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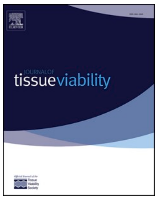

How susceptible are our Achilles Tendons? Sonoanatomical assessment. A cross-sectional study

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ABSTRACT

Objective: the aim of this study is to observe whether there are ultrasound changes between men and women in the Achilles tendon at rest, at maximum passive force is applied and during walking.
Material and methods: it was a cross-sectional study involving 27 healthy young participants recruited as volunteers between April to July 2022. A variety of data was recorded: (age, Body Mass Index, sex, smoking, current injury status, allergies, medications, previous surgeries, type of sport, and number of weekly workouts) and ultrasound measurements at rest and at passive force (Cross Sectional Area Achilles Tendon length, tendon thickness, Cross Sectional Area and pennation angle of the soleus muscle to the Achilles Tendon).
Results: women demonstrated a statistically significant lower proximal and median thickness both at rest (4.5 vs 5.1 mm with $p < 0.001$ for proximal thickness; 4.4 vs 5.3 mm with $p < 0.001$ for median thickness) as well as during maximum eccentric contraction (4.3 vs 4.8 mm with $p=<0.001$ for proximal thickness; 4.1 vs 4.8 mm with $p < 0.001$ for median thickness).
Conclusion: there are significant sonoanatomical differences *in vivo* Achilles tendon between men and women.

1. Introduction

The Achilles tendon (AT) is the thickest, strongest and largest tendon in the human body [1]. It originates from the gastrocnemius muscle and soleus muscle until it inserts into the calcaneal bone. Its characteristics (TA free length, thickness and area) are highly variable between individuals [2] and its mechanical properties store and release energy. When a deficit in this structure appears, there is a direct alteration in the biomechanics of walking, running and jumping [3]. For this reason, the strength, tension and stiffness of the TA is closely related to muscle activity, joint activity and tendon characteristics themselves [1–3].

Prospective studies indicate that risk factors for tendinopathy include female gender, black race, higher body mass index, previous tendinopathy or fracture, higher alcohol consumption, lower plantar flexion strength, higher weekly running volume, more years of running, use of spiked or cushioned shoes, cold weather training, use of oral contraceptives and/or hormone replacement therapy, reduced or excessive ankle dorsiflexion range of motion, and use of antibiotics in the fluoroquinolone class [4–6].

Achilles tendinosis is described to be a more frequent disorder in male runners who perform long duration and high intensity training [7, 8]. Deng et al. [9] evaluated the myotendinous junction of the medial gastrocnemius with the AT and their findings conclude that gender differences exist in individuals who do not have regular exercise habits. However, an updated systematic review on the prevalence of AT injury and physical exercise concluded that for gender there was no significant difference [8].

The mechanical properties of human tendons *in vivo* are based on

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tensile testing to assess tendon longitudinal elongations [10–12]. To obtain a measurement of the free TA, the anatomical reference point is the myotendinous junction of the soleus muscle, as this is a clear landmark that can be identified and traced by ultrasound scanning to its insertion with the calcaneus [11,13].

There are no known conclusive studies relating these morphological differences of the AT and gender in the general population (adult, healthy and with regular exercise habits) that provide clear data in the clinical management for the prevention of this pathology. The aim of this study is to observe whether there are ultrasound changes between men and women in the Achilles tendon when the tendon is at rest, at maximum passive force is applied and during walking.

2. Material and Methods

This study was conducted in full compliance with the provisions of the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects and was approved by the Experimental Ethics Committee. Written informed consent was obtained from all participants prior to data collection.

2.1. Design

Cross-sectional study.

2.2. Subjects

Participants were healthy young men and women who met the inclusion criteria: age between 18 and 30, physically active with an average of training sessions of 3 times a week, negative Lunge test [14]. They were recruited from *L'Institut National de Podologie*, Paris from April to July 2022.

Persons presenting any of the following criteria were excluded from the study: osteo-degenerative disease, metabolic disease, neurological problems, surgical intervention in the lower limb, processes of an infectious, neoplastic, or metastatic origin, cognitive impairment, pregnancy, musculoskeletal lesions in the lower limbs during the last 3 months, and use of corticosteroids and/or oral antibiotics.

2.3. Sample size

Sample size was determined via power analysis (G*power, version 3.1.9.6, Kiel University, Kiel, Germany). The effect size was calculated using the results of the comparison of Achilles tendon Cross Sectional Area (CSA) at rest between men and women in the study of Intziegianni et al. [15]. The results showed that 8 participants in each group was enough (effect size: 1.34, significance level: 0.05, statistical power: 80%).

2.4. Procedure

The subjects who were evaluated by the team member in face-to-face interviews where the procedure was explained, the informed consent was signed and demographic history forms were collected (age, height, weight, sex, smoking, current injury status, allergies, medications, previous surgeries, type of sport and number of weekly workouts).

Then, a brief exploration was carried out in which the Foot Posture Index (FPI) and the Lunge test were performed. The FPI assesses the multi-segmental nature of foot posture in all three planes and does not require the use of specialised equipment. Each FPI item is scored between −2 and +2, resulting in a total ranging from −12 (very supinated) to +12 (very pronated). Index items include palpation of the talar head, curves above and below the lateral malleolus, calcaneal angle, talonavicular protuberance, medial longitudinal arch and forefoot to hindfoot alignment. In all other aspects, the protocol described by Redmond et al. [16,17] was followed. The FPI has proven adequately reliable in varied clinical settings (Intraclass correlation coefficients (ICC) = 0.62–0.91) [18].

The Dorsiflexion Lunge test was performed to assess ankle dorsiflexion range of movement (ROM). The test protocol followed the procedure described by Bennell et al. [14], starting position of the participant in weight bearing with the big toe of the foot being tested 10 cm from the wall and the knee is in line with the second toe, can lean on the wall using two fingers of each hand to maintain balance. The test consists of pushing the knee until it touches the wall without the heel lifting off the ground, at which point the investigator recorded the angle of the tibia to the vertical (to the nearest tenth of a degree) as a measure of ankle dorsiflexion ROM. Three measurements were taken and the mean was used for statistical comparisons. This test has been shown to have an intra-rater reliability of ICC = 0.98 (SEM = 1.1°) and an inter-rater reliability of ICC = 0.99 (SEM = 1.4°) [14].

2.4.1. Gait analysis

For the gait analysis ten optoelectronic cameras (Motion Analysis Corporation, Santa Rosa, CA, USA) recorded the trajectories of reflective markers, positioned on the lower limbs of the participant following the methods of Davis et al. [19]. Markers were placed on the anterior superior iliac spines (ASIS), middle of posterior superior iliac spines (PSIS), greater trochanter, lateral thigh, medial and lateral femoral condyles, tibial tuberosity, head of fibula, lateral shank, medial and lateral malleoli, heel, first, second/third and fifth distal metatarsal heads. One AMTI force platform (Advanced Mechanical Technology Inc., Watertown, MA, USA) recorded the ground reaction forces. The 3D markers' trajectories and ground reaction forces were obtained synchronously with Cortex 1.3.0.562 software (Motion Analysis Corporation, Santa Rosa, CA, USA) with a sampling frequency of 100 Hz and 1000 Hz, respectively. The same operator positioned all the markers for each participant and carried out the whole 3D-gait analysis to limit variability [20]. During these gait analyses, participants computed an overground barefoot walking along a 10 m walkway at a self-selected speed. Five gait cycles were processed, and gait events (heel strike and toe off) were detected when the vertical ground reaction force exceeded or fell below 10 N [21]. The trajectories of skin markers were filtered using a fourth-order zero-lag Butterworth low-pass-filter with a cut-off frequency of 6 Hz. Joint kinematic were established using standard criteria [22]. Briefly, the three orthogonal axes of the shank and foot coordinate system are defined from the coordinates of skin markers. The ankle joint coordinates system is defined from a sequence of successive rotations about mobile axes to obtain the ankle joint kinematics through Euler angles. In this study, we focused the kinematic analysis on two key parameters: the ankle dorsiflexion peak and range of motion during stance phase.

2.4.2. Ultrasound measurements

Measurements were taken by a single investigator with more than 5 years of ultrasound experience. Scans were obtained using a MyLab Sigma Elite portable musculoskeletal ultrasound system (Esaote, Italy) and a high-density linear probe (4–15 MHz). The depth of the image field was set at 3.5 cm, the gain was set at 85 dB, the probe frequency at 14 MHz and a single focal zone (set at a depth of 0.5 cm) was placed at the level of the AT. All other options (e.g., compress, mapping, smoothing, X-resolution) were maintained in all examinations performed for all participants in order to standardize the recorded images across all participants.

Ultrasound measurements of AT length, tendon thickness, CSA and pennation angle of the soleus muscle to the AT were performed. The first measurement of these values was in prone position with both legs under the stretcher at rest. After 5 min of rest, the same ultrasound measurements were taken with the knee flexed on the stretcher (to annul the force of the gastrocnemius) and exerting an active force on the sole of the foot until complete dorsiflexion of the ankle (Fig. 1).

The AT insertion over the calcaneus was located by ultrasonography.

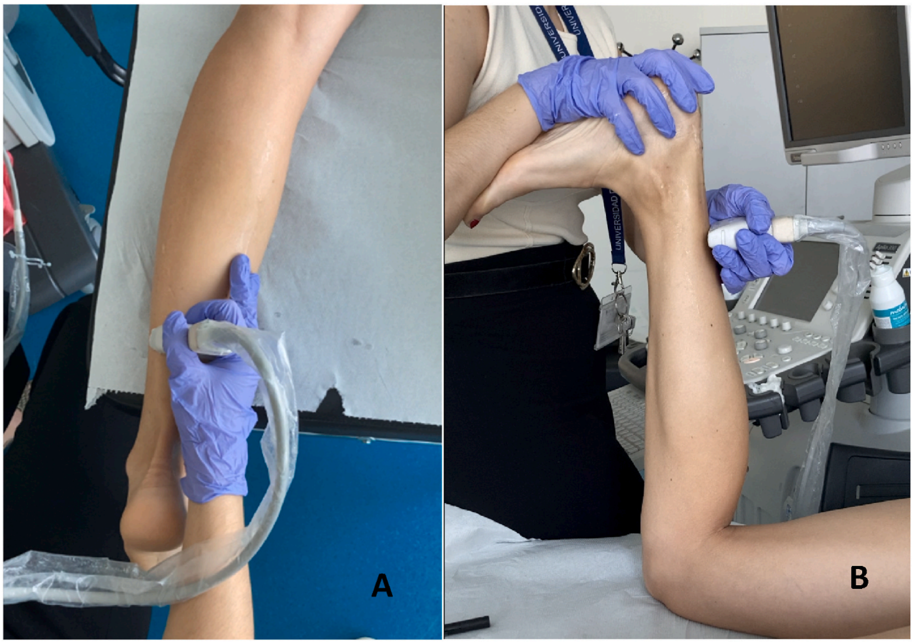

Fig. 1. A) Ultrasound measurements at rest. B) Ultrasound measurements with passive force.

A mark was made on the skin at that level, 4 cm from the calcaneus and at the proximal level when soleus musculature is no longer observed to record ultrasound images for the length of the AT [23]. For thickness measurement the assessor aligned the transducer at the precise location marks (proximal and insertional) and recorded three images in longitudinal view by placing the caliper at the superior and inferior edges of the Achilles tendon [4]. CSA was performed by positioning the transducer in a transverse view at the marks made and three images in transverse view the proximal CSA, medial CSA, insertional CSA were recorded [24]. The mean of 3 repeated values was collected for each measurement. For each image the transducer was removed and repositioned over the skin tags. Following Padhiar et al. [13] for the measurement of the pennation angle of the soleus- Achilles tendon, the probe was placed in a transverse view in the distal third of the leg, a sweep was made towards the foot, until the end of the insertion of the soleus muscle was observed, at this level a 90° turn of the ultrasound probe was made, positioning the probe in a longitudinal view oriented along the medial longitudinal axis determined by the soleus fibers to the Achilles tendon, from there the measurement of the pennation angle was extracted.

The ICC of the ultrasound examiner was used with 10 participants to evaluate the reproducibility of measurement of the thickness and CSA of the AT and angle pennation of the soleus-AT measured at baseline and after 24 h. They were estimated by calculating the ICC using a 1-way random effect model and found to be excellent (ICC = 0.94; 95% confidence interval, 0.90–0.96) at 0 (baseline) and (ICC = 0.87; 95% confidence interval, 0.80–0.92) at 24 h.

2.5. Statistical analysis

The SPSS v.25.0 program (IBM Inc., Chicago, IL, USA) was used for statistical calculations using descriptive statistical tests. A Shapiro–Wilk test was used to test the normality of all data distribution. An independent *t*-test was used to quantify the gender differences in the architectural properties of the Achilles tendon and gait biomechanics if the data were normally distributed. On the other hand, a Mann–Whitney *U* test was used. All the results are shown as mean ± standard deviation. The significance level was set as 0.05.

3. Results

Twenty-seven patients, who were Sixteen healthy women (thirty-two feet) and eleven healthy men (twenty-two feet) with normal Body Mass Index (BMI) (Table 1) [15].

Gender differences in the architectural properties of AT gait biomechanics are given in Table 2. The women demonstrated a statistically significant lower proximal and median thickness both at rest (4.5 vs 5.1 mm with $p < 0.001$ for proximal thickness; 4.4 vs 5.3 mm with $p < 0.001$ for median thickness) as well as during maximum eccentric contraction (4.3 vs 4.8 mm with $p=<0.001$ for proximal thickness; 4.1 vs 4.8 mm with $p < 0.001$ for median thickness). Moreover, the women demonstrated a statistically significant lower proximal and median CSA both at rest (52.4 vs 70.3 mm^2 with $p < 0.001$ for proximal CSA; 55.3 vs 72.0 mm^2 with $p < 0.001$ for median CSA) as well as during maximum eccentric contraction (55.4 vs 69.5 mm^2 with $p < 0.001$ for proximal CSA; 53.0 vs 71.3 mm^2 with $p < 0.001$ for median CSA). On the other hand, the women demonstrated a statistically significant higher ankle dorsiflexion range of motion during the stance phase of gait (Women: 19.4°; Men: 17.2°; $p = 0.01$, Fig. 2).

4. Discussion

The aim of this study was to describe and observe whether there were ultrasound changes in Achilles tendon tissue between men and women, without previous pathology, for clinical management in the prevention of Achilles tendinopathy.

The findings showed larger or longer measurements in men than in women; length and CSA of the AT at rest and at maximal passive force

Table 1
Anthropometric characteristics of participants.

Variable	Groups		p-value
	Women (n = 16)	Men (n = 11)	
Age (yr)	22.5 (2.4)	21.1 (1.7)	***0,022***
Height (cm)	163.0 (7.3)	178.7 (5.8)	***< 0.001***
Weight (kg)	56.3 (5.7)	72.8 (5.0)	***< 0.001***
BMI (kg/m^2)	21.1 (1.5)	22.8 (1.5)	***< 0.001***

BMI: Body Mass Index.

Table 2
Architectural properties of Achilles tendon and gait biomechanics.

Variable	All (n = 27)	Groups		p-value (SD)
		Women (n = 16)	Men (n = 11)	
Ultrasound Mesurements				
Proximal thickness at rest	4.7 (0.7)	4.5 (0.5)	5.1 (0.8)	**< 0.001**
Median thickness at rest	4.7 (0.8)	4.4 (0.5)	5.3 (0.7)	**< 0.001**
Proximal thickness with passive force	4.5 (0.5)	4.3 (0.4)	4.8 (0.6)	**0.001**
Median thickness with passive force	4.4 (0.6)	4.1 (0.4)	4.8 (0.6)	**< 0.001**
Proximal CSA at rest	59.7 (14.6)	52.4 (9.3)	70.3 (14.4)	**< 0.001**
Median CSA at rest	62.1 (14.6)	55.3 (9.9)	72 (14.9)	**< 0.001**
Proximal CSA with passive force	61.1 (13.7)	55.4 (10.3)	69.5 (14)	**< 0.001**
Median CSA with passive force	60.50 (15.2)	53 (9.8)	71.3 (15.3)	**< 0.001**
Achilles tendon free length at rest	73.6 (14.6)	71.9 (11.3)	76.1 (18.5)	0.310
Achilles tendon free with passive force	91.6 (19.8)	90.2 (14.5)	93.6 (25.9)	0.543
Angle pen at rest	13.1 (2.9)	13.2 (2.8)	13 (3.1)	0.791
Angle pen with passive force	10.6 (2.6)	10.7 (2.5)	10.4 (2.9)	0.758
Foot Posture and Gait Parametres				
FPI	5.8 (3.1)	5.5 (2.7)	6.3 (3.5)	0.321
Lunge test	38.5 (5.4)	39.4 (5.4)	37.2 (5.2)	0.139
FD MAX gait	13.3 (3.1)	13.9 (2.7)	12.4 (3.5)	0.091
FD RoM gait	18.5 (3.2)	19.4 (3.1)	17.2 (2.9)	**0.01**

CSA: cross sectional area; FPI: Foot posture index; FD MAX: maximal dorsiflexion; FD RoM: Joint Range of Motion during gait.

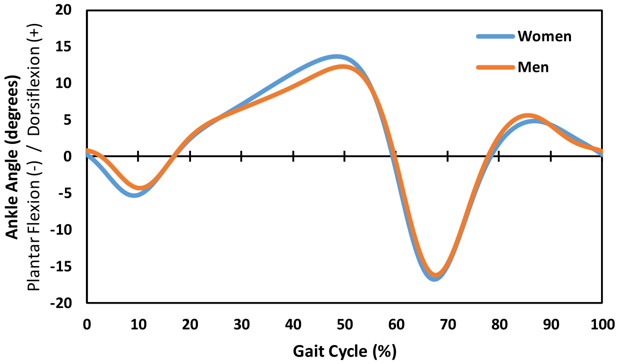

Fig. 2. Comparison between groups of ensemble mean of ankle kinematic during the gait cycle.

and, in terms of mechanical properties, more joint stiffness in the RoM of dorsal flexion of the ankle (women: 19.4°; Men: 17.2°; $p = 0.01$) (Fig. 1).

Of the 54 Achilles tendons analyzed, the women demonstrated statistically significant lower proximal and medial thickness both at rest ($p < 0.001$ for proximal thickness; $p < 0.001$ for medial thickness) as well as during maximal eccentric contraction ($p = 0.001$ for proximal thickness; $p < 0.001$ for medial thickness). It is argued that these anatomical differences in tendon structure properties may be due to physical function [8,11,15] and/or tissue adaptations to the subjected load [25].

The scientific literature describes that there is a positive correlation between muscle strength, maximal strength capacity and maximal power [1–3,25–27]. This could explain the anatomical and mechanical differences in gender given that individuals with greater muscle mass have greater connective tissue volume [27,28].

Blackburn et al. [29]assessed triceps sural plantarflexion strength and active ankle joint stiffness with a loading device between men and women. Apart from clear anthropometric differences (height, weight and BMI), men possessed greater muscle mass than women and therefore the structural stiffness and material modulus (the ratio of stress to strain) was higher in men than in women [29]. Higher force capacity as a function of muscle mass volume will be important in the active endurance capacity of the muscle-tendon unit [27,30]. Less movement in the RoM of dorsal ankle flexion may generate less stress-strain as our findings show. The difference in free AT was 18.3 mm for women versus men's free AT 15.5 mm; and proximal CSA was 3mm2 versus 0.8 mm2, with the women obtaining more deformation between rest and maximal passive force versus the men. Studies that investigated [9,15,31] gender-related differences in tendon properties under load are in line with our findings since they came to the same conclusion that there is a greater elongation/deformation in the tendon of females. There are morphological changes on ultrasound if we compare the tendon at rest and when in maximal dorsal flexion, showing less tendon stiffness, higher degrees of dorsal flexion in gait, compared to men. On the other hand, lower resting CSA was observed for proximal (52.4mm2 vs. 70.3mm2 with $p < 0.001$) and medial (62.1mm2 vs. 72mm2 with $p < 0.001$) slices of females vs. males. There are correlations between muscle-tendon properties and reproductive hormones [32]. Generally, men present higher levels of testosterone, which leads to greater muscle hypertrophy due to the anabolic stimulation of the muscle [30]. This muscle hypertrophy leads to tissue adaptation [33], and hence an increase in tendon thickness and CSA. Women have higher levels of estrogen, a hormone related to collagen synthesis. Higher quality collagen implies more compact and stiffer tissues that transmit force more quickly with greater elasticity and lower dissipated energy loss [15,32]. However, Lemoine et al. in their histological study observed that women had significantly lower dry mass per tendon wet weight (37.6 ± 0.9% vs. 34.3 ± 0.5% dry mass) and a strong trend toward less collagen per tendon wet weight (33.9 ± 1.4% and 30.6 ± 1.1% collagen for men and women, respectively, $P = 0.08$). Females may synthesize less tendon material overall per tendon size, resulting in lower tendon dry mass and collagen content.

The strength of our study is the measurement protocol carried out which ensures the validity and reliability of the ultrasound measurements in living individuals.

Clinical implications. Our study contributes that gender should be considered in the clinical management of AT pathology given that there are significant differences with respect to the difference in CSA, thickness and greater ankle dorsiflexion range of motion during the stance phase of gait in the physically active, healthy population, with a normal BMI and IPF, ruling out the aging factor and collagen alterations that would be observed with age.

Limitations of the study. Categorization by age range was not performed. Future research is needed to broaden the age range, as well as the sports activity performed, and even to be able to make a comparative control of the diet.

5. Conclusion

The results obtained from this study show that the biomechanical properties and biological behaviour of the Achilles tendon are not the same in men and women. There are differences in the sonoanatomical characteristics of the Achilles tendon both at rest and at maximum passive force. The female gender presents a greater strain deformation of the Achilles tendon in the ROM of dorsal flexion of gait.

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Author contributions

Raquel Alabau-Dasi and Gabriel Dominguez-Maldonado contributed to the conception of this study. Gabriel Gijon-Nogueron and Ana Belen Ortega-Avila did the statistical analysis. Data collectors were collected by Raquel Alabau-Dasi and Sebastian Delacroix. Raquel Alabu-Dasi, Gabriel Dominguez-Maldonado, Ana Belen Ortega-Avila and Gabriel Gijon-Nogueron were involved in the writing all in the review of the manuscript.

Ethical approval

The study protocol complied with the established ethical principles for human research. Written informed consent for participation and publication was given by each patient. The Institutional Review Board approved the study protocol: this was also approved by the Ethics Committee, Nº. 144-2021-H. All patients agreed to participate by signing an informed consent form.

Declaration of competing interest

All the authors declare that they have no conflict of interest derived from the outcomes of this study.

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