

Supplementary File 1. Detailed Measurement and Data-Processing Procedures

Achilles tendon thickness and stiffness

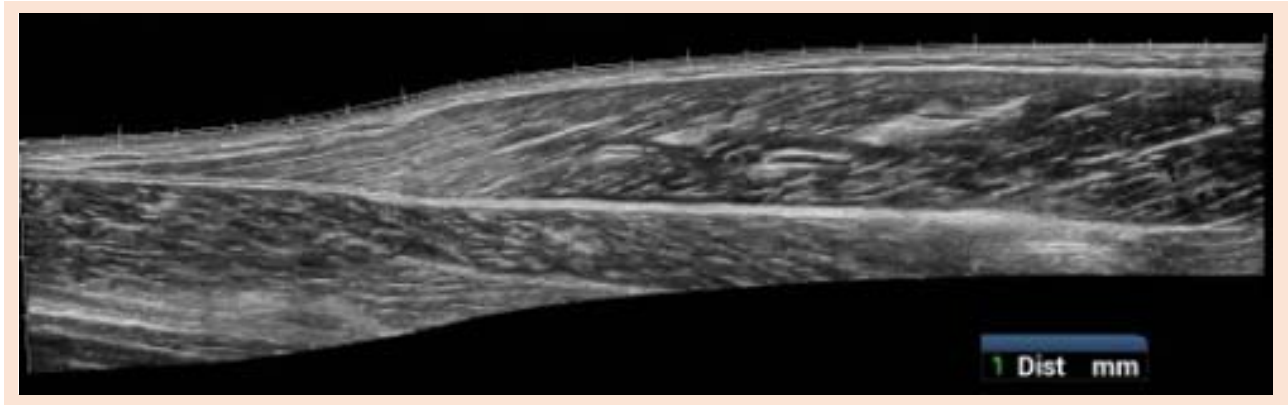
Ultrasound measurements were executed using an ultrasound device (Resona 7, Mindray, China). While performing ultrasound measurements, the participants were lying in a prone position with their ankle over the edge of the therapeutic table. All measurements were carried out using the L11-3U ultrasound probe, with ultrasound set to musculoskeletal mode. All images were taken in a longitudinal plane. In the case of the Achilles tendon, we measured the tendon thickness and stiffness (three measurements for each parameter). They were measured approximately five cm above the insertion of the Achilles tendon on the heel, in accordance with the recommendations of previous studies (note the markings on Figure 1A) (Mifsud et al., 2023). Tendon thickness was manually determined using the distance function (Figure 1B). Meanwhile, the stiffness of the tendon was measured using the shear wave quantitative mode with a fixed region of interest measuring 3 mm x 3 mm, positioned within the tendon tissue (Figure 1C). Additional care was taken to maintain minimal probe pressure and a stable hand position to avoid artefacts. After allowing the elastography image to stabilise, eight consecutive shear wave images were acquired and automatically combined by the system to generate a median stiffness value for the region of interest.



Supplementary Figure S1. Markings for Achilles tendon measurements and gastrocnemius panoramic imaging (A); B-mode images of Achilles tendon, used to determine tendon thickness (B); Achilles tendon in shear-wave elastography mode with region of interest marked as white square (C).

Gastrocnemius muscle architecture

All muscle measurements were performed using iScope View mode, which allowed us to capture panoramic images of both the medial and lateral gastrocnemius. From these images, we manually determined the fascicle length, pennation angle, and muscle thickness (two measurements for each parameter) using distance and angle functions. An example of a panoramic image is shown in Figure 2. All panoramic images of both the medial and lateral gastrocnemius muscles were taken along the same line, as shown in Figure 1A. To ensure greater accuracy, all muscle scans were performed jointly by two researchers, with one conducting the measurement using the aforementioned ultrasound probe. At the same time, the other monitored the scan on the screen, supervised the measurement process and image quality, and recorded the obtained values.



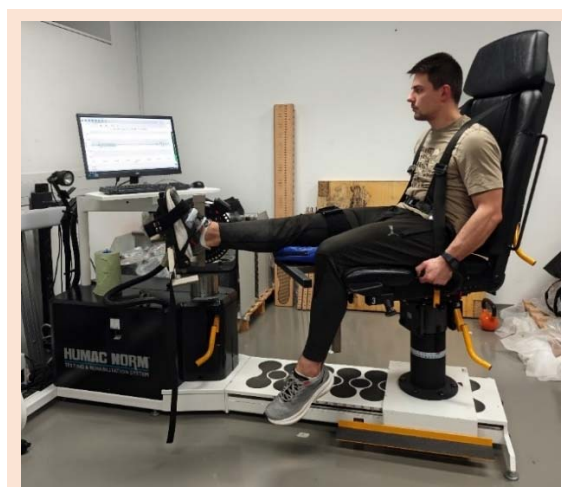
Supplementary Figure S2. An example of a panoramic image (medial gastrocnemius) used for muscle architecture measurements.

Open- and closed-chain ankle range of motion

Ankle range of motion (RoM) in the sagittal plane (ankle plantar and dorsiflexion) was measured using a manual goniometer. The measurement was taken in two different positions: lying in the supine position with the ankle over the edge of the therapeutic table (in an open kinetic chain) (Jakovljević and Hlebš, 2023) and standing on a higher surface (in a closed kinetic chain). In both cases, the goniometer axis was slightly distal to the medial malleolus while the stationary arm was parallel to the longitudinal axis of the tibia (towards the medial condyle of the tibia) and the movement arm directed towards the head of the first metatarsal bone, respectively (Jakovljević and Hlebš, 2023). To minimise errors, two researchers each performed the ankle mobility measurements independently.

Isokinetic dynamometer settings

The backrest of the isokinetic dynamometer (HumacNorm, Computer Sports Medicine Inc., Stoughton, MA, USA) was set at an angle of 80°, i.e., slightly tilted backwards – 10° from an upright sitting position in the direction of the trunk extension. The lumbar region of all participants was supported with an additional pad, since the dynamometer does not allow separate adjustment of the lumbar tilt. The aforementioned pad ensured that the lumbar region was slightly curved, which resulted in maintaining the natural arch of the lower back. Meanwhile, the participant's right leg was aligned with the dynamometer plantar support, with the knee slightly flexed and supported with additional pads (i.e., approximately 10-15°; to minimise any negative impact of a potentially uncomfortable position that could influence the results). The axis of rotation of the ankle was aligned with the axis of the dynamometer plantar support. The subjects were strapped across the shoulders, pelvis, distal part of the right thigh, and across the proximal and distal parts of the right foot, which was placed on the footrest. The participant's position and fixation in the dynamometer are shown in Figure 3. The position of the dynamometer was recorded for each subject to be repeated at post-measurement. Based on pilot testing, the fixations were modified to minimise the involvement of the knee extensors, knee flexors and gluteal muscles. Participants performed the protocol in sports shoes.



Supplementary Figure S3. Participant's position and fixation in the dynamometer.

Concentric, eccentric and isometric strength testing

Before the beginning of the strength measurements, all participants executed warm-up that consisted of three minutes of light jogging (on a treadmill) and four minutes of stepping onto a 10-centimeter-high platform (alternating with each leg

for 60 seconds, increasing the stepping velocity in the second series) followed by dynamic stretching exercises and ten repetitions of active strengthening exercises (i.e., jumping jacks, squats, push-ups, and toe raises with both feet without additional weight). After the participants finished with a warm-up, they moved to the dynamometer, where we initially adjusted the settings of the seat part of the dynamometer where they were strapped. Then an additional RoM assessment was carried out on the dynamometer, followed by the gravity effect correction in the neutral ankle position (i.e., 0°). The measurement protocol began with two warm-up sets that consisted of five isokinetic ankle plantar flexor repetitions (dynamometer was configured to isokinetic mode). The participants were instructed to perform the first set at ~50 % and the second set at ~70 - 80 % of maximal effort. The break between series lasted three minutes. Warm-up repetitions were followed by performing five consecutive maximal voluntary consecutive concentric and eccentric (ECC) contractions of the ankle plantar flexors. All mentioned repetitions were performed at a movement velocity of 60 °/s. Three minutes after the concentric and ECC maximal voluntary contraction (MVC) measurement, the dynamometer was configured to isometric mode, so the participants were able to perform three additional maximal voluntary isometric ankle plantar flexor contractions in the neutral ankle position. The break between each repetition lasted 30 s. Participants received continuous real-time visual feedback in the form of a time-torque curve, and verbal encouragement was given during every repetition as shown in Figure 3. Between repetitions, the thigh and both foot straps were loosened and removed, and then securely fastened again before the next contraction.

Eccentric quasi-isometric testing on the isokinetic dynamometer

The dynamometer was then set to isotonic mode to provide constant external resistance in one movement direction, which in this case was ankle dorsiflexion, since after measuring isometric MVC, all participants performed three eccentric quasi-isometric (EQI) actions, with a three-minute break in between. The load they had to overcome during the EQI action was set at 75 % of the predetermined isometric MVC, based on the findings of a previous study (Križaj et al., 2025b). During the EQI actions, participants resisted the load through alternating phases of isometric holding and slow ECC lengthening. Each EQI action consisted of a single repetition that began at the participants' maximal dorsiflexion position. At the start of the contraction, the participants performed an ankle plantar flexor concentric burst against applied resistance, followed by holding the position and resisting the ECC contraction by maintaining the load for as long as possible. Once the participants could no longer generate enough torque to counter the dynamometer at that specific angle, the ankle was pulled into a slightly more dorsiflexed position. Due to the increased stretch of the ankle plantar flexors, this resulted in greater ankle plantar flexion torque capacity. Such a change enabled the participants to sustain the new position for a longer period, even though they had previously been unable to tolerate the load at a greater plantarflexed angle. The cycle of slow ECC lengthening and isometric holding continued until the participants reached their maximal dorsiflexion, predetermined before the first warm-up isokinetic contraction. Although the final ankle angle matched the participants' maximal voluntary dorsiflexion RoM, the total EQI action RoM was smaller than the predetermined total RoM. This occurred because the participants were unable to push the dynamometer plantar support through the full RoM against applied resistance, since isometric MVC, from which we calculated the load for the EQI action, was measured with the ankle in a neutral position (i.e., 0°), whereas muscle capacity decreases with greater shortening (in the direction of the ankle plantar flexion). Throughout all the EQI actions, participants received verbal encouragement and could also follow visual signals of the screed (the time-torque curve).

Pain and effort outcomes

During the interventions, all EQI and ECC group participants reported their pain level before the beginning of the protocol (delayed onset muscle soreness) and the effort level after the end of the exercise session, which we documented. Both outcomes were assessed on a 10-point numeric rating scale, where a higher value meant a higher level of pain or effort that the person felt. We also recorded the EQI exercise sessions, which we analysed in the Kinovea tool. By measuring the starting and final ankle angle in the sagittal plane and the time under tension of a single contraction, we calculated the angular velocity at which the participant performed the contraction. We repeated this for all repetitions within the exercise unit. We also documented the number of repetitions for each exercise session.

Processing of ultrasound, strength and EQI outcomes

Each ultrasound measurement was recorded, and during data processing, we only took the mean values of individual variables (thickness and stiffness of the tendon, and fascicle length, pennation angle and muscle thickness for the medial and lateral heads of the gastrocnemius). As mentioned above, ankle mobility measurements were carried out by two independent researchers. If the deviation for any value exceeded 3°, both researchers repeated the procedure. The average value measured by the researchers for each position was recorded. Meanwhile, signals of time, ankle position (ankle plantarflexion and dorsiflexion RoM), torque and angular velocity, obtained using an isokinetic dynamometer, were sampled at 100 Hz frequency and analysed without prior filtering (Oranchuk et al., 2021a). In case of maximal concentric, ECC and isometric MVC measurements, we considered the maximum torque of the best repetition as the only dependent variable. The start of EQI action was determined at the first local maximum for angle data (in ankle plantar flexion direction), while the end of contraction was determined when: 1) the end of RoM (the greatest ankle dorsiflexion position) was reached; or 2) when the torque values fell below 50 % of the specified threshold. Each EQI action was visually inspected to remove any artefacts that might affect the accurate identification of contraction onset or terminal. The analysis of EQI actions executed on isokinetic dynamometer focused on the following four primary outcomes: 1) time under tension (s); 2) total RoM (°); 3) total angular impulse (area under the torque-time curve, determined via trapezoidal

integration; Nm·s); and 4) average angular velocity (°/s). RoM was included as a dependent variable for the EQI actions to capture overall differences between repetitions, since fatigue from earlier trials might limit the participants' ability to reach the same initial plantarflexion angle at the concentric part of the EQI measurement. Data processing of strength, EQI and RoM outcomes data was performed using MATLAB software (version R2024a; The MathWorks Inc., Natick, MA, USA).

Supplementary File 2. Summary of pilot phase of the research

In the pilot phase (n = 8; 4 male and 4 female), within-between-subjects comparisons were used to determine the most appropriate load that would enable the highest quality eccentric quasi-isometric (EQI) contraction of ankle plantar flexors (slowly lowering from the heel raise position to the end of the range of motion [RoM]) on the Smith machine. The Smith machine was selected due to the fact that, outside laboratory settings (i.e., electromechanical isokinetic dynamometers), it enables a more accurate and controlled execution of EQI action than free weights or elastic bands.

Laboratory measurement protocol

All participants of the pilot study executed the entire strength measurement protocol described in the manuscript and Supplementary file 1, with the exception of the concentric and eccentric maximal voluntary contraction. The intensity used when executing EQI action dynamometer measurement was determined based on the findings of previous studies and was equal to 75 % of the predetermined isometric maximal voluntary contraction (Križaj et al., 2025a; 2025b). The dynamometer measurements were all for both legs separately, with a five-minute rest in between.

Smith machine measurement protocol

After one week, all participants executed the same warm-up protocol as during laboratory measurement, followed by three repetitions of unilateral EQI action on the Smith machine with each leg separately. Four subjects (two male and two female) performed the protocol at a load of predetermined unilateral one-repetition maximum (1 RM) with 20 - 30° of ankle plantar flexion limited RoM on one leg and without any ankle plantar flexion RoM limitations on the other leg. Whereas the others performed the task at a load of 80 % of the predetermined unilateral 1 RM on one leg and at a load of 110 % of unilateral 1 RM on the other leg. In the case of a second group, the 20 - 30° ankle plantar flexion RoM limit was used only at the 110 % 1 RM load. In all cases, the determined ankle position was achieved by bilateral heel rise. The distribution of the intensity between the left and right legs and the sequence of measurements were quasi-random, using a Latin square.

Table S1. Characteristics (load and range of motion limitation) of pilot study.

	First group (n = 4)		Second group (n = 4)	
	Lower limb one	Lower limb two	Lower limb one	Lower limb two
Load	100 % 1 RM	100 % 1 RM	80 % 1 RM	110 % 1 RM
APF limitation	20 - 30°	/	/	20 - 30°
ADF limitation	/	/	/	/

ADF – ankle dorsiflexion; APF – ankle plantar flexion; 1 RM – one-repetition maximum

Data processing

The data, obtained by EQI exercise using an isokinetic dynamometer, were, for the purpose of the comparison, processed in the same way as described in the manuscript and Supplementary file 1. Meanwhile, all Smith machine EQI exercises were videorecorded and analysed using the Kinovea tool (version 0.9.5). We then recorded the ankle RoM in the sagittal plane, the duration of each EQI action, and based on the data obtained, we also calculated the angular velocity of the movement. We compared the obtained data (i.e., ankle RoM, time under tension and angular velocity) for all three consecutive repetitions (within and between repetitions) among the two variations performed on an isokinetic dynamometer and a Smith machine.

Results of pilot testing

This procedure indicated that the most appropriate way to perform EQI action of ankle plantar flexors on the Smith machine to achieve optimal dynamics compared to EQI action performed using an isokinetic dynamometer is through the entire RoM, at an intensity equal to unilateral 1 RM.

Supplementary File 3. Detailed eccentric quasi-isometric and eccentric exercise protocols

Intervention protocols

After the baseline measurement, the participants were equally divided into one of three groups: the control group, the eccentric quasi-isometric (EQI) group and the eccentric (ECC) group.

Control group

Participants in the control group were told to continue their normal physical activity, but without any direct training that would affect changes in the ankle plantar flexors (i.e., exercises for direct strengthening or stretching of the ankle plantar flexors; plyometric exercise, etc.) until the execution of post-measurement, which was performed after seven weeks. Meanwhile, the EQI and ECC group participants had to start the exercise protocol five days after the baseline testing. In both cases, the exercise protocol lasted six weeks, with participants having to perform two exercise sessions per week, with at least a two-day break between them. All exercise sessions were supervised by researchers, who corrected the correctness of the movement task, recorded the repetitions and measured the time under tension and resting time (especially in the EQI group).

Intervention groups

Regardless of whether it was an exercise session for the EQI or ECC group, it began with the same warm-up protocol pre-described in the manuscript and Supplementary file 1 (Figure 1). Then, in both protocols, heel raise repetitions had to be performed on a Smith machine. The repetitions began in a standing position on a higher platform (or stepper) with an Olympic barbell (and additional weights) placed across the trapezius muscle. The subjects had the distal third of their feet (including the footpads) in contact with the higher platform. In the starting position, their knees had to be extended.

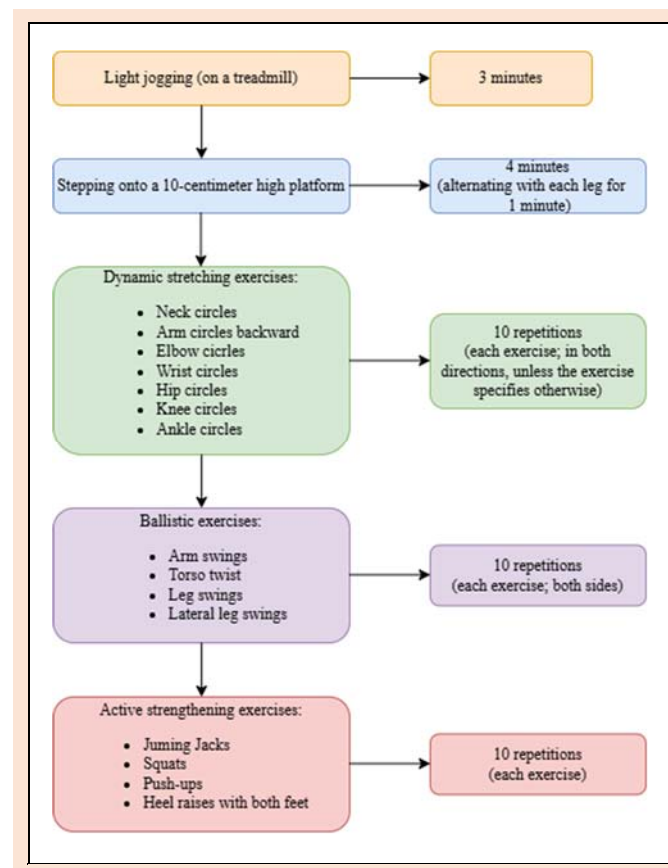


Figure S4. Warm-up chart.

Eccentric quasi-isometric group

In the EQI group, participants started the contraction by a bilateral heel raise to the highest position and then a flexion of the left hip (Figure 2A) and an attempt to hold the position statically on the right leg. During the repetition, the ankle gradually moved into an increasingly greater dorsal flexion, which the subjects resisted (Figure 2B). The repetition ended when the ankle, due to fatigue and the inability of the ankle plantar flexors to overcome the load, reached its final RoM (the participants touched the ground with their heel or began to overcome the load with the help of the other, passive leg) (Figure 2C). If participants were able to maintain the EQI action for a predetermined amount of time during a repetition, they completed the exercise set; however, if the time under tension was too short, they had to repeat it until they achieved a sufficient cumulative time under tension. The rest between each repetition in that case lasted three minutes.

Eccentric group

The participants in the ECC group started the contraction with a bilateral heel raise to the highest ankle plantarflexion position (Figure 2A), followed by a hip flexion of the left leg and three seconds of slow, controlled lowering of the right heel in the direction of ankle dorsal flexion (Figure 2B). The participants then bilaterally raised their heels again to the highest point and performed another three seconds of resisted lowering to the final RoM of the dorsal ankle flexion. They repeated these repetitions until they had completed a sufficient number of repetitions within the set. There were no breaks between repetitions, while the rest between sets lasted three minutes.

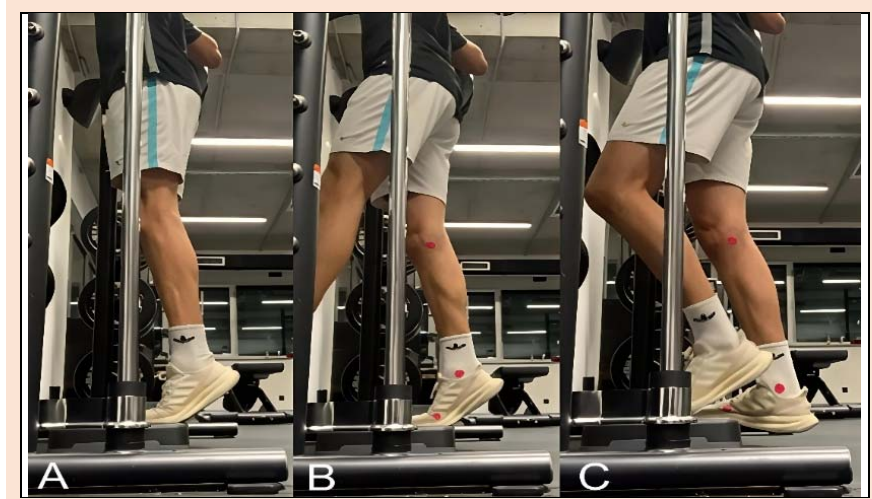


Figure S5. Bilateral heel rise on the Smith machine (A); the beginning of the unilateral EQI or ECC contraction on the Smith machine (B); the end of the unilateral EQI or ECC contraction on the Smith machine (C).

Load and time under tension characteristics of the protocols

Both intervention protocols consisted of 12 supervised sessions over six weeks, performed twice weekly, and were matched for load and time under tension (Table 3). The load corresponded to the individual's 1 RM and was re-assessed before sessions 1, 5, and 9; therefore, Load 1 was used in sessions 1 - 4, Load 2 in sessions 5 - 8, and Load 3 in sessions 9 - 12. Time under tension progressively increased from 90 s in sessions 1 - 2 to 135 s in sessions 3 - 6 and 180 s in sessions 7 - 12. In the EQI group, participants repeated contractions with two-minute breaks until the prescribed time under tension was achieved. In the ECC group, the corresponding volume was 2×15 repetitions in sessions 1 - 2, 3×15 repetitions in sessions 3 - 6, and 4×15 repetitions in sessions 7 - 12.

Table S2. Characteristics (time under tension or repetitions and load) of experimental groups.

Exercise session (week / exercise session)	EQI group [time under tension]	ECC group [repetitions]	Load
1 / 1	90 s	2 x 15	X
1 / 2	90 s	2 x 15	X
2 / 3	135 s	3 x 15	X
2 / 4	135 s	3 x 15	X
3 / 5	135 s	3 x 15	Y
3 / 6	135 s	3 x 15	Y
4 / 7	180 s	4 x 15	Y
4 / 8	180 s	4 x 15	Y
5 / 9	180 s	4 x 15	Z
5 / 10	180 s	4 x 15	Z
6 / 11	180 s	4 x 15	Z
6 / 12	180 s	4 x 15	Z

ECC – eccentric; EQI – eccentric quasi-isometric; X – load 1, measured before first exercise session; Y – load 2, measured before fifth exercise session; Z – load 3, measured before ninth exercise session

Table S3. Changes in muscle–tendon morphology, ankle range of motion, strength, and EQI performance from baseline (PRE) to post-intervention (POST) in the EQI, ECC, and control groups.

Outcome	EQI PRE	EQI POST	p (η^2)	ECC PRE	ECC POST	p (η^2)	Control PRE	Control POST	p (η^2)
Achilles tendon thickness (cm)	4.782 ± 0.526	5.124 ± 0.493	<0.001 (0.79)	4.880 ± 0.496	5.276 ± 0.496	<0.001 (0.78)	4.760 ± 0.648	4.775 ± 0.644	0.070 (0.20)
Achilles tendon stiffness (kPa)	127.0 ± 22.9	115.4 ± 12.1	0.065 (0.22)	117.6 ± 15.4	112.7 ± 16.7	0.305 (0.08)	115.6 ± 12.8	115.5 ± 13.1	0.818 (0.00)
MG fascicle length (cm)	5.118 ± 0.911	5.689 ± 0.934	<0.001 (0.69)	4.983 ± 0.703	5.513 ± 0.616	<0.001 (0.70)	5.210 ± 0.606	5.213 ± 0.609	0.528 (0.03)
MG pennation angle (°)	20.90 ± 2.06	21.40 ± 1.72	0.316 (0.07)	20.03 ± 2.31	20.73 ± 2.78	0.358 (0.06)	19.44 ± 2.49	19.63 ± 2.53	0.211 (0.10)
MG thickness (cm)	1.959 ± 0.337	2.133 ± 0.303	<0.001 (0.75)	1.843 ± 0.340	2.057 ± 0.370	<0.001 (0.60)	1.876 ± 0.262	1.876 ± 0.260	0.941 (0.00)
LG fascicle length (cm)	5.339 ± 0.560	5.802 ± 0.525	<0.001 (0.71)	4.993 ± 0.737	5.451 ± 0.633	<0.001 (0.76)	5.499 ± 0.883	5.510 ± 0.877	0.048 (0.24)
LG pennation angle (°)	14.00 ± 3.79	16.77 ± 4.16	<0.001 (0.60)	14.10 ± 2.12	16.57 ± 2.03	0.001 (0.53)	13.19 ± 1.95	13.28 ± 2.02	0.270 (0.08)
LG thickness (cm)	1.407 ± 0.446	1.696 ± 0.399	<0.001 (0.76)	1.178 ± 0.243	1.565 ± 0.260	<0.001 (0.71)	1.279 ± 0.249	1.283 ± 0.245	0.127 (0.15)
Open-chain dorsiflexion (°)	10.60 ± 3.02	12.53 ± 3.14	0.014 (0.36)	12.67 ± 4.78	15.13 ± 2.67	0.019 (0.33)	12.56 ± 4.99	13.13 ± 4.91	0.208 (0.10)
Closed-chain dorsiflexion (°)	18.93 ± 5.82	22.67 ± 4.40	<0.001 (0.61)	20.13 ± 4.17	24.07 ± 3.90	0.003 (0.47)	21.56 ± 7.87	22.63 ± 7.50	0.045 (0.24)
Concentric strength (Nm)	228.4 ± 51.5	266.8 ± 47.7	0.001 (0.58)	237.4 ± 58.9	271.6 ± 67.9	<0.001 (0.70)	228.6 ± 62.3	232.6 ± 60.5	0.193 (0.11)
ECC strength (Nm)	221.4 ± 50.1	260.7 ± 48.3	0.001 (0.59)	232.7 ± 62.8	266.1 ± 63.7	<0.001 (0.76)	223.0 ± 64.0	225.1 ± 59.0	0.620 (0.02)
MVIC (Nm)	194.3 ± 49.3	216.7 ± 46.6	<0.001 (0.77)	195.8 ± 47.7	218.1 ± 50.1	<0.001 (0.63)	194.2 ± 51.2	194.3 ± 51.0	0.954 (0.00)
EQI time under tension (s)	122.6 ± 46.7	144.3 ± 66.0	0.098 (0.18)	113.1 ± 34.7	135.5 ± 50.7	0.020 (0.33)	109.4 ± 44.9	113.2 ± 41.7	0.273 (0.08)
EQI total torque impulse (Nm·s/MVIC)	93.2 ± 37.1	111.9 ± 52.9	0.084 (0.20)	86.2 ± 26.3	105.5 ± 39.1	0.012 (0.37)	82.7 ± 34.6	85.3 ± 31.6	0.382 (0.05)
EQI angular velocity (°/s)	-1.18 ± 0.71	-1.09 ± 0.48	0.440 (0.04)	-1.08 ± 0.24	-1.00 ± 0.25	0.371 (0.06)	-1.17 ± 0.46	-1.29 ± 0.51	0.240 (0.09)

Values are mean ± SD. p and η^2 refer to within-group time effects. ECC – eccentric; EQI – eccentric quasi-isometric; LG – lateral gastrocnemius; MG – medial gastrocnemius; MVIC – maximal voluntary isometric contraction