effects, and thus may have a bearing on the outcome of the reconstruction procedure. In addition, as a static stretch has been shown to differentially affect tendon properties between genders, it is also likely that other acute loading interventions such has cyclical loading, which has been shown to effect tendon properties in males,[34] may also have a gender-dependent effect.

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