Title:
Effects of eccentric training on mechanical properties of the plantar flexor muscle-tendon complex.

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Running title: Eccentric training effects on muscle and tendon stiffness.
Abstract

Eccentric training is a mechanical loading classically used in clinical environment to rehabilitate patients with tendinopathies. In this context, eccentric training is supposed to alter tendon mechanical properties but interaction with the other components of the muscle-tendon complex remains unclear. The aim of this study was to determine the specific effects of 14 weeks of eccentric training on muscle and tendon mechanical properties assessed in active and passive conditions \textit{in vivo}. Twenty-four subjects were randomly divided into a trained group (n=11) and a control group (n=13). Stiffness of the active and passive parts of the series elastic component of plantar flexors were determined using a fast stretch during submaximal isometric contraction; Achilles tendon stiffness and dissipative properties were assessed during isometric plantar flexion; and passive stiffness of \textit{gastrocnemii} muscles and Achilles tendon were determined using ultrasonography while ankle joint was passively moved. A significant decrease in the active part of the series elastic component stiffness was found (P < 0.05). In contrast, a significant increase in Achilles tendon stiffness determined under passive conditions was observed (P < 0.05). No significant change in \textit{triceps surae} muscles and Achilles tendon geometrical parameters was shown (P > 0.05). Specific changes in muscle and tendon involved in plantar flexion are mainly due to changes in intrinsic mechanical properties of muscle and tendon tissues. Specific assessment of both Achilles tendon and plantar flexor muscles allowed a better understanding of the functional behavior of the muscle-tendon complex and its adaptation to eccentric training.

\textbf{Keywords:} \textit{triceps surae} muscles, Achilles tendon, passive stiffness, CSA, Ultrasound.
Introduction

Eccentric training is a mechanical loading often used in clinical context to rehabilitate patients with tendon injuries. Eccentric loading consists in muscle-tendon complex (MTC) lengthening while muscle is contracting, thus combines effects of stretching and strengthening (3). It was shown that tendinopathies alter tendon mechanical properties when determined in active condition (6) and many positive effects in tendon rehabilitation were found after clinical program including eccentric loadings (2, 28, 42, 47, 52, 81). Most studies examining eccentric effects on tendon have utilized ultrasonography in vivo to determine tendon stiffness during muscle contraction, however, results differ in several previous studies (12, 54, 61) considering the type of the tested population (e.g. healthy volunteers, subjects with tendinopathies) and training characteristics (i.e. intensity, volume, duration). To better understand the efficiency of eccentric exercises in rehabilitation, adaptations of tendon mechanical properties linked to tendon mechanobiology processes were investigated (85). For example, physiological mechanisms such as collagen turn-over were shown to be altered after eccentric training (48, 49, 50).

Most of the studies, determining the effects of eccentric training, only assessed tendon stiffness during isometric contraction (12, 54, 61). Yet, tendon dissipation coefficient is representative of a storage-recoil process and was shown to be altered by strength (75) and plyometric (18) training in vivo. In addition, other structures included in the series elastic component (SEC) also play a role in tension transmission and influence the elastic energy storage-recoil process (1). Effects of eccentric training on the global SEC stiffness were already assessed (73). From our results, a specific adaptation of muscle and tendon can be hypothesized (73). Due to technological limitations of non-invasive investigation of muscle stiffness during contraction in vivo, this hypothesis remains to be verified. However, a method developed for isolated muscle (15, 57) allows the determination of specific stiffness for the
force dependent component (SEC₁) and force independent component (SEC₂), constituting the plantar flexors SEC during isometric contraction. This method was recently adapted in vivo (17). With regard to previous studies, SEC₁ and SEC₂ were considered to be the active and passive part of the SEC respectively (17, 19). To our knowledge, no study has yet investigated the effects of eccentric training on the active part of SEC stiffness in vivo even though a decrease in muscle stiffness of the elbow flexors was hypothesized after eccentric training (73). This potential is supposed to be linked to structural changes in muscle such as architecture and/or fibre type composition and has already been shown to result from eccentric training programs (7, 38).

In addition, only a few studies concentrated on adaptations of passive mechanical properties of MTC to eccentric training in vivo (35, 39, 72, 87). Yet, it was shown that the passive mechanical properties of MTC play an important role in postural, walking and running activities (e.g. 30, 80). Mahieu et al. (54) showed that eccentric training decreases the passive torque produced by ankle joint during dorsi flexion. A specific change in muscle and tendon passive stiffness after eccentric training was hypothesized but specific adaptation between muscle and tendon was not assessed. By means of a recent method determining the stiffness of muscle and tendon of gastrocnemii MTC using ultrasonography during passive motion of ankle joint (36, 37), specific adaptation of muscle and tendon passive stiffness could be analyzed.

The determination of specific adaptations of muscle and tendon mechanical properties assessed in active and passive conditions to eccentric training may provide important information concerning the functional behavior of MTC and the underlying mechanisms to successfully apply eccentric training in rehabilitation programs. Based on previously described methods (17, 18, 19, 20, 21, 22, 69), the aim of the present study was to determine the effects of eccentric training on mechanical properties of plantar flexors considering: i) the
stiffness of SEC₁ and SEC₂, ii) the stiffness and dissipative properties of the Achilles tendon during isometric plantar flexion and, iii) the stiffness of gastrocnemii muscles and the Achilles tendon determined during passive motion of the ankle joint.

Methods

Subjects

Twenty-four males volunteered to participate in this study and were randomly assigned to trained [n = 11, 21.2 (2.7) years, 177.1 (6.1) cm, 71.1 (5.8) kg] and control groups [n = 13, 20.5 (1.7) years, 178.0 (6.5) cm, 68.7 (6.9) kg]. All subjects were involved in regular sport practices (8.8 (6.5) h.wk⁻¹) and did not change their usual activity during the period of the study. Subjects were fully informed about the nature and the aim of the study before they signed a written informed consent form. Approval for the project was obtained from the local ethics committee. All procedures used in this study were in conformity with the Declaration of Helsinki.

Eccentric training

The eccentric training program was based on different kinds of exercises, as defined in the literature (e.g. 2, 54). More precisely, the subjects performed: i) eccentric contraction of the plantar flexor muscles with the leg fully extended as described in Alfredson et al. (2); ii) eccentric contractions by going down from a box of different heights (i.e. from either low (35 cm), medium (50 cm) or high (65 cm) height) performed on one or both feet. All eccentric actions of the plantar flexors were performed either by the right leg or both legs and concentric actions with the left leg only. The intensity level was increased by an elevated number of exercises (i.e. the number of eccentric plantar flexion per exercise) and the jump
height. The training program lasted for 14 weeks and included 34 sessions of one hour for a total of approximately 6800 eccentric actions (from 200 to 600 per session).

**Experimental design**

Subjects were tested over three sessions performed on different days in a randomized order:  
i) a session to assess Achilles tendon and triceps surae muscles geometry;  
ii) a session to determine Achilles tendon mechanical properties and SEC stiffness in active conditions;  
iii) a session to assess ankle joint range of motion, and the stiffness of gastrocnemii muscles and Achilles tendon during passive motion of the ankle joint.

Subjects performed the three test sessions before (pretest) and one week after the end of the eccentric training period (posttest). Reproducibility of all defined parameters was assessed. Methods were already used in previous studies to determine effects of plyometric exercises (16, 18, 19).

**Achilles tendon and triceps surae muscles geometry**

Measurements of the Achilles tendon CSA were performed by ultrasonographic imaging scans (Philips HD3, Philips Medical Systems, Andover, MA, USA) with an electronic linear array probe (7.5-MHz wave frequency; L9-5, Philips medical systems, Andover, MA, USA). Measurement of Achilles tendon CSA (CSA<sub>T</sub>) and length was already described in a previous study (18). Intraclass coefficient correlation (ICC) of tendon geometrical parameters ranged from 0.95 to 0.99 (n = 8).

The measurements of the triceps surae muscles cross sectional area and architecture were previously described (19) and performed using the same ultrasonographic device and the same probe. Subjects stood on one leg with the ankle and knee joints of the other leg flexed at 90°, and fully immersed in a water-filled container. The cross sectional area of triceps surae
and *gastrocnemius* muscles (CSA<sub>TS</sub> and CSA<sub>GAS</sub> respectively) was averaged across points at 50%, 60% and 70% from proximal extremity of lower leg length. Using longitudinal ultrasonographic images captured along the belly of each muscle as described in previous studies (40, 41), fascicle length and pennation angle were measured. ICC of muscle architecture and cross sectional area ranged from 0.81 to 0.97 (n = 13 and n = 16 respectively) (19).

**Series elastic component stiffness**

The experimental design was already described in previous studies (16, 17, 19). A Biodex system 3 research® (Biodex medical, Shirley, NY, USA) isokinetic dynamometer was used to measure the external torque, ankle joint angle and ankle joint angular velocity. Subjects were seated on the isokinetic dynamometer with legs fully extended and their thighs, hip and shoulders secured and held in position by adjustable lap belts. The ankle joint angle was fixed at 75° (the foot perpendicular to the tibia = 90° with angles less than 90° being in plantar flexion), and hip angle was flexed to 140° (full extension = 180°).

Surface electromyographic (sEMG) signals of the *gastrocnemius medialis*, *gastrocnemius lateralis*, *soleus* and *tibialis anterior* muscles were recorded as described previously (19, 20) using active surface electrodes with an inter-electrode distance of 10 mm (DE-2.1, Delsys Inc, Boston, MA, USA) placed on the belly of muscles according to SENIAM recommendations (33). sEMG and mechanical signals were recorded simultaneously and sampled at 1000 Hz using an A/D converter (National Instrument, Delsys Inc, Boston, MA, USA), and saved on a computer hard drive using EMGWorks 3.1 software (Delsys Inc, Boston, MA, USA). In particular, EMG values were determined for a 100 ms period prior to and after the stretching trials to detect potential effects of short latency reflex on EMG signals (19).
Subjects performed: i) a warm up which consisted of submaximal isometric plantar flexions; ii) two maximal voluntary contractions under isometric condition in plantar flexion performed at 75° with two minutes of rest between each trial; iii) a familiarization to the short range stiffness experiment in which subjects had to sustain two submaximal torques at 40 and 80% of their maximal torque. During each trial, a fast stretch into dorsi flexion was applied through a range of motion of 20° (i.e., from 75° to 95°). The acceleration of this stretch was controlled by the Biodex Research Tool kit software. The angular velocity during the stretch reached 250°/sec and was constant 60ms after the beginning of the motion; iv) the short range stiffness experiments (14 trials) described above were then performed at 7 levels of submaximal torque in a random order (two trials at each 10% of maximal torque from 30% to 90% of maximal torque). Between each trial, two minutes of rest were provided.

The torque measured by the dynamometer was corrected with regard to inertia and the weight of the dynamometer attachment (i.e. without the foot being included) to obtain the external torque at the ankle joint. The external torque and the ankle angle were determined when the joint started to move (i.e., when velocity > 0) and 60ms thereafter. A maximal velocity (i.e. ~250°/s) was obtained 60ms after the beginning of the stretch with a range of motion of about 7°. The MTC stretching velocity and range of motion estimated from published anthropometric data (31) were about 90-110 mm.s⁻¹ and ~2% of the initial length of MTC respectively. Considering that muscle fibers slack length of gastrocnemii estimated in Humans (36) is very close to those reported by Morgan et al. (58, 60) for isolated muscle, it was assumed that stretching velocity was in the same range to those used previously on animals. Since the starting angle was 15° in plantar flexion, the contribution of the parallel elastic component of agonists and antagonists was neglected. Thus, the joint compliance (i.e. inverse of joint stiffness) was considered as the compliance of two springs placed in series, representing compliance of the SEC₁ and SEC₂. The active component of the series elastic
component compliance was thought to be inversely proportional to the torque (57) and the passive component of the series elastic component compliance \( C_{\text{SEC2}} \) was assumed to be constant across the range of torque investigated. The relationship between ankle joint compliance and torque can be written as follows:

\[
\alpha = C \cdot T = \alpha_0 + C_{\text{SEC2}} \cdot T
\]  

(1)

where \( \alpha \) is calculated as the product between external torque \( T \) produced before the stretch and ankle joint compliance \( C \), and \( \alpha_0 \) representing the stiffness index (i.e. the slope of the torque-stiffness relationship) of the SEC active part.

A linear regression was applied on the relationship alpha \( \alpha \) – torque \( T \). Then, \( \alpha_0 \) and \( C_{\text{SEC2}} \) were extracted as the Y-intercept, and the slope respectively. These two parameters (i.e. \( \alpha_0 \) and \( C_{\text{SEC2}} \)) were used to calculate the joint stiffness \( S \) using Eq. (2):

\[
S = \frac{T}{T \cdot C_{\text{SEC2}} + \alpha_0}
\]  

(2)

The joint stiffness-torque relationship was assessed for each subject and each session (i.e., pretest and posttest). A stiffness index of the active component of the SEC \( S_{\text{SEC1}} \) and a stiffness of the passive component of the SEC \( S_{\text{SEC2}} \) were calculated as the inverse of \( \alpha_0 \) and \( C_{\text{SEC2}} \) respectively. Joint stiffness was determined as the ratio of changes in external torque and ankle angle between the beginning of the stretch and 60ms after the stretch (the determination of instantaneous compliance with regard to the torque-angle relationship during the stretch would have been also possible. This alternative method for the assessment of joint stiffness is developed in Appendix A and provides similar results as compared to those calculated by the ratio of changes). Joint stiffness and SEC1 stiffness \( S_{\text{SEC1}} \) were determined for two relative torque levels (i.e. 30% and 60% of MVC) and 60N.m. ICC of series elastic component parameters ranged from 0.88 to 0.96 \((n = 14)\) (17).
Subjects were in prone position with their legs fully extended. Thighs, hips and shoulders were secured and held in position by adjustable lap belts as previously described (18).

The session that assessed the mechanical properties of Achilles tendon included the following protocols: i) A warm up consisting of constant submaximal isometric plantar flexion. ii) Two maximal voluntary isometric contractions in plantar flexion and dorsi flexion, with the knee fully extended at an ankle angle of 90°. The maximal isometric torque in plantar flexion (MVC) was then determined as the maximal value for the two trials. The maximal rate of torque development in plantar flexion (RTD_max), defined as the maximal slope of the torque-time relationship, was also characterized for the two MVC trials. iii) Familiarization trials to perform a linear increase in isometric torque in plantar flexion, from a relaxed state to 90% of MVC within 5 s, followed by a linear decrease in isometric torque, from 90% of MVC to the rest state within 5 s. Visual feedback was used to regulate and train the subjects for this task. iv) Five trials of constant isometric torque development in plantar flexion as described in iii) were then performed by each subject with 2 min of rest between each trial. Displacement of the distal myotendinous junction of the gastrocnemii was measured during the test using ultrasonography. The linear array probe mounted on an externally fixed bracket was strapped onto the skin of subjects to obtain longitudinal ultrasonic images of the distal myotendinous junction of the gastrocnemii.

Ultrasonographic videos were recorded on a hard disk at 25 Hz. In order to synchronize the torque signal and ultrasonographic images from the videos, the signal of the switch used to start the video was also recorded using the Delsys® system. A selection of 40 images, including 20 images equally spaced from both the loading and unloading phases of torque development were collected (Adobe Premiere Elements, Adobe Systems Inc., San
Jose, CA, USA) from each trial. Displacements of the myotendinous junction were manually measured on these 40 images using open source digital measurement software (Image J, NIH, USA).

The ankle joint torque measured by the dynamometer was converted to tendon force (Ft) using Eq. (3) (6):

$$F_t = \frac{T}{m_g}$$  \hspace{1cm} (3)

where $m_g$ is the moment arm length of gastrocnemii at 90° of ankle joint and knee fully extended, which was estimated from the limb length of each subject (31, 34). Myotendinous junction displacement was corrected with the passive displacement due to joint rotation during the “isometric” contraction as previously described (18, 22). The ratios of the calculated tendon force ($F_t$) and the corrected elongation ($\Delta L$) (i.e., displacement of myotendinous junction) were used to calculate the stiffness of the Achilles tendon.

The $F_t$–$\Delta L$ relationship is usually curvilinear, consisting of an initial region (toe region), characterized by a large increase in $\Delta L$ with increasing force, and a linear region after the toe region (85). In the present study, the $F_t$ and $\Delta L$ values from the ascending curve between 50% and 90% of minimal MVC between pretest and posttest were fitted to a linear regression equation, the slope of which was defined as the Achilles tendon stiffness ($S_T$) (45). A stiffness index ($SI_T$) using an exponential model was also determined as previously described (18). The maximal elongation was defined as $\Delta L_{\text{max}}$.

Areas under the ascending and descending phases of the curve were calculated and represent the potential elastic energy stored ($E_S$) and recoiled energy ($E_R$) respectively (53, 65, 66). From these parameters, a dissipation coefficient (DC) was calculated as follows:

$$DC = \frac{(E_S - E_R)}{E_S}$$  \hspace{1cm} (4)
sEMG signals of the *gastrocnemius medialis*, *gastrocnemius lateralis*, *soleus* and *tibialis anterior* muscles were recorded during isometric contraction using active surface electrodes. ICC of tendon mechanical properties ranged from 0.94 to 0.99 (n = 7) (18).

Ankle joint range of motion, passive stiffness of gastrocnemii muscles and Achilles tendon

Range of motion and passive torque-angle relationship

The method used to assess passive mechanical properties of the musculo-articular complex was similar to previous studies (e.g. 16, 67, 76). Isokinetic dynamometer was used to measure the torque produced in resistance to passive stretch (T), ankle joint angle (θ<sub>a</sub>) and ankle joint angular velocity. Subjects were in prone position with legs fully extended and with their thighs, the hip and shoulders secured and held in position by adjustable lap belts. The device enabled us to change the knee angle (θ<sub>k</sub>) as described previously (16, 20, 69). The reference angle of the knee joint (θ<sub>k</sub> = 0°) corresponded to the knee fully extended. The reference angle of the ankle joint (θ<sub>a</sub> = 90°) was defined as the foot perpendicular to the tibia.

sEMG signals of *triceps surae* muscles and *tibialis anterior* were recorded to ensure that no muscle activity occurred during the passive stretching procedures. sEMG signals were also visualized in real time by the subject and the principal investigator during tests. If during the passive measurements, sEMG values were found to be greater than 1% of the maximal sEMG value determined during maximal voluntary contractions for a given subject, the data were disregarded for analysis (e.g., 56, 66, 68).

Four different tests were performed: i) Assessment of the maximal ankle joint range of motion in plantar flexion with leg fully extended (RoM<sub>PF</sub>), in dorsi flexion with leg fully extended (RoM<sub>DFext</sub>) and with the knee joint flexed at 80° (RoM<sub>DFflex</sub>). During this test, the foot was passively and manually moved from neutral position (θ<sub>a</sub> = 90°) to the maximal RoM determined by the subject when the maximal tolerable stretch was perceived. The foot was
then immediately put back into the starting position. In each condition, three measurements were performed and the best trial was considered as the maximal RoM. \textit{ii)} Five cyclic passive motions at 5°/s from 80\% of RoM\textsubscript{PF} to 80\% of RoM\textsubscript{DF\text{ext}} with knee fully extended. \textit{iii)} Five cyclic passive repetitions at 5°/s for five randomly tested knee flexion angles (θ\textsubscript{k} = 15°, 30°, 45°, 60° and 80°). The range of stretch was increased in proportion with the increase in knee flexion up to passive motions performed from 80\% of RoM\textsubscript{PF} to 80\% of RoM\textsubscript{DF\text{flex}} with knee flexed at 80° in order to apply Hoang’s model (34) improved by Nordez \textit{et al.} (69). Five minutes of rest between each stretching series of tests were respected. \textit{iv)} Two maximal voluntary contractions under isometric conditions (θ\textsubscript{k} = 0°; θ\textsubscript{α} = 90°) for sEMG normalization purposes.

All of the data were processed using standardized programs computed with Matlab\textsuperscript{®} (The Mathworks, Natick, USA). External passive torque and angle position data were filtered using a Butterworth second-order low pass filter (10Hz), and recorded torque was corrected from gravity. ICC and variation coefficient of RoM parameters ranged from 0.77 to 0.93 and from 3.3\% to 4.4\% respectively (n = 6).

\textit{Force-length relationship of the gastrocnemii MTC}

To determine the passive force of the \textit{gastrocnemii} MTC, an optimization procedure was performed on the differences between the torque-angle relationships obtained at 0°, 15°, 30°, 45°, 60° and 80° of knee angle. Thus, the contribution of the \textit{gastrocnemii} could be determined directly according to the Eq. (5) (69):

\[ T_k - T_{80} = m_g (F_{Gk} - F_{G80}) \tag{5} \]

with \( T_k \) and \( F_{Gk} \) the ankle passive torque and the \textit{gastrocnemii} passive force determined at different knee angles (0°, 15°, 30°, 45° and 60°); \( T_{80} \) and \( F_{G80} \) the ankle passive torque and the \textit{gastrocnemii} passive force determined at 80° of knee angle, respectively, \( m_g \) is the
gastrocnemii moment arm assessed using model of Grieve et al. (31). The exponential model used in the present study was similar to the model of Sten Knudsen (64, 69, 82) where $F_G$ could be calculated as follows:

$$F_G = \frac{1}{\beta} (e^{\beta(l-l_0)} - 1) \quad \text{for } l > l_0 \quad (6)$$

$$F_G = 0 \quad \text{for } l < l_0$$

where $l$ is the gastrocnemii length (i.e. assessed using Grieve’s model), $\beta$ is the parameter determined using the optimization that concerns MTC stiffness, and $l_0$ the gastrocnemii slack length determined using the optimization procedure. Then, the $\beta$ parameter was identified as a stiffness index of the gastrocnemii MTC ($S_{F-1}^{FSI-MTC}$). In addition, the gastrocnemii MTC length was determined for a force production of 1N ($L_{F=1}^{MTC}$) (20). Using this exponential model, the maximal gastrocnemii stiffness ($S_{max}^{FS-I}$) can be determined using Eq. (7) at the maximal passive force ($F_{max}$) common for both tests:

$$S_{max}^{FS-I} = \beta F_{max} + 1 \quad (7)$$

Force-length relationships of gastrocnemii muscle and Achilles tendon

The method used to separate muscle and tendon properties was similar to previous works (20, 36). During experiments in passive conditions described above, the ultrasonographic linear array probe, mounted on an externally fixed bracket, was strapped onto the skin of the subjects to obtain longitudinal ultrasonic images of the distal myotendinous junction of the GM. Preliminary experiments using hypoechogenic wire glued on the skin ensured that the probe remained in a fix position during passive stretching and isometric plantar flexion. The probe was placed at the same position on the lower leg between pretest and posttest. Ultrasonographic videos were recorded on a hard disk at 25 Hz. In order to synchronize the torque signal and ultrasonographic images from the videos, the signal of the switch used to start the video was also recorded using the Delsys® system. Thirty images
equally spaced from the loading phases of the fifth cycle of passive stretches performed at 0°,
15°, 30°, 45°, 60° and 80° of knee flexion were extracted from ultrasonographic videos.

During passive stretching, passive external torque, ankle angle and displacement of the
distal myotendinous junction of the GM were determined during the loading curve of the fifth
cycle (i.e. from plantar flexion to dorsi flexion) performed at each knee angle. The MTC
length of the gastrocnemii was calculated using published anthropometric data (31).
Elongation of the gastrocnemii muscles was determined as the total displacement of the
myotendinous junction of the GM.

Length of the gastrocnemii MTC and Achilles tendon was determined for $\theta_a = 90°$ and
$\theta_k = 0°$ (i.e. calculated using Grieve et al. (31) model and measured using ultrasonography
respectively). Muscle length was calculated for this position as the difference between MTC
and tendon lengths. Length of MTC was determined as function of knee and ankle angles
using the model of Grieve et al. (31) on full range of motion for each stretch cycles. Changes
in muscle length were determined using displacement of the myotendinous junction of the
GM (i.e. elongation of the gastrocnemii muscles determined by ultrasonography) during
passive stretches. Thus, tendon length was calculated as the difference between MTC and
muscle length on the full range of motion of each stretch cycles.

Relationships between $F_G$ and length of the gastrocnemii muscles and Achilles tendon
were characterized. Then, Sten Knudsen model (Eq. 6) was fitted on these two relationships in
order to determine stiffness index and length for a force development of 1N by both
gastrocnemii muscles and Achilles tendon (i.e. $SI_{\text{muscle}}^{F=1}$, $L_{F=1}^{\text{muscle}}$, $SI_{\text{tendon}}^{F=1}$ and $L_{F=1}^{\text{tendon}}$
respectively). ICC of gastrocnemii passive mechanical properties ranged from 0.82 to 0.98
(n = 15).

Statistics
After checking the distribution of data, parametric statistical tests were performed using Statistica® software (Statsoft Inc., Tulsa, OK, USA). Descriptive data included means ± standard deviation. Two-way multivariate analyses of variance (ANOVA) (group × time) were performed to assess the statistical significance of changes. The critical level of significance in the present study was set at $P < 0.05$.

Results

No significant change was found in control group between pretest and posttest whatever the parameter assessed (some individual raw data determining MTC mechanical properties under active and passive conditions are shown in Appendix B).

Achilles tendon and triceps surae muscles geometry

No significant change was found in MVC, CSA$_T$, CSA$_{GAS}$ and CSA$_{TS}$ ($P > 0.05$) (Table 1). Although a decrease of 1.7% in GM tendon length was found in trained group ($P = 0.002$), no significant change was found in triceps surae muscles architecture ($P > 0.05$) (Table 1).

Active and passive parts of the series elastic component stiffness

Mean sEMG activity did not significantly change until a latency of 45 ms following the onset of motion (Fig. 1). At this point, sEMG activity from gastrocnemii and soleus muscles increased for a period of 20-30ms. Activity from tibialis anterior was unchanged through the recording period. Concerning sEMG data, similar results were observed in a short range experiment performed at baseline and after the eccentric training period.

Although the global angular joint stiffness determined at 60N.m was significantly lower for the trained group after the eccentric training period (-5.4%, $P = 0.006$) (Fig. 2), no
significant change was found in $S$ determined at 30% and 90% of MVC ($P > 0.05$) (Table 2 and Fig. 3).

Mean alpha-torque relationships of trained subjects determined at baseline and after 14 weeks of eccentric training are shown in Fig. 3. A significant interaction ($P = 0.018$) was found between “group” and “time” factors for $SI_{SEC1}$. For the trained group, a significant decrease of 10.4% in $SI_{SEC1}$ ($0.16 \pm 0.02$ to $0.14 \pm 0.02 \text{°}^{-1}$) without any change in $S_{SEC2}$ was observed after training (Fig. 4). No significant change was found in maximal $S_{SEC1}$ ($P > 0.05$) whereas a significant decrease was determined for $S_{SEC1}$ calculated at 60N.m ($P > 0.05$) (Table 2).

**Achilles tendon mechanical properties**

For the trained group, no significant change in $RTD_{max}$ was found (1536 ± 297 to 1515 ± 319 N.m.s$^{-1}$, $P > 0.05$). Torque-time and force-elongation relationships are presented in Fig. 5. Linear regression applied on individual $Ft-\Delta L$ and mean sEMG-torque curves showed a very good correlation coefficient (mean $R^2 = 0.97 \pm 0.02$) allowing the calculation of $ST$. Mean sEMG-time and sEMG-torque relationships did not significantly change for all muscles between measurements performed at baseline and after eccentric training period (Fig. 6). Thus, no significant change was found in sEMG signals, $\Delta L_{max}$, $S_T$, $SI_T$ and DC ($P > 0.05$) (Table 3).

**Ankle range of motion, passive stiffness of gastrocnemii muscles and Achilles tendon**

No significant interaction was found between “group” and “time” factors for $RoM_{DFext}$, $RoM_{PF}$ and $RoM_{DFflex}$ was determined ($P > 0.05$) (Table 4). Also, no significant change could be shown for passive torque-ankle angle relationships whatever the knee flexion angle (Fig. 7-A). In addition, no significant change in $S_{max}^{F-l}$ (trained group: 62451 ± 25975 to...
Mean passive force-length relationships of the muscle-tendon complex of the gastrocnemii for the trained group at baseline and after eccentric training period are shown in Fig. 7-B.

The mean force-length relationships of the Achilles tendon and the gastrocnemii muscle for trained group in pretest and posttest are shown in Fig. 8-A and 8-B respectively. No significant change in $S_{\text{FSI}}^{F=L}$ and $L_{F=1}^m$ ($P > 0.05$) was determined (Table 4) whereas significant increases in $S_{\text{FSI}}^{F=L}$ and $L_{F=1}^t$ of 21.8% and 6.4% respectively were found in the trained group ($P = 0.044$ and $P = 0.040$ respectively) (Table 4).

**Discussion**

The aim of the present study was to examine whether the mechanical properties of plantar flexors muscle and tendon structures were altered with eccentric training. The main purpose was to assess specific mechanical properties of MTC structures in both active and passive conditions by using innovative methods. The effects of eccentric training showed a decrease in the active part of the SEC stiffness of plantar flexors and an increase in Achilles tendon stiffness during passive motion of ankle joint, although training did not change stiffness and dissipative properties of the Achilles tendon during isometric contraction. Furthermore, no significant changes in MTC geometrical properties and sEMG parameters were found indicating that changes were mainly due to modified intrinsic properties of muscular and tendinous tissues.

The results of this study showed that ankle range of motion measured under passive condition was unchanged after the eccentric training program. Mahieu et al. (54) found an increase in dorsi flexion range of motion with the leg fully extended in a healthy population despite no significant interaction for statistical analysis between “group” and “time” factors.
Our results are in accordance with Silbernagel et al. (79) who showed no significant change in ankle joint range of motion after 12 weeks of eccentric training in subjects with Achilles tendinopathies.

Only one study assessed the effects of eccentric training on passive stiffness of the ankle joint (54). It was shown that passive ankle joint torque plays an important role in daily activities (63, 80, 88) and that functional performances are influenced by elastic properties of the passive musculo-articular complex, including structures such as muscles, tendons, skin, subcutaneous tissue, fascia, ligaments, joint capsule and cartilage (76, 89). During passive stretches, the intrinsic sub-cellular cytoskeletal proteins (i.e. titin, desmin) (86), the associated connective tissues (epimysium, perimysium and endomysium), and the tendons (32) were probably the main tissues lengthened (29). The measurement of the passive force-length relation of human muscle *in vivo* is challenging as the muscle must be “isolated” from other structures (34, 36, 37). Thus, force obtained during passive stretches is lower than during slow walking when exclusively the passive behavior of the *gastrocnemii* muscle-tendon is considered. In the current study, a decrease in passive torque was shown for dorsiflexion of the ankle joint indicating a specific adaptation of muscle and tendon stiffness for passive conditions. The specific passive stiffness of *gastrocnemii* muscles and Achilles tendon were examined. An increase in Achilles tendon stiffness was determined whereas no significant change in stiffness of *gastrocnemii* muscle was found.

Eccentric training had no significant effect on tendon stiffness and dissipative properties for isometric contraction. This result is in accordance with the study of Mahieu *et al.* (54) using a similar training program. Other studies showed an increase in tendon stiffness (e.g. 12), however, additional loads during eccentric loadings have been used. Appropriate mechanical loading can result in positive changes in tendinous structures and may lead to improved functional behavior, whereas excessive loading may induce tendon degeneration...
To our knowledge, no previous study assessed the effects of eccentric loading on dissipative properties of tendon. No change in the tendon’s DC was found in the present study indicating that eccentric training did not change the capacity to dissipate potential elastic energy during lengthening. Yet, a previous study showed a decrease in the tendon DC after strength training in elderly subjects (75). However, eccentric training combines stretching and strengthening effects (3). Our results are in accordance with those findings obtained in studies assessing effects of chronic stretching and low-load resistance training (45, 46). Interestingly, in a previous study, a decrease in DC of Achilles tendon was found after 14 weeks of plyometric training (18). Plyometric contraction consists in an eccentric action followed by a concentric action in order to restore potential elastic energy stored during the first phase. Eccentric contraction performed alone consists in the storage of elastic energy which is dissipated. Changes in tendon DC after plyometric and eccentric trainings were shown to be coherent with the functional behavior of MTC induced by plyometric and eccentric contractions.

During contraction a decrease in joint stiffness for an absolute level of torque (i.e. 60 N.m) was assessed which is in accordance with the results of Pousson et al. (73) who found an increase in SEC compliance of elbow flexors. Specific adaptations of active and passive parts of the SEC were characterized more precisely in the current study. As it was hypothesized in a former study (73), a decrease in the stiffness of the active part of the SEC was determined whereas no significant change was found in the SEC passive part stiffness. Our results could be explained by an increase in muscle sarcomere in series which has already been shown in animal studies (51, 59) and put forward in recent studies determining the effects of eccentric training in vivo (10, 54). In addition, structural changes in muscle (e.g. fiber type transition) were taken into account as a possible factor in former studies (4, 23, 73) as well as the fact that fast muscle fibers are more compliant than slow ones (71).
However, some methodological considerations have to be considered with regard to the assessment of the mechanical properties of MTC. Assumptions linked to the alpha method were abundantly discussed in previous studies (17, Supplementary material of 20). As mentioned before, the stiffness of SEC$_2$ was assumed to be constant and SEC$_1$ stiffness was considered as proportional to torque on the full range of torque investigated (i.e. between 30% and 90% MVC). Studies have shown that the onset of a change in EMG activity, as a result of the short latency stretch reflex, generally occurs between 40 and 60 ms (11, 70). An analysis on sEMG signal was performed before and after the beginning of the stretch using a method similar to that described by Cronin et al. (11). Compared to the results of Cronin et al., the short latency reflex occurred later. This difference could be explained by the ankle angle used before the stretch (i.e. 15° in plantar flexion, whereas 0° was used in the study of Cronin et al.). In the current study, reflex activity was shown to start at least 45 ms or more after the beginning of the stretch. In the literature, the electromechanical delay for the plantar flexors is between 16 ms (at 15° in plantar flexion) during electrically evoked contractions (62) and 24 ms (at 0°) during voluntary contractions (90). Therefore, the influence of reflex activity on external torque and stiffness assessed in the present study is likely to be minimal. Furthermore, no significant change at the onset of the reflex activity was shown after our eccentric training protocol.

In addition, the assumption that tendon stiffness remains constant at high forces might be inconsistent when using an exponential equation for the length-tension properties of the tendon. Nevertheless, the force level for passive stretches was lower than for the contraction. It is well known that the tendon force-length relationship can be considered as biphasic consisting of a toe region (non linear part, characterized by a large increase in length with small increments in force) and followed by a linear region (85). Most notably, Proske and Morgan (74) concluded that “above 20-30% of maximal isometric tension, tendon stiffness is
more nearly constant than proportional to the tension” (74), indicating that the toe region comprises this range of force. In our study, torque ranged from 0 to 25% of MVC in passive condition, thus, corresponding to the toe region. This justifies the use of an exponential model for the length-tension properties of the tendon for passive conditions (27). On the contrary, in the short range stiffness experiment, torque ranged from 30% to 90% of MVC corresponding to the linear region. This justifies the hypothesis of the constant tendon compliance for the alpha method (Supplementary material of 20). Note that this last assumption is in accordance with the linear regression to model the tendon force-length relationship obtained by using ultrasonography during contractions.

In addition, we assume that $S_{SEC1}$ is proportional to torque. Linear relationships were reasonably plotted between sEMG and torque for plantar flexor muscles ($R^2 = 0.96$ and 0.98 for the trained group on pretest and posttest respectively) on the range of torque investigated (i.e. from 30% to 90% MVC) and no significant difference in muscle activation levels was found between pretest and posttest. Thus, the results of eccentric training are likely not to be influenced by our hypothesis that $S_{SEC1}$ is proportional to torque.

Moreover, recent studies observed a potential lateral transmission of tension laterally between muscles. Bojsen-Moller et al. (9) indirectly showed some inter-muscle force transmission between gastrocnemii and soleus in humans (9). However, in this last study, displacements were measured instead of force. Due to the non-linear force-displacement relationship of the muscle, large displacements can be observed at low force transmission. Only one recent study quantified the inter-muscle force transmission between gastrocnemii and soleus in humans (83). Using an elegant model, this study estimated the magnitude of force transmitted from the gastrocnemii to the soleus, and showed that this magnitude is negligible (about 5 N). In addition, another study stated that the shear elastic modulus measured using supersonic imaging provide an estimate of passive muscle tension. Using this
technique, it was shown that shear elastic modulus-length relationships were similar whatever
the knee angle during passive dorsi flexion (55). Even though further studies are required to
confirm this finding, the hypotheses used in the Hoang’s model can be considered as being
reasonable because there is currently not enough evidence to contest its validity.

Another methodological consideration concerns the relative contribution of the triceps
surae to the active ankle torque which is challenging to determine non-invasively in vivo. To
our knowledge, the only studies assessing the inclusion of the triceps surae are invasive in
nature, for instance by using optic fiber sensors (e.g. 5). Arndt et al. (5) indicate that the
relative contribution of the triceps surae is increased when the knee is extended (i.e. the same
position as for the present study). The authors indicate that under those conditions the relative
contribution of the triceps surae is approximately 80%. Due to limitation of accuracy by using
this method and the inclusion of only one subject (13, 14), these results should be considered
with caution. Nevertheless, the finding (i.e. ~80% triceps surae contribution to ankle torque)
is in accordance with estimations provided by other studies (24, 77, 84). Thus, the
contribution of the five other muscles (i.e. flexor hallucis longus, tibialis posterior, flexor
digitorum longus, peroneus longus, peroneus brevis) to active ankle plantar flexion torque
could be considered as negligible in comparison to the triceps surae muscles contribution
(84). The muscle contribution to active torque is supposed to be almost proportional to the
physiological cross-sectional area (PCSA) (24, 25, 26). In addition, both muscle CSA and
muscle architecture (i.e., muscle thickness, fascicle length and pennation angle) and sEMG
activity level did not significantly change after the training period for the trained group,
indicating that the relative contribution of muscles remain unchanged. Thus, for instance, a
change in PCSA of the other muscles of 10% (to the best of our knowledge no study
investigated changes in geometric parameters of other muscles after chronic intervention)
should induce a change in relative contribution of about 2%. Therefore, its influence on the
contribution of the total plantar flexion torque production is likely to be minimal. Thus, it
could be assumed that changes in the dissipative coefficient were mainly associated to
changes in the dissipative properties of the tendon rather than being linked to muscle function.

Interestingly, specific adaptations of tendon were found in stiffness determined in
active and passive conditions. Indeed, no significant changes in $S_T$ and $SI_T$ were found while
an increase in $SI_{F-L}^{tendon}$ occurred after 14 weeks of eccentric training. Although the mechanical
properties of the same structure were assessed, different adaptations occurred specifically
when tendon stiffness is assessed in active and passive conditions. Similar results were found
following plyometric training showing an increase in Achilles tendon stiffness for the active
condition but without any changes during passive motion of the ankle joint (18, 20). Two
main hypotheses could explain the different findings for active and passive tendon stiffness.
Firstly, during contraction of the triceps surae muscle group, higher stress is applied on
tendon structures than during passive motion and changes in tendon stiffness due to the
training can depend on the stress level. Secondly, the stress applied can be qualitatively
different. Indeed, shearing movements were shown between the soleus and gastrocnemii
during an isometric plantar flexion (8). Since different muscle structures are implied in
tension production for muscle contractions and passive joint motion (i.e. mainly cross-bridges
during contraction and parallel elements during passive motion), it could be expected that
components of Achilles tendon are not identically stressed due to their specific mechanical
behavior. Several studies focused on the role of the extracellular matrix (ECM) is playing
with regard to mechanical properties of the tendon (e.g. 43, 44). The ECM is made of a
variety of substances (i.e. mainly collagen fibrils and proteoglycans). In addition to the
proteoglycans, the hydrophilic ECM consists of a variety of other proteins such as
noncollagen glycoproteins (e.g. 78). It is known that the force transmission of the muscle-
tendon complex is dependent on the structural integrity between individual muscle fibers and
the ECM as well as the fibrillar arrangement of the tendon and its allowance for absorption
and loading of energy (43). Thus, considering the fact that change in tendon stiffness is
specific to the stress level and/or tendon stretching velocity, an increase in stiffness of
different sub-structures within ECM could be effective. However, further studies are needed
to compare mechanical tendon properties at the same level of tension in active and passive
conditions and to determine more precisely the specific adaptations of these sub-structures
according to the type (e.g. higher shearing stress during contraction) and level of applied
stress.

In summary, the present study concentrated on the effects of eccentric training on
specific mechanical properties of plantar flexor muscles and the Achilles tendon in both active
and passive conditions. The major effect of eccentric training can be seen in the decrease in
the active part of the SEC stiffness leading to an increase in energy that could be stored during
eccentric contraction. Eccentric loading abundantly used in tendinopathies rehabilitation
programs did not significantly affect tendon mechanical properties. Adaptations in mechanical
properties of plantar flexor muscles and the Achilles tendon seem to be more protective to
lower the risk of muscle and/or tendon injuries (i.e. no change in dissipative properties of
tendon and decrease in SEC₁ stiffness). However, differences in adaptation to training of the
Achilles tendon stiffness determined in active and passive conditions remain unclear. Further
studies are needed to precise the specific adaptations of tendon structures with regard to the
type and level of stress applied to the tendon during passive motion and muscle contraction.
Such an approach might allow a better understanding of specific physiological mechanisms
potentially involved in changes of tendon mechanical properties.
Acknowledgements

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Appendix A

In active condition, the joint stiffness was calculated as the ratio of changes in external torque and ankle angle between the beginning of the stretch and 60ms after. It would have been also possible to determine instantaneous compliance considering the torque-angle relationship during the stretch. For the only time point for which no significant angular acceleration occurred (i.e. 60ms after the beginning of the stretch in our experimental design), instantaneous stiffness was determined with regard to the torque angle relationship. However, the instantaneous stiffness was slightly noisy with the filters used which are usually considered to be appropriate (17). Thus, the alpha torque relationship obtained using this calculation was not appropriate. Therefore, instantaneous stiffness was averaged on time ranges to limit the influence of noise but also of inertia and reflex influence during the stretch. This additional processing was performed for the trained group before and after eccentric training. The mean R² values of the alpha-torque relationships are shown in Figure A1. The values indicate that time range between 50 and 70ms after the beginning of the stretch reached a good compromise considering inertial and reflex influences. In addition, analyses performed in this time range provide similar quality of fits than those exposed in the present study when determining the alpha-torque relationship. Thus, the influence of training was determined using this additional approach (Figure A2).

Results showed that changes are similar for both $\alpha_0$ and $S_{sec2}$. Therefore, the method using the instantaneous stiffness is an interesting alternative and the results presented in
Figure A2 strengthen the results concerning the effects of eccentric training obtained in the present study.

Appendix B

In addition to mean relationships, some raw data and traces of (i) angle and torque during the rapid stretch used in the alpha method (Figure B1, B2 and B3), (ii) EMG and measures of myotendinous junction displacement during isometric contractions (Figure B4 and B5), and (iii) the passive force-length relationships (Figure B6) of a representative trained subject.
References


### Table 1: Geometrical parameters of Achilles tendon and *triceps surae* muscles

<table>
<thead>
<tr>
<th></th>
<th>Trained Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretest</td>
<td>Posttest</td>
</tr>
<tr>
<td><strong>CSAₜ (mm²)</strong></td>
<td>67.6 ± 7.2</td>
<td>66.6 ± 10.4</td>
</tr>
<tr>
<td><strong>CSAₜS (mm²)</strong></td>
<td>3952 ± 750</td>
<td>3960 ± 735</td>
</tr>
<tr>
<td><strong>CSA₉ₙAS (mm²)</strong></td>
<td>1686 ± 415</td>
<td>1668 ± 411</td>
</tr>
<tr>
<td><strong>Tendon length (cm)</strong></td>
<td>22.2 ± 1.9</td>
<td>22.0 ± 1.7</td>
</tr>
<tr>
<td><strong>GL Pennation angle (°)</strong></td>
<td>13.3 ± 1.6</td>
<td>12.6 ± 3.5</td>
</tr>
<tr>
<td><strong>Fascicle length (cm)</strong></td>
<td>8.6 ± 1.8</td>
<td>9.5 ± 2.8</td>
</tr>
<tr>
<td><strong>Tendon length (cm)</strong></td>
<td>19.7 ± 2.5</td>
<td>19.2 ± 2.2*</td>
</tr>
<tr>
<td><strong>GM Pennation angle (°)</strong></td>
<td>22.7 ± 2.3</td>
<td>21.6 ± 1.9</td>
</tr>
<tr>
<td><strong>Fascicle length (cm)</strong></td>
<td>5.9 ± 0.9</td>
<td>6.2 ± 1.0</td>
</tr>
<tr>
<td><strong>Tendon length (cm)</strong></td>
<td>4.9 ± 1.4</td>
<td>4.4 ± 1.5</td>
</tr>
<tr>
<td><strong>SO Pennation angle (°)</strong></td>
<td>33.5 ± 8.5</td>
<td>31.9 ± 8.4</td>
</tr>
<tr>
<td><strong>Fascicle length (cm)</strong></td>
<td>3.1 ± 0.7</td>
<td>3.1 ± 0.8</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. **CSAₜ**: Achilles tendon cross sectional area; **CSAₜS** and **CSA₉ₙAS**: mean cross sectional area of the triceps surae and gastrocnemius muscles respectively calculated from measurements at 50, 60 and 70% from proximal extremity of the lower leg length, tendon length, pennation angle and fascicle length for each muscle of the triceps surae (soleus [SO], gastrocnemius medialis [GM] and gastrocnemius lateralis [GL]). *: P < 0.05.
Table 2: Active stiffness of plantar flexors muscle-tendon complex mechanical properties

<table>
<thead>
<tr>
<th></th>
<th>Trained Group</th>
<th></th>
<th>Control Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretest</td>
<td>Posttest</td>
<td>Pretest</td>
<td>Posttest</td>
</tr>
<tr>
<td>30% MVC</td>
<td>3.6 ± 0.5</td>
<td>3.6 ± 0.6</td>
<td>3.5 ± 0.4</td>
<td>3.4 ± 0.5</td>
</tr>
<tr>
<td>90% MVC</td>
<td>6.0 ± 0.8</td>
<td>6.0 ± 1.1</td>
<td>6.0 ± 0.8</td>
<td>6.0 ± 0.7</td>
</tr>
<tr>
<td>60 N.m</td>
<td>4.7 ± 0.3</td>
<td>4.4 ± 0.3 *</td>
<td>4.6 ± 0.4</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>S (N.m.°⁻¹)</td>
<td>9.2 ± 1.4</td>
<td>10.2 ± 2.8</td>
<td>9.7 ± 1.9</td>
<td>9.8 ± 1.8</td>
</tr>
<tr>
<td>SSEC2 (N.m.°⁻¹)</td>
<td>9.9 ± 1.3</td>
<td>8.4 ± 1.5 *</td>
<td>9.2 ± 1.5</td>
<td>9.3 ± 2.4</td>
</tr>
<tr>
<td>SSECı max (N.m.°⁻¹)</td>
<td>19.8 ± 3.7</td>
<td>17.4 ± 4.2</td>
<td>18.5 ± 3.2</td>
<td>18.6 ± 5.4</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. S: global angular joint stiffness determined at 30%, 90% of maximal voluntary contraction and for an absolute level of contraction (60N.m), SSEC2: passive part of the series elastic component stiffness, SSECı: active part of the series elastic component stiffness determined at 60N.m and for maximal voluntary contraction (SSECı 60N.m and SSECı max, respectively).

*: P < 0.05.
Table 3: Achilles tendon mechanical properties

<table>
<thead>
<tr>
<th></th>
<th>Trained Group</th>
<th>Control Group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pretest</td>
<td>Posttest</td>
</tr>
<tr>
<td>△L&lt;sub&gt;max&lt;/sub&gt; (mm)</td>
<td>14.4 ± 2.6</td>
<td>15.2 ± 2.9</td>
</tr>
<tr>
<td>S&lt;sub&gt;T&lt;/sub&gt; (N.mm&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>215.8 ± 55.0</td>
<td>251.1 ± 109.2</td>
</tr>
<tr>
<td>S&lt;sub&gt;IT&lt;/sub&gt; (mm&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.076 ± 0.049</td>
<td>0.096 ± 0.050</td>
</tr>
<tr>
<td>DC (%)</td>
<td>27.8 ± 13.7</td>
<td>26.3 ± 11.2</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. △L<sub>max</sub>: maximal Achilles tendon elongation; Achilles tendon stiffness (S<sub>T</sub>); dissipation coefficient (DC).
Table 4: Ankle joint range of motion and passive stiffness of the gastrocnemii muscle and Achilles tendon

<table>
<thead>
<tr>
<th></th>
<th>Trained Group</th>
<th></th>
<th>Control Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretest</td>
<td>Posttest</td>
<td>Pretest</td>
<td>Posttest</td>
</tr>
<tr>
<td>RoMPF (°)</td>
<td>61 ± 6</td>
<td>63 ± 4</td>
<td>59 ± 10</td>
<td>58 ± 8</td>
</tr>
<tr>
<td>RoMDFext (°)</td>
<td>49 ± 4</td>
<td>52 ± 6</td>
<td>51 ± 8</td>
<td>50 ± 7</td>
</tr>
<tr>
<td>RoMDFflex (°)</td>
<td>60 ± 6</td>
<td>62 ± 7</td>
<td>58 ± 10</td>
<td>58 ± 12</td>
</tr>
<tr>
<td>$SI_{\text{MTC}}^{F-L}$ (m$^{-1}$)</td>
<td>87.5 ± 12.9</td>
<td>89.2 ± 11.2</td>
<td>84.6 ± 11.0</td>
<td>83.8 ± 16.4</td>
</tr>
<tr>
<td>$LF_{1\text{MTC}}$ (m)</td>
<td>0.372 ± 0.019</td>
<td>0.373 ± 0.018</td>
<td>0.371 ± 0.026</td>
<td>0.366 ± 0.031</td>
</tr>
<tr>
<td>$SI_{\text{muscle}}^{F-L}$ (m$^{-1}$)</td>
<td>168.1 ± 44.0</td>
<td>153.8 ± 27.7</td>
<td>150.6 ± 34.8</td>
<td>142.0 ± 39.9</td>
</tr>
<tr>
<td>$LF_{1\text{M}}$ (m)</td>
<td>0.247 ± 0.026</td>
<td>0.246 ± 0.027</td>
<td>0.251 ± 0.017</td>
<td>0.248 ± 0.020</td>
</tr>
<tr>
<td>$SI_{\text{tendon}}^{F-L}$ (m$^{-1}$)</td>
<td>152.9 ± 16.6</td>
<td>185.9 ± 32.6*</td>
<td>165.6 ± 21.8</td>
<td>169.9 ± 38.4</td>
</tr>
<tr>
<td>$LF_{1\text{T}}$ (m)</td>
<td>0.170 ± 0.023</td>
<td>0.174 ± 0.024*</td>
<td>0.168 ± 0.025</td>
<td>0.167 ± 0.028</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. RoMPF: Maximal range of motion of the ankle joint in plantar flexion with leg fully extended, RoMDFext: Maximal range of motion of the ankle joint in dorsiflexion with leg fully extended, RoMDFflex: Maximal range of motion of the ankle joint in dorsiflexion with knee flexed at 80°. Ankle joint angle = 0° with foot perpendicular to the tibia. Knee angle = 180° with leg fully extended. Stiffness index and length at a passive force of 1N of the gastrocnemii muscle-tendon complex, Achilles tendon and gastrocnemii muscle (SI$^{F-L}_{\text{MTC}}, LF_{1\text{MTC}}$; SI$^{F-L}_{\text{tendon}}, LF_{1\text{T}}$ and SI$^{F-L}_{\text{muscle}}, LF_{1\text{M}}$ respectively) determined from force-length relationships. *: $P < 0.05$. 
Figure legends

Figure 1
Mean sEMG-time relationships for (A) Gastrocnemius Lateralis [GL], (B) Gastrocnemius Medialis [GM], (C) Soleus [SO] and (D) Tibialis Anterior [TA] obtained on 100ms before and after the stretch beginning [vertical dotted line] for the trained group before (solid line) and after (dotted line) the 14 weeks of eccentric training.

Figure 2
Mean joint stiffness-torque relationships (A) and joint stiffness-relative torque relationship (B) for the trained group before (filled circles) and after (empty squares) the 14 weeks of eccentric training. Error bars were removed for clarity. *: $P < 0.05$.

Figure 3
Mean alpha-torque relationship for the trained group before (filled circles - solid line) and after (empty squares - dotted lined) the 14 weeks of eccentric training.

Figure 4
Stiffness index of the active part of the series elastic component ($SI_{SEC1}$) (A) and passive part of the series elastic component stiffness ($S_{SEC2}$) (B) determined before (filled area) and after (empty area) the 14 weeks of eccentric training for both trained and control groups. Results are presented as mean ± standard deviation. *: $P < 0.05$. 
**Figure 5**

Mean torque-time relationships (A) and tendon force-elongation relationships (B) obtained during isometric contraction in plantar and dorsi flexion for the trained group before (solid line) and after (dotted lined) the 14 weeks of eccentric training.

**Figure 6**

Mean sEMG-time relationships for (A) Gastrocnemius Lateralis [GL], (B) Gastrocnemius Medialis [GM], (C) Soleus [SO] and (D) Tibialis Anterior [TA] obtained during isometric contraction in plantar and dorsi flexion for the trained group before (solid line) and after (dotted lined) the 14 weeks of eccentric training.

**Figure 7**

Mean relationships between passive torque and ankle angle obtained during passive stretching of ankle joint performed at 0°, 45° and 80° of knee flexion angle (A) and mean force-length relationships of the gastrocnemii muscle-tendon complex determined in passive condition (B) for the trained group before (solid line – filled circles) and after (dotted lined – empty squares) the 14 weeks of eccentric training.

**Figure 8**

Mean force-length relationships of the Achilles tendon (A) and the gastrocnemii muscle (B) determined in passive condition for the trained group before (solid line – filled circles) and after (dotted lined – empty squares) the 14 weeks of eccentric training.
Mean values of regression coefficient ($R^2$) of the linear fit on alpha-torque relationships using stiffness calculated as exposed in the present study, instantaneous stiffness determined 60ms after the beginning of the stretch, mean instantaneous stiffness values calculated between 40 and 60ms, and between 50 and 70ms after the beginning of the stretch.

Mean values of $SI_{SEC1}$ and $S_{SEC2}$ determined considering instantaneous stiffness averaged on the range 50-70ms after the beginning of the stretch and considering method exposed in the manuscript for the trained group before (black) and after (white) training.

Typical raw data obtained during a trial of the short range stiffness experiment with (A) ankle angle, (B) angular velocity and (C) external torque. After the isometric contraction, triceps surae was quickly stretched. The first 60 ms [vertical dotted line] of the stretch were used to determine the joint stiffness.

Joint stiffness-torque relationships (A) and joint stiffness-relative torque relationship (B) for a representative trained subject before (filled circles) and after (empty squares) the 14 weeks of eccentric training. Error bars were removed for clarity.

Alpha-torque relationship for a representative trained subject before (filled circles - solid line) and after (empty squares - dotted lined) the 14 weeks of eccentric training.
Figure B4

Elongation-time relationships (A) and torque-time relationships (B) obtained during isometric contraction in plantar and dorsi flexion for a representative trained subject before (solid line) and after (dotted lined) the 14 weeks of eccentric training.

Figure B5

Tendon force-elongation relationships obtained during isometric contraction in plantar and dorsi flexion for a representative trained subject before (solid line) and after (dotted lined) the 14 weeks of eccentric training.

Figure B6

Force-length relationships of the gastrocnemii muscle-tendon complex (A) and the Achilles tendon (B) determined in passive condition for a representative trained subject before (solid line – filled circles) and after (dotted lined – empty squares) the 14 weeks of eccentric training.
Pretest: $y = 0.097x + 6.962$
$R^2 = 0.86$

Posttest: $y = 0.090x + 8.276$
$R^2 = 0.79$
Pretest: $y = 0.107x + 6.034$
$R^2 = 0.89$

Posttest: $y = 0.084x + 8.843$
$R^2 = 0.89$