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RELIABILITY AND RELATIONSHIPS BETWEEN PLANTAR FLEXOR MUSCULOTENDINOUS UNIT ASSESSMENTS: ULTRASONOGRAPHY, MYOTONOGRAPHY, AND ISOMETRIC STRENGTH

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ABSTRACT

Objectives: The objective of this study was to determine the test-retest reliability of gastrocnemius muscle thickness and stiffness measurements, maximal isometric force using seated and standing plantar flexion, and Achilles tendon length, thickness, and stiffness. Additionally, we aimed to assess the correlations between musculotendinous unit properties and unilateral isometric force assessments of the plantar flexor muscles.

Methods: Twenty physically active males (23.79 ± 3.64 years, 175.56 ± 9.63 cm, and 83.41 ± 16.09 kg) completed the study. Participants underwent anthropometric assessments, ultrasound imaging for gastrocnemius muscle thickness and Achilles tendon properties, and myotonography for mechanical tissue characteristics. Unilateral standing and seated isometric plantar flexion tests were conducted using a force plate. Reliability was determined using intraclass correlation coefficients ($ICC_{3,k}$) and coefficients of variation (CV). Stepwise regression assessed relationships between musculotendinous properties and isometric peak force.

Results: Achilles tendon length, calf muscle thickness, and calf circumference exhibited excellent reliability (ICC >0.9), while Achilles tendon thickness (ICC = 0.89), calf echo intensity (ICC = 0.83), and seated isometric peak force (ICC = 0.93) demonstrated good reliability. Standing isometric peak force (ICC = 0.83) also showed good reliability, while calf stiffness (ICC = 0.96), calf frequency (ICC = 0.97), and Achilles tendon stiffness (ICC = 0.78) exhibited good to excellent reliability. Calf decrement correlated with standing peak force (R^2 = 0.23, p = 0.018), while Achilles decrement was associated with gastrocnemius muscle thickness (R^2 = 0.374, p = 0.004).

Conclusion: Achilles tendon length, gastrocnemius muscle thickness, and stiffness are reliable for plantar flexor assessment. Relationships between muscle-tendon properties and force production highlight their structural influence. Standardized protocols are recommended for improved consistency.

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KEYWORDS
Plantar flexor, muscle thickness, Achilles tendon, reliability, isometric force

INTRODUCTION

Plantar flexor muscles are important for forward movement and support during walking in humans[1]. The triceps surae muscle group comprised of the soleus, the two-headed (medial and lateral) gastrocnemius and the plantaris muscles, play a crucial role in locomotion. Even though the triceps surae muscles are relatively small compared to the hip musculature, they are essential during accelerating the trunk movement forward and backwards, as well as, walking, running, and climbing stairs[2]. Conversely, limited plantar flexion function has been associated with decreased walking speed and mobility, as well as deficits in calf muscle endurance and strength in young adults who have experienced Achilles tendon injuries[3].

Skeletal muscle strength is influenced by muscle mass and architecture. Muscle thickness, often measured as the distance between fascial layers, correlates with cross-sectional area and strength, though relationships are inconsistent[4]. However, previous studies have shown varying degrees of correlation between muscle thickness and strength measures. Inami et al.[5] examined the relationship between isometric exercise of the ankle plantar flexors and gastrocnemius muscle thickness at four different intensities (25, 50, 75, and 100%), observing no correlation at rest but a significant correlation ($R^2 = 0.68$, $p < 0.05$) at 100% intensity. Similarly, Drazan et al.[6] reported that gastrocnemius muscle thickness measured via B-mode ultrasound was positively, but weakly, correlated with peak torque and isometric max ($R^2 = 0.161$). These low correlation coefficients suggest that muscle thickness alone may not fully explain variations in muscle strength.

Isometric assessments offer an opportunity to evaluate the functional capacity of specific muscle groups, such as the plantar flexors[5]. While the isometric mid-thigh pull provides a general measure of lower-body strength, it does not isolate plantar flexors. More specific dynamometer and force plate methods have been developed to quantify isometric plantar flexion in standing or seated positions[7].

Recent advances in ultrasound and myotonography have enabled assessment of structural and mechanical properties of muscle and tendon in vivo. Myotonography applies brief mechanical impulses to quantify tissue stiffness, elasticity, and tone, with good reliability in muscle stiffness and elasticity measures[8]. Muscle stiffness reflects resistance to deformation and has been linked to reduced range of motion and impaired force generation[8]. However, the reliability of these newer measures for plantar flexor musculotendinous properties, and their relationship to isometric force, remains unclear.

Therefore, the purpose of this study was to determine the test-retest reliability of the gastrocnemius muscle thickness and stiffness measurements, maximal isometric force using seated and standing plantarflexion, and Achilles tendon length, thickness, and stiffness. Additionally, we aimed to assess the correlations between musculotendinous unit properties and unilateral isometric force assessments of the plantar flexor muscles.

METHODS

Participants

Twenty-two physically active males between the ages of 18-30 years (age: 23.79 ± 3.64; height: 176.31 ± 9.80 cm; weight: 83.1 ± 16.1 kg; body fat: 17.31 ± 7.84%) were recruited to participate in this muscle damage study. However, two participants dropped out after the first visit due to scheduling conflicts, leaving 20 participants who completed the study. Only males were included to limit confounding factors such as hormonal fluctuations associated with the menstrual cycle, which can influence exercise-induced muscle damage (EIMD)[9]. All participants were required to be recreationally active (defined as participating in exercise at least 3 times per week), free of physical limitations that would prevent them from performing any assessments, and answer “no” to all questions on the Physical Activity Readiness Questionnaire (PAR-Q+). All methods were approved by the institutional review board for human subects safety and all participants signed a document of informed consent prior to any assessments.

Sample size estimation

A priori sample size estimation using power analysis software (G*Power 3.1.9.4, HHU, Dusseldorf, Germany) revealed that for a two-tail correlation t-test, with power of 0.80, p-value of 0.05, and an effect size (f) of 0.56 derived from a similar study[6] using gastrocnemius muscle thickness and plantar flexion torque, the minimum necessary sample size was approximately 19 participants. Additionally, sample size analysis estimated that for an intraclass correlation coefficient between 0.6 and 0.7, a minimum of 15 participants is needed to achieve a power of 0.80-0.90,with an α-value of 0.05[10].

PROCEDURES

Anthropometric measurements

Height and weight were assessed using a stadiometer and scale (Health-o-meter Professional Patient Weighing Scale, Model 500 KL, Pelstar, Alsip, IL, USA) measured to the nearest one hundredth. Body composition was assessed using bioelectrical impedance analysis (BIA; InBody 770,

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Biospace Co, Ltd. Seoul, Korea) and estimated values for body fat percentage were evaluated.

Muscle thickness and echo intesnity

Portable B-mode Ultrasound (GE Logiq e BT12, GE Healthcare, Milwaukee, WI, USA) with a multi-frequency linear- array probe (12 L-RS, 5–13 MHz, 38.4-mm field of view, GE Healthcare, Milwaukee, WI, USA) was used to determine gatronemius muscle thickness of the randomized leg. Ultrasound gel was applied on the measurement location at 70% of the distance between the medial point of the knee joint space and the central point of the medial malleolus, as described by Yanagisawa et al.[11]. The probe was held perpendicular to the skin without applying excessive pressure. Ultrasound settings were standardized for all measurements: gain at 45 dB, depth at 3.5 cm, and frequency at 10.0 MHz. The highest and mean values of 3-5 muscle thickness measurements (in centimeters) of the randomized leg were recorded for each assessment *(Fig. 1)*. The images were saved for later analysis.

Echo intensity was evaluated using greyscale analysis on an arbitrary scale of 0 (black) to 255 (white) using Image-J software (version 1.8.0, National Institute of Health, Bethesda, MD, USA). A single trained technician, blinded to the participants' numbers and visit order, analyzed echo intensity of all images. The region of interest (ROI) was carefully traced to include the entire muscle area, avoiding bone and surrounding fascia, following the protocol suggested by previous studies[12]. ROI was standardized across participants by maintaining consistent ultrasound probe positioning and a skin mark was applied and reapplied at 70% of the distance to ensure precise probe placement for each measurement, following established protocols. Consistent settings were used to minimize variability due to probe tilt or other factors.

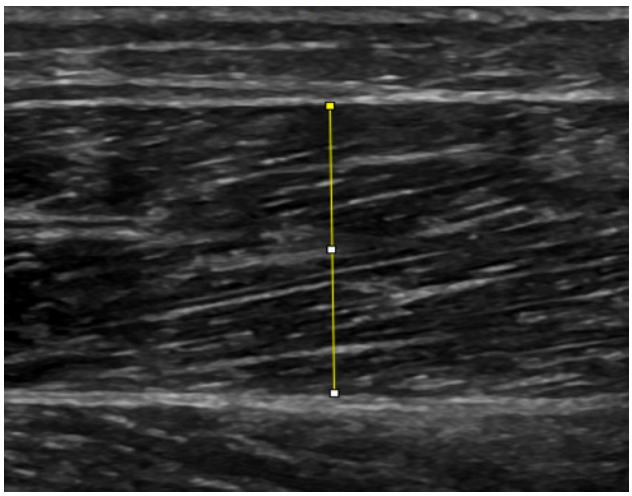


Figure 1. *Ultrasound image: gastrocnemius muscle thickness.*

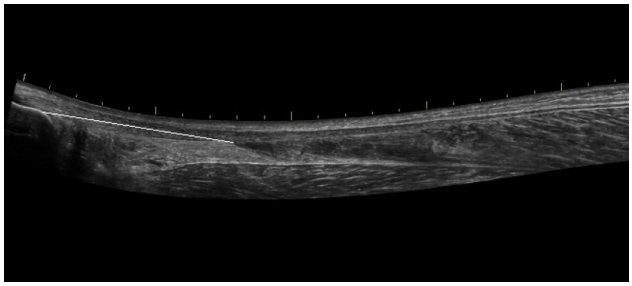


Figure 2. *Ultrasound image: Achilles tendon length.*

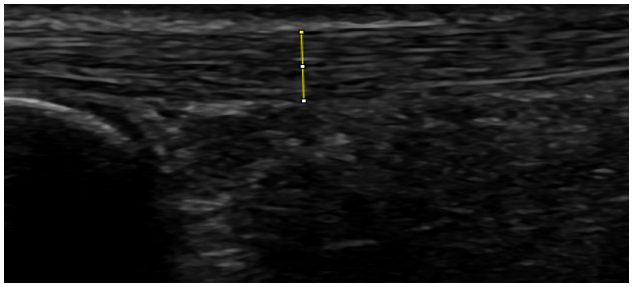


Figure 3. *Ultrasound image: Achilles tendon thickness.*

Tendon length and thickness

As described by Zallers et al.[13], participants were lying prone with their knee passively flexed at 90 degrees. Tendon length (in centimeters) was measured from the calcaneal insertion to the gastrocnemius myotendinous junction using extended field-of-view ultrasound imaging[13]. The ultrasound settings were standardized: gain at 45 dB, depth at 2.5 cm, frequency at 10.0 MHz. The foot was positioned at 90 degrees to control for foot positioning, as variations can influence tendon measurements[14] *(Fig. 2)*.

Prior to the assessment, participants rested for approximately 10 minutes in order to account for potential fluid shifts. The Achilles tendon was scanned using a B-mode ultrasound and a multi-frequency linear-array probe. The same researcher performed the assessments. All ultrasound imaging analyses were performed using Image-J software. Achilles tendon length was determined using the segmented line function between calcaneal notch and muscle-tendon junction along the midline of the Achilles tendon[15]. Additionally, sagittal images of the Achilles tendon were collected with the ankle passively dorsiflexed to approximately 90 degrees. Tendon thickness was measured at a reference point 2 cm proximal to the superior aspect of the calcaneus[16] *(Fig. 3)*.

Mechanical tissue and achilles tendon properties

The mechanical properties of tissue stiffness in the randomly selected leg were assessed using a MyotonPRO device (Myoton AS, Tallinn, Estonia).

As described by Garcia-Bernal et al.[17], the probe of the device was placed on the gastrocnemius muscle at 70% of the length between the head of the fibula and the lateral malleolus. The device applied a brief impulse to the muscle lasting 15 milliseconds, with a mechanical force of 0.6 N and the movement of the muscle was evaluated as oscillatory decay. The results of the measurement included mechanical stiffness of the tissue expressed in Newtons per meter (N/m) indicating the tissue's resistance to deformation, the oscillation frequency (or muscle tone) in Hertz (Hz) representing the tissue's natural tension, the logarithmic

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decrement indicating elasticity (D) and reflecting tissue elasticity through the rate at which oscillations diminish, the mechanical stress relaxation time (R) measuring how quickly the tissue returns to its original position after displacement, and the ratio of relaxation to deformation time (C) which describes the gradual lengthening of tissues under sustained tensile stress.

Participants rested for approximately 10 minutes prior to the assessment to account for potential fluid shifts. All measurements were taken with the participant in a relaxed prone position, and the same researcher performed all assessments to ensure consistency.

A unilateral isometric standing and seated plantarflexion

As described by Mattiussi et al.[18], during the standing isometric plantarflexion *(Fig. 4)* assessment, participants were instructed to adopt a knee position of 170-180 degrees and a hip position of 170-180 degrees. The measurement of body positions involved the use of goniometer prior to the beginning of the test with the fulcrum of the goniometer aligned over the greater trochanter and the stabilization arm aligned with the glenohumeral joint in order to ensure uniform starting position. For the ankle, the measurements were taken at approximately 130 degrees of plantarflexion, with 90 degrees being a baseline for neutrality. Participants were instructed to place their arms over their across their shoulders in order to avoid assistance from the upper limbs. In order to ensure consistency of the measurements, participants were asked to wear the same type of athletic shoes for each visit. Participants placed the ball of their foot directly under the barbell with the opposite limb positioned at 90 degrees of hip flexion in order to ensure neutral hip stance.

In the seated isometric plantarflexion *(Fig. 5)* assessment, the barbell was positioned approximately two-thirds of the way along the length of the thigh, just above the knee the knee on the quadriceps, while participants maintained a seated position with one knee bent at 90 degrees while the other knee o (non-exerecising leg) was relaxed and out of the way. The techniques for measuring knee and hip angles were the same as those used in the standing plantarflexion protocol, while the non-exercising limb was kept off the force platform to avoid any unintentional support.

Prior to testing, participants underwent a familiarization session to practice the isometric contractions and ensure proper technique. They performed two submaximal trials at 50% and 70% of their maximal effort before attempting maximal efforts. This was intended to minimize learning effects and improve the reliability of the measurements.

The exercises were conducted on a limb chosen at random. Participants performed two sets of maximal isometric muscle actions, each lasting 5 seconds. Between each 5-second muscle action, there was a 20-second rest period. A 2-minute recovery period was observed between the standing and seated assessments. Seated and standing plantarflexion were executed on a portable force plate (Accupower; AMTI, Watertown, MA) using a sampling rate of 1,000 Hz. Isometric peak force and rate of force development were recorded (AccuPower Software 4.0, ACCUPOWER SOLUTIONS, Dickinson, ND, USA)[19] and normalized to body mass (N/kg and N/second/kg).



Figure 4. *Standing isometric plantarflexion.*



Figure 5. *Seated isometric plantarflexion.*

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Statistical analysis

Descriptive statistics were calculated to determine group demographics. Test-retest reliability values were calculated using the two-way mixed intraclass correlation coefficient ($ICC_{3,k}$) with 95% confidence intervals (CI) to determine relative reliability and the coefficient of variation (CV) for absolute reliability. Relative reliability was classified based on the lower bound of the CI as poor (ICC <0.5), moderate (0.5 ≤ ICC < 0.75), good (0.75 ≤ ICC < 0.9), and excellent (ICC ≥0.9)[20]. Individual CV values were calculated for each participant using the data from visits 1 and 2 with ranges interpreted as low variability (<10%), moderate variability (10–20%), and high variability (>20%)[21].

Normality of the data was assessed using the Shapiro-Wilk test. If the data were normally distributed, paired samples t-tests were utilized to compare variables between visit 1 and visit 2. If normality was violated, non-parametric tests (e.g., Wilcoxon signed-rank test) were used.

Exploratory stepwise regression was performed to assess the relationships between calf muscle thickness, Achilles tendon length, Achilles tendon thickness, and isometric peak force in seated and standing positions. Explanatory myotonography variables, including calf frequency, stiffness, decrement, relaxation, and creep, were included in the analysis only if they met predetermined thresholds for reliability (good to excellent) and variability (low to moderate). For each outcome variable, only the single independent variable that demonstrated the most significant association with it was retained in the final model.

Statistical significance was set at p <0.05. Data are presented as mean ± SD unless otherwise stated. The calculations were performed using JASP (Version 0.15.0.0, University of Amsterdam, Netherlands), and custom Excel spreadsheets (Microsoft, Redmond, WA) were used to compute minimal detectable change (MD), and standard error of measurement (SEM) values.

RESULTS

Achilles tendon and calf measurements

Detailed results for the Achilles tendon and calf measurements, including ICC and CV values, are in *Table 1*. The measurements for Achilles tendon length, calf muscle thickness, and calf circumference showed *excellent* relative reliability, with the lower bound of ICCs being greater than 0.9 while Achilles tendon thickness showed *good* relative reliability. Absolute reliability evaluation exhibited *low* variability for calf circumference (8.7%), *moderate* variability for Achilles tendon length (16.28%) and thickness (15.15%), and *high* variability for calf thickness (24.84%).

Calf echo intensity exhibited good relative reliability but *high* variability with a CV of 30.2%. Achilles tendon echo intensity showed poor relative reliability, with the lower bound of the ICC being less than 0.5 and *high* variability (29.74%).

Calf endurance and isometric force

Detailed results for calf endurance, isometric force, and rate of force development, including ICC and CI values, are presented in *Table 1*. The measurements for standing and seated isometric peak force demonstrated *moderate* and *good* relative reliability, respectively. Absolute reliability evaluation showed *high* variability for both standing (20.3%) and seated (36.56%) peak force. The number of repetitions performed during calf endurance testing demonstrated *good* relative reliability, but absolute reliability evaluation resulted in *high* variability for the number of repetitions

Variable	Visit 1 mean ± SD	Visit 2 mean ± SD	t	p value	$ICC_{3,k}$	Lower 95% CI	Upper 95% CI	SEM	MD
AT length (cm)	8.25 ± 1.40	8.15 ± 1.27	1.65	0.11	0.99	0.97	0.99	0.18	0.52
AT thickness (cm)	0.50 ± 0.08	0.49 ± 0.07	0.74	0.76	0.89	0.76	0.94	0.02	0.07
Calf thickness (cm)	2.08 ± 0.49	2.09 ± 0.54	0.56	0.57	0.98	0.97	0.99	0.08	0.22
Calf circumference (cm)	37.92 ± 3.36	37.95 ± 3.23	1.26	0.94	0.97	0.94	0.98	0.73	2.03
Calf echo intensity (au)	35.83 ± 11.10	38.32 ± 11.31	-1.75	0.09	0.83	0.62	0.92	4.53	12.51
Achilles echo intensity (au)	42.63 ± 16.62	43.20 ± 8.71	-0.19	0.85	0.59	0.04	0.72	10.1	28.10
Standing isometric peak force (N)	1857.32 ± 406.08	2031.10± 380.17	-2.19	0.04	0.83	0.63	0.91	213.71	592.35
Seated isometric peak force (N)	730.85 ± 270.94	795.31± 286.68	-1.45	0.16	0.93	0.84	0.96	103.13	286.00
Number of repetitions	40.45 ± 11.06	49.65± 13.4	-5.77	<.001	0.86	0.72	0.92	4.60	12.72

Table 1. *Average Achilles tendon length, Achilles tendon thickness, calf muscle thickness, calf circumference, calf endurance, and standing and seated isometric values for visit 1 and 2.*

Abbreviation: cm, centimeters; au, arbitrary units; N, newtons; SD, standard deviation; t, t-test; $ICC_{3,k}$, intraclass correlation coefficient for consistency; CI, confidence interval; SEM, standard error of measurement; MD, minimal detectable difference.

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Variable	Visit 1 mean ± SD	Visit 2 mean ± SD	t	p value	$ICC_{3,k}$	Lower 95% CI	Upper 95% CI	SEM	MD
Calf frequency (Hz)	16.99 ± 2.17	17.37 ± 1.92	-2.23	0.03	0.97	0.97	0.94	0.47	1.30
Calf stiffness (N/m)	332.68 ± 64.21	339.30 ± 56.54	-1.53	0.14	0.96	0.95	0.92	16.60	46.13
Calf decrement (au)	0.92 ± 0.13	0.90 ± 0.13	1.78	0.09	0.97	0.97	0.94	0.03	0.08
Calf relaxation (ms)	16.55 ± 2.63	15.92 ± 2.15	2.69	0.01	0.93	0.93	0.86	0.84	2.32
Calf creep (au)	1.04 ± 0.16	0.99 ± 0.13	2.81	0.01	0.93	0.93	0.85	0.05	0.14
Achilles frequency (Hz)	33.85 ± 4.53	33.57 ± 4.61	0.89	0.38	0.84	0.84	0.66	2.43	6.73
Achilles stiffness (N/m)	1067.78 ± 191.39	1063.72 ± 199.36	0.73	0.46	0.78	0.78	0.55	117.22	325.12
Achilles decrement (au)	0.98 ± 0.11	1.01 ± 0.16	-0.92	0.37	0.46	-0.14	0.74	0.12	0.32
Achilles relaxation (ms)	6.65 ± 0.66	6.71 ± 0.71	-1.03	0.31	0.82	0.58	0.91	0.39	1.09
Achilles creep (au)	0.55 ± 0.04	0.87 ± 1.39	-1.04	0.31	0.03	-1.03	0.54	0.97	2.71

Table 2. *Average calf and Achilles mechanical property values for visit 1 and 2.*
Abbreviations: Hz, oscillation frequency in hertz; N/m, newtons per meter; ms, milliseconds; au, arbitrary units; SD, standard deviation; t, t-test; $ICC_{3,k}$, intraclass correlation coefficient for consistency; CI, confidence interval; SEM, standard error of measurement; MD, minimal detectable difference.

(27.16%). Notably, there was a significant increase in the number of repetitions performed during calf endurance testing from visit 1 to visit 2 ($p < 0.001$), indicating a possible learning effect or improvement due to familiarization with the test protocol.

Calf and achilles mechanical properties

Detailed results for the calf and Achilles tendon mechanical properties, including ICC and CI values, can be found in *Table 2*. The measurements for mechanical properties of the calf muscle, including frequency, stiffness, decrement, and relaxation, showed *excellent* relative reliability with lower bound of ICC being greater than 0.9. Absolute reliability was *moderate* for calf frequency (11.95%), stiffness (17.98%), decrement (14.34%), and relaxation (14.64%). Achilles tendon mechanical properties showed *good* relative reliability for frequency and stiffness, while Achilles tendon decrement and creep exhibited *moderate* relative reliability. Absolute reliability evaluation exhibited *moderate* variability for Achilles tendon frequency (13.51%), stiffness (18.33%), decrement (13.09%), relaxation (10.18%), and *high* variability for creep (83.52%).

Exploratory regression

Stepwise regression revealed that calf decrement was the independent variable most associated with standing peak force ($\beta = 0.52$, $p = 0.018$), accounting for 23% of the variance ($R^2 = 0.23$). Calf frequency was the independent variable most associated with Achilles tendon thickness ($\beta = 0.53$, $p = 0.017$), accounting for 27.6% of the variance ($R^2 = 0.276$). Finally, Achilles decrement was the independent variable most associated with calf muscle thickness ($\beta = 0.61$, $p = 0.004$), accounting for 37.4% of the variance ($R^2 = 0.374$). None of the exploratory myotonography variables were associated with Achilles tendon length or sitting peak force.

DISCUSSION

This study demonstrated good to excellent reliability for several measures of plantar flexor musculotendinous properties, particularly Achilles tendon length, calf muscle thickness, calf circumference, and mechanical properties such as frequency and stiffness. These findings support their use in both research and applied settings for monitoring plantar flexor structure and function.

In contrast, Achilles tendon thickness and calf echo intensity showed only moderate reliability, with higher variability. Achilles tendon echo intensity, in particular, demonstrated poor reliability, likely due to sensitivity to probe tilt and operator error, as noted by Dankel et al.[22]. Small deviations from perpendicular alignment can substantially alter echo intensity, emphasizing the need for strict standardization if EI is to be used in tendon assessments. The present results align with prior work showing high reliability for tendon length and muscle thickness when body position is controlled[17,23]. Methodological differences in ankle position or joint support likely explain some variability across studies. Similarly, our excellent reliability for calf muscle thickness and circumference is consistent with prior research in both young and older populations[24]. Together, these findings reinforce the importance of consistent positioning and measurement protocols to maximize reliability.

Myotonography proved highly reliable for muscle stiffness

and frequency, consistent with previous reports[25]. However, Achilles decrement and creep demonstrated only moderate reliability, echoing systematic reviews that highlight stiffness as the most stable parameter, while other indices show greater variability[8,26]. These results suggest stiffness and tone may be the most useful mechanical markers of plantar flexor muscle and tendon properties, whereas decrement and creep require further refinement.

Functional measures showed mixed reliability. Standing isometric peak force demonstrated good reliability, whereas seated isometric peak force displayed higher variability despite a strong ICC. The higher coefficient of variation for the seated condition suggests that stabilization demands or limited participant familiarity may have influenced performance consistency. This contrasts with Mattiussi et al.[18], where trained participants (e.g., dancers) showed lower variability across conditions, highlighting the influence of training status and task familiarity on measurement error. Similarly, the heel raise endurance test demonstrated good relative reliability but a clear learning effect, with significantly more repetitions performed on the second visit. Future protocols should include additional familiarization sessions to mitigate this effect.

Exploratory regression analysis revealed that calf decrement was associated with standing isometric force, suggesting that elastic and damping properties of the muscle contribute to postural force production. In contrast, no associations were observed for seated force, likely because external support reduces reliance on elastic energy transfer and balance control. Additional associations between calf frequency and Achilles tendon thickness, and between Achilles decrement and calf muscle thickness, suggest potential interactions between resting muscle tone, tendon adaptation, and muscle morphology. These exploratory findings should be interpreted cautiously but provide a basis for future research on the interplay between mechanical properties and force production.

LIMITATIONS

The sample was limited to young, recreationally active males, reducing generalizability to females, older adults, or clinical populations. Variability in seated force testing and the observed learning effects highlight the need for additional familiarization. Finally, while regression analyses identified associations between mechanical and structural variables, the cross-sectional design prevents causal interpretation.

CONCLUSION

This study supports the use of Achilles tendon length, calf muscle thickness, calf circumference, and certain calf mechanical properties, such as frequency and stiffness, as reliable measures for assessing the plantar flexors muscles. Standing isometric peak force showed good reliability, while seated isometric peak force exhibited higher variability, likely due to differing stabilization demands and participant familiarity with the assessments. The identified relationships between muscle-tendon unit size and mechanical properties suggest that specific muscle and tendon characteristics may differentially impact force production and structural adaptation.

These findings contribute to the existing literature by identifying reliable assessment methods for the plantar flexor musculotendinous unit. Practitioners and researchers should consider using measures that demonstrated good to excellent reliability, such as Achilles tendon length, calf muscle thickness, calf circumference, and mechanical properties like stiffness and frequency, in their evaluations. Caution is advised when interpreting mesurements with higher variability, such as Achilles tendon echo intensity and decrement. Standardized protocols, detailed instructions, and enhanced participant familiarization with isometric assessments are recommended to improve measurement consistency.

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