

## RESEARCH ARTICLE

# The Achilles tendon is mechanosensitive in older adults: adaptations following 14 weeks versus 1.5 years of cyclic strain exercise

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**ABSTRACT**

The aging musculoskeletal system experiences a general decline in structure and function, characterized by a reduced adaptability to environmental stress. We investigated whether the older human Achilles tendon (AT) demonstrates mechanosensitivity (via biomechanical and morphological adaptations) in response to long-term mechanical loading. Thirty-four female adults (60–75 years) were allocated to either a medium-term (14 weeks;  $N=21$ ) high AT strain cyclic loading exercise intervention or a control group ( $N=13$ ), with 12 participants continuing with the intervention for 1.5 years. AT biomechanical properties were assessed using ultrasonography and dynamometry. Tendon cross-sectional area (CSA) was investigated by means of magnetic resonance imaging. A 22% exercise-related increment in ankle plantarflexion joint moment, along with increased AT stiffness ( $598.2 \pm 141.2$  versus  $488.4 \pm 136.9$  N mm<sup>-1</sup> at baseline), Young's modulus ( $1.63 \pm 0.46$  versus  $1.37 \pm 0.39$  GPa at baseline) and about 6% hypertrophy along the entire free AT were identified after 14 weeks of strength training, with no further improvement after 1.5 years of intervention. The aging AT appears to be capable of increasing its stiffness in response to 14 weeks of mechanical loading exercise by changing both its material and dimensional properties. Continuing exercise seems to maintain, but not cause further adaptive changes in tendons, suggesting that the adaptive time–response relationship of aging tendons subjected to mechanical loading is nonlinear.

**KEY WORDS:** Aging, Strength training, Tendon stiffness, Tendon cross-sectional area, Tendon Young's modulus

**INTRODUCTION**

Tendons play a crucial role in movement by transmitting muscle forces to the skeleton in order to cause motion (Biewener and Roberts, 2000; Ettema et al., 1990; Hof et al., 2002). However, the mechanical properties of tendons may change depending on their exposure to loading (Butler et al., 1978; Tkaczuk, 1968; Woo et al.,


1980, 1982), and research about the mechanosensitivity of tendinous tissue *in vivo* has proliferated in recent years. In order to maintain tissue homeostasis, tendinous tissue deforms in response to the mechanical load of a muscle contraction, and the resultant tendon strain is directly transferred to its cellular cytoskeleton via the extracellular matrix, causing structural changes (Wang, 2006). Structural changes at the tendon's cellular level trigger complex signal transductions within the cell, leading to various catabolic and anabolic molecular responses, such as synthesis of matrix proteins (Arnoczky et al., 2002), gene expression of proteoglycans and collagen (Robbins and Vogel, 1994; Yang et al., 2004), improvement in the density of collagen matrix and its alignment (Pins et al., 1997) and expression of different growth factors (Olesen et al., 2006). These changes following long-term mechanical loading have been associated with modifications in tendon mechanical properties (Galloway et al., 2013; Heinemeier and Kjaer, 2011; Kjaer, 2004; Wang, 2006).

Recent *in vivo* cross-sectional studies with younger adults have examined the effects of different habitual mechanical loading patterns on the mechanical, material and morphological properties of tendons (Couppé et al., 2008; Fukutani and Kurihara, 2015; Kongsgaard et al., 2005; Magnusson and Kjaer, 2003; Westh et al., 2008). For instance, in young badminton players and fencers with between-leg strength differences in quadriceps femoris muscle (on average 22%), an asymmetrical adaptation of patellar tendon (PT) cross-sectional area (CSA) and stiffness has been identified (Couppé et al., 2008). Although not statistically significant, habitual exercise has been suggested to affect the Young's modulus (on average up to 16%) and may possibly trigger structural adaptive modifications in human tendons (Seynnes et al., 2013; Westh et al., 2008; Wiesinger et al., 2015). In addition, diverse exercise training interventions for 12–14 weeks in younger adults have led to increased PT and AT stiffness, Young's modulus and tendon CSA, concurrent with increments in muscle strength (Arampatzis et al., 2007a; Bohm et al., 2014; Kongsgaard et al., 2007; Kubo et al., 2001, 2007a; Malliaras et al., 2013). These study results therefore suggest that the PT and AT of younger adults are sensitive to mechanical loading and respond to changes in the mechanical environment by adapting their mechanical, material and morphological properties.

Aging tendons experience many biochemical, cellular, mechanical and pathological changes which lead to a general deterioration in their structure and function (Kjaer, 2004; Komatsu et al., 2004; Noyes and Grood, 1976; Vogel, 1991). These degradations are often characterized by a reduced ability to adapt to environmental stress through a loss of tissue homeostasis (Tuite et al., 1997). Studies reporting on the adaptability of human tendons to exercise interventions *in vivo* have been predominantly conducted in younger adults. Nonetheless, several exercise intervention studies

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(e.g. strength training) have confirmed that tendons preserve their adaptability to mechanical loading even in older age (Grosset et al., 2014; Karamanidis et al., 2014; Maganaris et al., 2004; Onambele-Pearson and Pearson, 2012; Reeves et al., 2003a,b). One common finding of the limited number of exercise training intervention studies in older participants is a post-intervention increase in tendon stiffness and Young's modulus, in the absence of any changes in tendon dimensions (Grosset et al., 2014; Onambele-Pearson and Pearson, 2012; Reeves et al., 2003a). The lack of changes in tendon CSA may have been caused by the limited number of transversal scans (typically from 3 regions) taken along the tendon, which may exclude regional CSA changes as reported in studies with younger adults in response to strength training (Arampatzis et al., 2007a; Kongsgaard et al., 2007; Magnusson and Kjaer, 2003; Seynnes et al., 2009).

One other possibility is that tendon material and dimensional adaptations occur over different time frames. In younger adults, exercise-induced increments in tendon stiffness in the first months of exercise seem to be primarily triggered by changes in material properties, whereas tendon hypertrophy may be a long-term (years) effect of mechanical loading (Bohm et al., 2015a; Heinemeier and Kjaer, 2011; Kjaer et al., 2009; Wiesinger et al., 2015). Therefore, one might suggest that because of the diminished responsiveness to mechanical loading, compared with younger tendons, the morphological adaptation processes in elderly tendinous tissues may be more prolonged (Tuite et al., 1997). Yet, to our knowledge, previous studies examining older adults have mainly investigated the PT after medium-term exercise (for ~3 months). There is limited information about the long-term (years) mechanosensitivity of tendon material, mechanical and morphological properties, and it is unknown whether these properties are associated with the maximum muscle force production capacity within older individuals. Examining the effect of long-term increases in mechanical loading and different mechanical environments on tendon properties could improve our understanding of tendon mechanosensitivity in old age.

The purpose of the current work was (1) to examine whether medium-term (14 weeks) or long-term (1.5 years) exercise interventions are adequate to cause adaptive changes in the mechanical, morphological and material properties of the *in vivo* AT in the elderly to a similar degree as previously reported in younger adults (Arampatzis et al., 2007a), and (2) to determine whether AT stiffness, Young's modulus and CSA along the entire free AT length are associated with the maximal force production capacity of the triceps surae muscle (TS) in a group of older female adults. Based on previous observations in young adults, we hypothesized that older adults will show an adaptive increment in tendon stiffness after medium-term exercise, mainly due to changes in tendon material properties, whereas after the long-term exercise (post 1.5 years), an adaptive hypertrophy of the tendon might also play a role in the changes in tendon mechanical properties. Regarding our cross-sectional investigation, we hypothesized that a greater free AT CSA, higher tendon stiffness and higher Young's modulus would be associated with a higher TS muscle strength in the older adults. These findings would indicate that the human AT preserves its mechanosensitivity at older age, and that the AT of older adults can respond and adapt to the mechanical environment over a longer time period, by changing both tendon material properties and size.

## MATERIALS AND METHODS

### Participants and experiment design

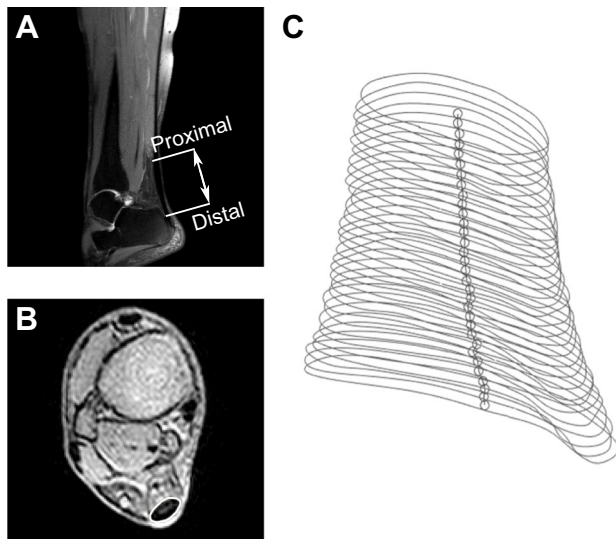
Thirty-four older female adults (age:  $65 \pm 7$  years; body mass:  $65 \pm 9$  kg; body height:  $165 \pm 6$  cm; means  $\pm$  s.d.) from a large-scale knee

osteoarthritis study ( $N=38$ , K-L score: 2–3) voluntarily participated in the study. Exclusion criteria were any previous AT rupture, AT pain or injury (e.g. tendinopathy) within the last 5 years, any other musculoskeletal impairments of the lower limbs (e.g. ankle joint pain), or pain during the exercise training that could influence the findings of the study. The subjects were generally healthy for their age, according to the SF-36 general health questionnaire (average scale value at baseline over all participants was 73.5%) and to clinical functional tests such as SLS (single leg stance; average of 39.6 s; with a test duration of 45 s) and TUG (timed up and go test; average of 7.2 s). For the interventional part of the study 21 subjects (age:  $65 \pm 7$  years; body mass:  $66 \pm 10$  kg; body height:  $165 \pm 8$  cm) were recruited for the experimental group (exercise intervention) and the remaining 13 participants (age:  $66 \pm 8$  years; body mass:  $64 \pm 9$  kg; body height:  $165 \pm 5$  cm) formed the no-contact control group (no exercise intervention). For the experimental group, the training program was approved by a physician, who was consulted before the beginning of training. The study was approved by the responsible ethics committees (German Sport University Cologne and University of Bonn) and written informed consent was obtained from all participants.

In order to examine whether tendon properties are associated with the maximal force production capacity, a cross-sectional investigation was performed by analyzing the AT mechanical, material and morphological properties in all of the participating older adults before the start of the intervention. In an attempt to induce adaptive changes in AT mechanical, material and morphological properties in the older adults, a longitudinal investigation was implemented by extending a mechanical loading exercise protocol (intensity at 90% of that day's MVC) previously conducted over 14 weeks with younger adults by Arampatzis and colleagues (Arampatzis et al., 2007a) to a longer time period of 1.5 years. Each subject was tested on three occasions [before the start of the intervention (baseline), and 14 weeks (medium-term exercise) and 1.5 years (long-term exercise) after the intervention began] to obtain the following main outcome measures: the CSA of the free Achilles tendon along the full length, and the AT stiffness and elastic modulus (Young's modulus).

### Analysis of Achilles tendon cross-sectional area

To determine the morphological properties (CSA and length) of the free AT, magnetic resonance imaging (MRI) was used to capture images of the lower limb of the preferred leg for step initiation in the examined subjects with a 3 Tesla MRI scanner (Ingenia 3.0T, Philips Healthcare; Best, NL). MRI sequences were acquired in transversal and sagittal orientation (Fig. 1) using a high-resolution single shot T1-weighted 3D gradient echo sequence (e-THRIVE) with an additional spectral attenuated inversion recovery (SPAIR) for fat suppression. The following parameters were used for the transversal sequences: acquisition matrix  $420 \times 372$ , acquired voxel size  $1.00 \times 1.00 \times 2.00$  mm, reconstructed voxel size  $0.58 \times 0.58 \times 1.00$  mm, time of repetition (TR) 3.6 ms, time of echo (TE) 1.7 ms, flip angle 10 deg, parallel imaging factor (SENSE)=2. During the scan process, the participants were measured in an unloaded supine position with the hip and knee fully extended and the ankle joint fixed at 20 deg plantarflexion. In the sagittal sequences, the borders of the free AT were detected by identifying the AT's most proximal attachment of the tuber calcanei and the most distal aspect of the soleus muscle. In every transversal image sequence along the free AT, the boundaries of the AT were manually outlined by investigators blind to the subjects' group and measurement time point using a custom routine for the image



**Fig. 1. Magnetic resonance images and calculated contours of the free Achilles tendon.** (A) Sagittal images were used as a reference to locate free AT boundaries (m. soleus: AT junction and AT most proximal attachment on the tuber calcanei). (B) On transversal images, the outlines of the Achilles tendon between the free AT boundaries were manually segmented (white oval). (C) The AT free length was determined as the curved path through the centroids of the digitized cross sections.

processing program ImageJ (ImageJ v.1.48; National Institutes of Health, USA). Acquired coordinates were exported as 3D coordinates and further processed using custom routines in MATLAB 2015a (The MathWorks, Natick, MA, USA). The length of the free AT was determined as the curved path through the centroids (calculated using Delaunay triangulation) of every outlined CSA between the two previously mentioned landmarks. In order to examine region-specific changes of the AT, the CSA was calculated in 10% intervals along the length of free AT.

#### **Analysis of triceps surae muscle strength, Achilles tendon stiffness and Young's modulus**

The mechanical and material properties of the TS MTU were analyzed in all subjects by using ultrasonography and dynamometry synchronously on a custom-made device, while performing maximal isometric voluntary ankle plantarflexion contractions (MVCs). The subjects were seated with their lower leg fixed and their foot on a custom-made strain gauge type dynamometer (1000 Hz) with the ankle and knee at 90 deg angle (thigh and foot perpendicular to the shank). The seating position of the subject relative to the force plate (foot, shank and thigh location) was obtained in order to reliably replicate the positions in the post measurements. Following a regulated warm-up to precondition the tendon (Maganaris, 2003), which consisted of 10 submaximal and three maximal isometric voluntary contractions (MVCs), the subjects performed six plantarflexion MVCs (three contractions without instructions and three controlled ramp contractions) with verbal encouragement. The resultant moments at the ankle joint were calculated using inverse dynamics by taking the gravitational moments into account using a passive (relaxed muscle) measurement. The resultant plantarflexion moment is an approximation of the moment produced by the TS muscle as it does not account for the moment contributions of the other synergistic agonist muscles or the antagonist dorsiflexors. The maximal plantarflexion moment (TS muscle strength) was assessed by considering all 6 MVCs, and the 3 MVC ramp contractions were

carried out to examine the force–length relationship of the tendon (mean values of the three ramp contractions were taken). AT force was calculated by dividing the resultant ankle joint moment by the tendon moment arm. The AT moment arm was individually assessed for each participant at baseline measurement by using the tendon excursion method (An et al., 1983, 1984; Maganaris et al., 1998). For this reason, we assessed tendon displacement due to ankle joint angular rotation of the foot during passive movement between 85 and 105 deg ankle joint angle, and estimated the AT moment arm from the slope of the tendon excursion versus ankle joint angular changes.

To analyse the displacement of the myotendinous junction (MTJ) of the m. gastrocnemius medialis during the passive movements as well as during ramp contractions, a 50 mm linear array ultrasound probe at a frequency of 29 Hz was used (ultrasound device: MyLab™Five, Esaote, Genoa, Italy). In several pilot studies, we attempted to analyze the free AT; however, this specific position was chosen for analysis because the more distal MTJ of m. soleus was difficult to reliably detect and track in this group of older individuals. To prevent any movement of the ultrasound probe in relation to the skin, the probe was fixed on the shank above the MTJ of the m. gastrocnemius medialis within a custom-made polyethylene foam casing using adjustable straps. The displacement of the MTJ in relation to a skin marker during the contraction was manually digitized in every ultrasound video frame by investigators blind to the subjects' group and measurement time point using the Simi Motion 5.0 video analysis software (SIMI Reality Motion System, Unterschleißheim, Germany). The effect of the inevitable ankle joint angular rotation on the measured tendon elongation during each contraction (Muramatsu et al., 2001) was taken into account by using a potentiometer located under the heel to measure any elevation and thereby calculate changes in the ankle joint angle via the inverse tangent of the ratio of the heel lift to the distance between the ankle joint axis (set exactly above the rotation axis of the dynamometer footplate) and the fifth metatarsal. A pilot study with nine healthy younger adults found this method to be in accordance with measured motion capture results showing absolute difference lower than 1.1 deg in maximal ankle joint angle changes during maximal isometric plantarflexion contractions (current method:  $3.8 \pm 0.2$  deg; motion capture:  $4.9 \pm 0.2$  deg). Thus, it was possible to estimate the actual tendon elongation due to the applied tendon force.

The stiffness of the AT tendon was assessed as the slope of the calculated tendon force and actual tendon elongation between 50% and 100% of maximum tendon force using linear regression. The elastic modulus (Young's modulus) of the AT was assessed by using a linear regression between the relationship of tendon stress and AT tendon strain from 50% to 100% of the maximal AT stress. Resting length of the tendon was determined with a flexible measuring tape along the skin surface in the same seated position at resting state (relaxed muscles) as the curved path from the most proximal point of the tuber calcanei to the MTJ of the GM (both defined using ultrasonography). For the AT stress (tendon force divided by AT tendon CSA) calculations, the average CSA of the free AT between 10% and 100% length were used, similar to previous studies in younger adults (Arampatzis et al., 2007a, 2010). Several previous studies have already examined the accuracy of ultrasound-based measurements and showed that, when the placement of the probe is precisely replicated, then the differences in maximal tendon length changes are under 1.0 mm, with within- and between-day correlation coefficients of 0.89–0.95 in tendon elongation and tendon stiffness values (Lichtwark and Wilson,

2005; Kubo et al., 2002). In the current study the US probe was positioned relative to distinct anatomical landmarks on each subject's leg (GM MTJ distance from the most proximal point of the tuber calcanei). Accordingly, the US probe was placed in the exact same position in relation to the GM MTJ at all measurement time points for each subject.

### Analysis of triceps surae muscle architecture

Muscle architecture measurements were taken from the m. soleus (SOL) and m. gastrocnemius medialis (GM) of the same leg as in all other MTU measurements. The subjects were set in an unloaded prone position with their hip and knee joints fully extended and the ankle joint fixed at a 90 deg angle using straps. The ultrasound probe specified above was used to obtain images along the mid-sagittal plane of the GM muscle belly, with the mid-point of the probe set at ~66% or 79% of the shank length for SOL and GM respectively, where the highest cross-sectional area of these muscles has been identified (Albracht et al., 2008). On the images recorded, fascicle length was measured as the length of a fascicle between the deeper aponeurosis, pennation angle was defined as the angle between the fascicle and its insertion into the deep aponeurosis, and muscle thickness was assessed as the distance between the deep and superficial aponeuroses at the centre of the acquired image. In cases where the fascicle insertions on the aponeuroses were not visible, a linear extrapolation was used to obtain the full length of the analyzed muscle fascicle. Owing to difficulties with respect to precise identification of the SOL muscle fascicles in ultrasound images in older adults, reliable measures of fascicle length and pennation angle were not possible for a considerable number of the examined elderly subjects. Therefore, for the SOL muscle only, thickness data are reported. The results were defined as mean values from three separate scans. All morphometric analyses were carried out by implementing custom routines in MATLAB 2015a (MathWorks).

### Medium- and long-term exercise intervention

The experimental group completed a supervised exercise intervention three times per week for 14 weeks (except for week 1 and 2, where training was conducted twice) and twice a week after the initial 14 week period for 1.5 years (approximately 50 min per session). In each session, five sets of four repetitions of isometric plantarflexion contractions were completed with each leg individually and then with both legs together (90% of MVC; 3 s loading, 3 s relaxation). This was carried out using eight custom-made training devices equipped with strain gauge type dynamometers (1000 Hz; ankle and knee angle set at 90 deg) and a visual feedback system developed with LabVIEW 2013 SP1 (National Instruments, Austin, TX, USA). The resultant moments at the ankle joint were calculated as described above. As a part of a large-scale clinical study, the intervention contained an additional motor skill training programme, where a series of curves presented on a computer screen had to be traced using the real time ankle plantarflexion moment signal as precisely as possible. The specific motor skill training programme was based on the previous work of Perez et al. (2004) and Jensen et al. (2005), and was intended to provoke increased excitability of the cortical representation of specific muscles. The motor skill training was conducted bilaterally with a low intensity (30–45% MVC), which could therefore not influence the tendon mechanical, morphological and material properties due to the low tendon strain magnitude (Arampatzis et al., 2007a; Grosset et al., 2014). At the start of each training session, following a standardized warm-up of 2–3 min of

submaximal isometric contractions, the participants performed unilateral and bilateral plantarflexion MVCs from which their maximal resultant ankle plantarflexion moments and the 90% intensities for the training session were calculated. With this method, the training load was individually adjusted for every session, and therefore gradually increased according to the subject's muscle strength over the 1.5 years.

### Statistics

All 34 subjects were additionally assigned to either a strong group (GroupStrong) or weak group (GroupWeak), according to the assessed TS muscle strength in the baseline measurement using a median split. Unpaired sample *t*-tests were used to examine possible differences between GroupStrong and GroupWeak in anthropometric characteristics (body height and mass), age and AT moment arm, TS muscle strength, muscle architecture (SOL and GM muscle thickness, GM pennation angle and fascicle length), maximal tendon elongation and strain, AT stiffness, AT CSA (mean value along the free AT) and Young's modulus. To check for potential tendon interval and subject group effects (GroupStrong versus GroupWeak) on AT CSA, the free AT was divided into ten 10% intervals along its length, which were then tested using a two-way repeated-measures ANOVA with the factors 'subject group' and 'interval'.

The experimental group ( $N=21$ ) was reduced after 14 weeks (medium-term intervention) and a subsample of 12 subjects continued exercise training for 1.5 years. The control group ( $N=13$ ) participated at all three measurement time points (baseline, after 14 weeks and after 1.5 years). Therefore, the statistical tests with the experimental group were conducted once with the 12 subjects from the long-term intervention for all measurement time points and once with the 21 subjects from the medium term intervention for the first two measurement time points. Firstly, a two-way ANOVA with repeated measures, with subject group (experimental versus control) and time point (baseline, post 14 weeks and post 1.5 years) as factors, was used to examine potential exercise-related effects on TS muscle strength, muscle architecture (SOL and GM muscle thickness, GM pennation angle and fascicle length), maximal AT elongation and strain, AT stiffness, AT CSA and Young's modulus. To identify potential region-specific effects on AT CSA, every 10% interval along the AT length was statistically analyzed using a three-way repeated-measures ANOVA (with subject group, time point and tendon interval as factors). If a significant effect was detected, a Bonferroni *post hoc* comparison was performed. Unpaired *t*-tests were used to identify possible differences in AT moment arm, age, body mass and body height between subject groups. The level of significance for all analyses was set to 0.05. All statistical analyses were performed using Statistica (v.7.1; Statsoft, Tulsa, OK, USA). All results provided in the text, tables and figures are presented as means $\pm$ s.d.

## RESULTS

### Cross-sectional investigation

The two subjects groups differed in TS strength by about 42% (GroupStrong,  $N=16$ ,  $138\pm 22$  Nm; GroupWeak,  $N=18$ ,  $97\pm 10$  Nm). When examining the muscle architecture, GroupStrong showed significantly greater SOL and GM muscle thickness compared with GroupWeak (SOL:  $13.3\pm 1.7$  versus  $11.9\pm 2.3$  mm;  $P=0.041$ ; GM:  $18.3\pm 1.8$  versus  $16.4\pm 1.7$  mm;  $P=0.005$ ) and pennation angle ( $21.4\pm 2.9$  versus  $19.3\pm 1.9$  deg;  $P=0.024$ ), but no differences in GM muscle fascicle length ( $57.3\pm 8.9$  versus

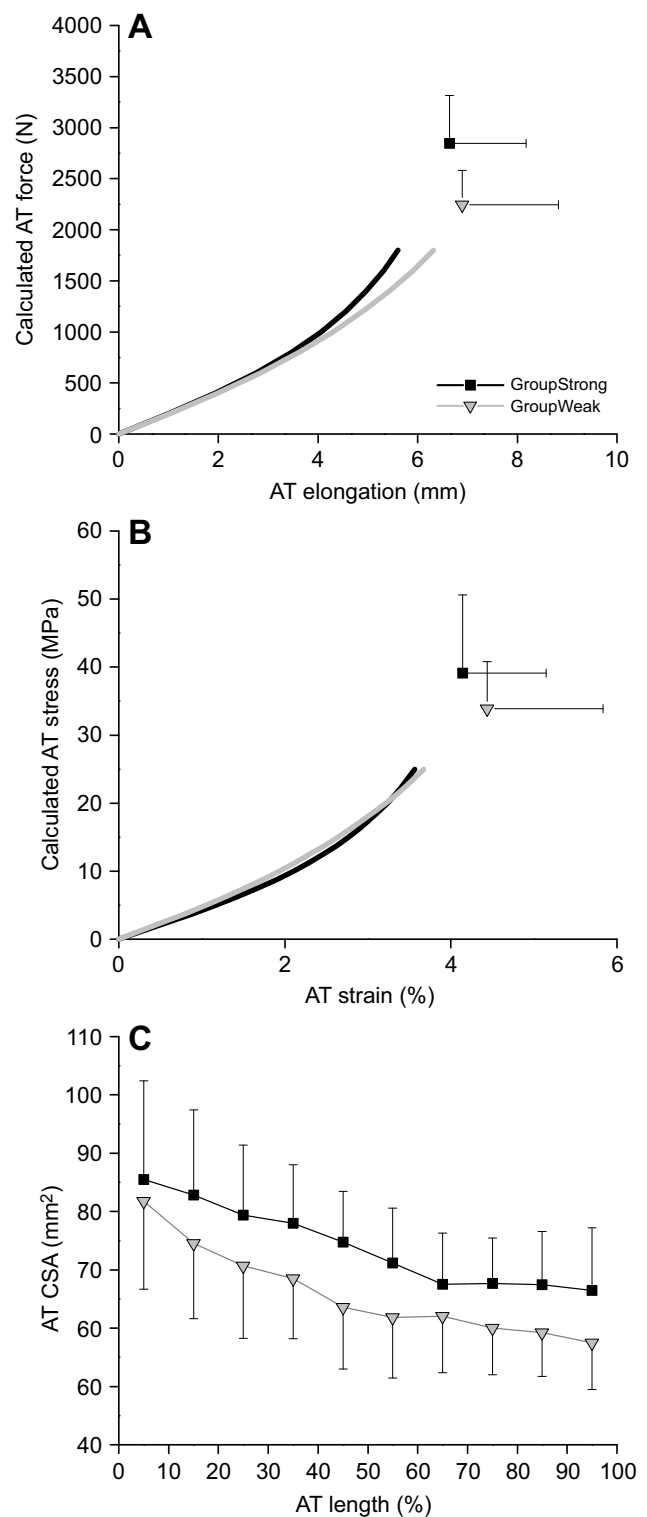
56.6±6.4 mm) were found between the two groups. GroupStrong also showed significantly higher AT stiffness (588±156 versus 441±129 N mm<sup>-1</sup>;  $P=0.008$ ; Fig. 2) and Young's modulus (1.40±0.25 versus 1.20±0.40 GPa;  $P=0.018$ ; Fig. 2). Analysis of the ten 10% intervals along the free AT revealed no significant interactions (Fig. 2), indicating that differences in the mean free AT CSA between the groups of elderly women were independent of the analyzed intervals (i.e. GroupStrong had a significantly higher CSA along the whole free AT). Accordingly, the average AT CSA was significantly higher for GroupStrong compared with GroupWeak (74.1±10.8 versus 66.0±10.5 mm<sup>2</sup>;  $P=0.033$ ). The mean length of the free AT (measured from most distal point of the soleus muscle to most proximal attachment to the calcaneus bone) did not differ between GroupStrong (34.6±4.8 mm) and GroupWeak (36.3±3.2 mm) and there were no significant differences between the groups' age (GroupStrong: 64.6±4.9 years, versus GroupWeak: 65.6±8.3 years), body mass (68.3±9.8 versus 63.0±8.8 kg), body height (167±7 versus 161±5 cm) or in AT moment arm values (40.6±0.4 versus 41.9±0.5 mm).

### Longitudinal investigation

Following the 14 week exercise intervention with 21 older female adults (medium-term group) completing the intervention, the use of the two-way ANOVA revealed a statistically significant group×time interaction for the TS muscle strength, muscle architecture, AT stiffness and Young's modulus. The *post hoc* analyses revealed significantly higher TS muscle strength ( $P<0.001$ ; Table 1), higher AT stiffness ( $P=0.014$ ; Table 1) and higher Young's modulus ( $P=0.025$ ; Table 1) for the experimental group (see also Fig. 3). In addition, the experimental group showed a significantly ( $P=0.007$ ) greater AT CSA (Table 1), with an average increment in tendon CSA of about 6% post exercise training. However, after examination of the tendon at different intervals, we did not find any group×time×interval interaction, meaning that the changes in tendon CSA post exercise were independent of the analyzed interval of the AT (Fig. 4). The experimental group showed a significantly higher SOL ( $P=0.032$ ) and GM muscle thickness ( $P=0.036$ ) and pennation angle ( $P=0.024$ ) following the first 14 weeks of exercise intervention in comparison to baseline (Table 1).

The 1.5 year exercise intervention (long-term group) resulted in a significant subject group×time interaction revealing higher TS muscle strength ( $P<0.001$ ), SOL ( $P=0.038$ ) and GM muscle thickness ( $P=0.020$ ), pennation angle ( $P=0.041$ ), AT stiffness ( $P<0.001$ ), Young's modulus ( $P<0.001$ ) and mean AT CSA ( $P<0.01$ ) along the entire free tendon length after 1.5 years compared with baseline measurements (changes in tendon CSA post exercise training were independent of the analyzed interval of the AT; Table 1). However, although there were significant changes in tendon properties after medium- and long-term exercise when compared with baseline, the experimental group did not show any further significant increment in tendon stiffness, Young's modulus, AT CSA, TS muscle strength, SOL muscle thickness or in GM muscle thickness and pennation angle following the long-term (1.5 years) exercise intervention when compared with the measurement after 14 weeks (Table 1). The GM fascicle length showed no differences to baseline values in response to the 14 week exercise intervention or following the 1.5 year exercise intervention.

The control group did not demonstrate any statistically significant differences in TS muscle strength, muscle architecture (SOL and GM muscle thickness, GM fascicle length and pennation angle) AT stiffness, Young's modulus and mean AT CSA between baseline,



**Fig. 2. Achilles tendon measurements in strong and weak subject groups.** Mean Achilles tendon (AT) force–length (A), stress–strain relationship (B) and cross-sectional area (CSA) in 10% intervals of the free tendon length (C) for the strong (GroupStrong) and weak (GroupWeak) subject groups. GroupStrong ( $N=16$ ) in comparison to GroupWeak ( $N=18$ ) showed on average 42% higher triceps surae muscle strength (138±22 versus 97±10 Nm;  $P<0.001$ ), 33% higher AT stiffness (588±156 versus 441±129 N mm<sup>-1</sup>;  $P=0.008$ ), 17% higher Young's modulus (1.40±0.25 versus 1.20±0.40 GPa;  $P=0.018$ ) and 12% higher mean AT CSA (74.1±10.8 versus 66.0±10.5 mm<sup>2</sup>;  $P=0.033$ ). AT CSA and all maximal values in the force–length and stress–strain relationship are expressed as means with s.d. (error bars).

**Table 1. Properties of the triceps surae muscle-tendon unit before (baseline), after a medium-term (14 weeks) and after a long-term (1.5 years) exercise intervention with high AT strain magnitudes for the experimental and the control group (N=13)**

	Experimental group			Control group		
	Baseline	Medium-term	Long-term	Baseline	Medium-term	Long-term
Maximal ankle joint moment (Nm)	116.3±30.8 (116.4±25.4)	141.5±36.2* (145.8±35.3)*	145.9±30.2*	117.5±28.6	124.3±29.5	126.9±28.9
AT maximal elongation (mm)	7.1±1.6 (7.0±1.6)	7.5±2.2 (7.5±2.3)	7.4±2.0	7.0±1.7	7.3±1.9	7.4±1.4
AT maximal strain (%)	4.3±1.1 (4.4±1.0)	4.6±1.3 (4.7±1.3)	4.5±1.2	4.3±1.2	4.6±1.3	4.7±1.3
AT stiffness (N mm <sup>-1</sup> )	488.4±136.9 (513.3±183.6)	598.2±141.2* (614.1±190.1)*	637.1±183.2*	499.3±121.0	463.3±148.2	466.1±130.8
AT E-modulus (GPa)	1.37±0.39 (1.27±0.39)	1.63±0.46* (1.55±0.45)*	1.69±0.44*	1.31±0.31	1.22±0.52	1.19±0.38
Mean AT CSA (mm <sup>2</sup> )	68.0±11.8 (69.4±12.3)	72.0±11.5* (73.5±13.5)*	71.5±11.3*	69.8±11.5	68.7±13.6	68.4±11.8
AT free length (mm)	36.5±9.6 (36.6±8.9)	37.4±9.8 (36.8±9.4)	37.8±9.6	36.8±8.9	36.7±9.5	37.9±9.0
SOL muscle thickness (mm)	12.8±2.1 (12.7±2.2)	13.9±1.9* (13.8±1.9)*	13.8±1.9*	12.8±2.0	12.6±1.8	12.4±1.7
GM muscle thickness (mm)	16.3±1.2 (17.0±2.1)	18.1±1.0* (18.6±1.7)*	18.6±1.3*	18.1±1.8	18.6±1.4	18.4±2.2
GM pennation angle (deg)	19.4±2.4 (19.5±2.8)	20.4±3.2* (20.7±3.4)*	20.8±2.6*	19.8±1.9	19.7±1.8	20.1±2.3
GM fascicle length (mm)	56.8±8.0 (56.9±8.9)	57.7±8.5 (58.1±9.5)	57.8±6.9	59.5±7.0	59.1±7.0	58.9±6.5

Note that there was a drop-out after 14 weeks and therefore we had one experimental group completing the long-term (N=12) and a second (in parentheses; N=21) completing only the medium-term intervention. CSA, cross-sectional area; AT, Achilles tendon; SOL, m. soleus; GM, m. gastrocnemius medialis. \*P<0.05 compared with baseline.

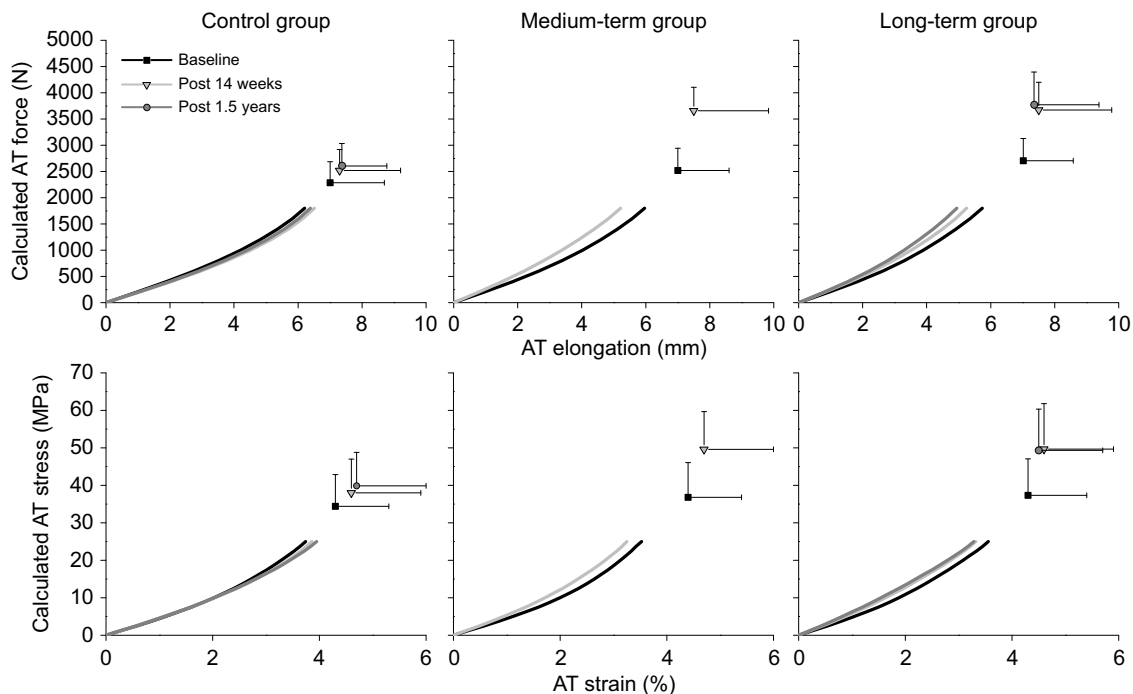
post 14 weeks and post 1.5 years measurements (Table 1). There were no statistically significant time point or subject group effects or interactions on maximal AT elongation or strain, or on the length of the free AT (Table 1). No significant differences in AT moment arm values were found between the control group (41.8±0.5 mm) and either experimental groups (medium-term 40.8±0.4 mm; long-term 39.6±0.3 mm). There were also no significant differences in subjects' age, body mass and body height between the experimental and control group (see 'Participants and experiment design').

## DISCUSSION

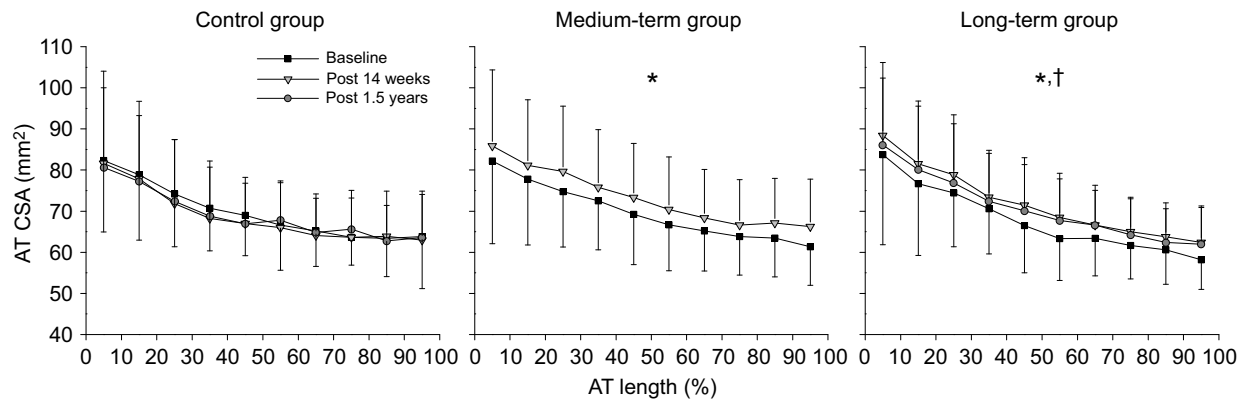
The current work aimed to examine the long-term (1.5 years) mechanosensitivity of AT biomechanical and morphological properties and their association with the muscle force production

capacity within a group of older individuals. Our hypotheses, that the tendon stiffness in older adults increases after medium-term exercise mainly due to changes in tendon material properties and that after long-term exercise, hypertrophy of the tendon might also induce changes in tendon mechanical properties, were partly confirmed, as tendon hypertrophy was identified after 14 weeks of an exercise intervention.

The cross-sectional study revealed a 33% higher tendon stiffness, 17% higher Young's modulus and a 12% higher mean free AT CSA along the entire tendon length for GroupStrong in comparison to GroupWeak (~42% difference in the TS muscle strength). These findings show that the higher tendon stiffness for the stronger subjects is caused by a larger tendon CSA in combination with an intrinsic tendon material improvement, indicating that the elderly AT may still be mechanosensitive as shown in earlier studies with



**Fig. 3. Achilles tendon measurements after exercise intervention.** Mean AT force–length (top row) and stress–strain (bottom row) relationships for the control (N=13) and medium-term group (N=21) at baseline and post 14 weeks, and for the long-term group (N=12) at baseline, post 14 weeks and post 1.5 years. Maximal values are shown as means and s.d. (error bars).



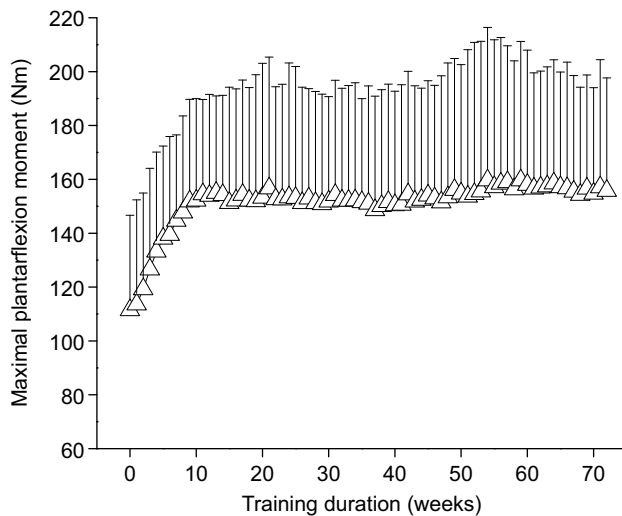
**Fig. 4. Achilles tendon measurements along the free tendon after exercise intervention.** AT cross-sectional area (CSA) in 10% intervals of the free tendon length for the control ( $N=13$ ) and medium-term group ( $N=21$ ) at baseline and post 14 weeks, and for the long-term group ( $N=12$ ) at baseline, post 14 weeks and post 1.5 years. All values are shown as means and s.d. (error bars). \* $P<0.05$  between baseline and post 14 weeks (medium-term) of exercise intervention values. † $P<0.05$  between baseline and post 1.5 years (long-term) of exercise intervention values.

younger adults (Arampatzis et al., 2007a; Bohm et al., 2014; Kongsgaard et al., 2007). However, one might argue that the above findings result from natural differences (genetic factors, diet and lifestyle etc.), not through mechanosensitivity. Therefore, in order to experimentally confirm whether tendons preserve their mechanosensitivity in old age and respond to an increased mechanical loading by inducing biomechanical and morphological changes, we conducted both medium- and long-term physical exercise interventions.

After the medium-term intervention period of 14 weeks, the experimental group demonstrated ~22% higher TS muscle strength along with a 9% increased SOL muscle thickness, 11% increased GM muscle thickness and 6% increased GM pennation angle, when compared with the baseline. In addition to the higher TS muscle strength, a 23% increase in AT stiffness was found in comparison to baseline. These findings, combined with results from the cross-sectional investigation, imply that the elderly AT remains capable of adapting its properties to the mechanical environment. Increases in tendon stiffness may be related to changes in the tendon's material, as well as its dimensions. In the current study, an increment in Young's modulus (material properties) by 20% was identified in the experimental group after the medium-term intervention. These exercise-related increases in tendon stiffness and Young's modulus are comparable with earlier medium-term (12–14 weeks) exercise studies with younger adults using similar loading magnitudes on the AT (increases of 16–36% and 15–45% respectively; Arampatzis et al., 2007a, 2010; Bohm et al., 2014; Fletcher et al., 2010; Foure et al., 2013; Kubo et al., 2007a). Moreover, we found a significant increase of 6% in average AT CSA after the medium-term (14 weeks) exercise intervention which is consistent with previous studies in younger adults (mean AT CSA increases of between 0.5 and 10%; Arampatzis et al., 2007a, 2010; Bohm et al., 2014; Kongsgaard et al., 2007). When investigating the possible region-specific effects, we did not find any significant group $\times$ time $\times$ interval interaction, indicating that the tendon hypertrophied uniformly along the whole tendon. Thus, using protocols with fewer scans (e.g. 3 scans: proximal, central and distal) would have been sufficient for the current longitudinal study. In a similar manner, our cross-sectional investigation showed, for GroupStrong in comparison to GroupWeak, a greater AT CSA along the whole tendon rather than region-specific differences. The above findings are somewhat surprising, as previous cross-sectional and longitudinal investigations with young adults reported region-specific changes

in tendon CSA (Arampatzis et al., 2007a; Kongsgaard et al., 2007; Magnusson and Kjaer, 2003; Seynnes et al., 2009), which may be related to regional differences in strain during muscular contractions for the tendon (Pearson et al., 2014). Whether older adults show less-pronounced regional strain variations during muscular contractions cannot be answered because the characteristics of localized human tendon strain *in vivo* are largely unknown. Additional investigations are needed to verify whether a uniform AT hypertrophy is more likely to occur in older compared with younger adults and to examine whether regional strain variation in the AT is affected by aging.

After the 1.5 year exercise intervention, the experimental group had a TS muscle strength of  $145.9\pm 30.2$  Nm (25% increase from baseline), AT stiffness  $637.1\pm 183.2$  N mm $^{-1}$  (30% increase), Young's modulus  $1.69\pm 0.44$  GPa (23% increase) and mean AT CSA of  $71.5\pm 11.3$  mm $^2$  (5% increase). However, the *post hoc* tests revealed no significant differences compared with the values after 14 weeks of exercise intervention, indicating maintenance of, rather than further increments in the analyzed parameters. This is confirmed by the muscle architecture results, as no changes in SOL or GM muscle thickness or GM muscle pennation angle were found (an enlargement would have implied increased muscle strength). The maintenance of material and morphological properties after 1.5 years of exercise intervention may be partly explained by the lack of any further increase in the TS muscle strength. When considering all TS muscle strength measurements prior to each training session, we identified a plateau after about 11–12 weeks of exercise, from where the increment of the maximal plantarflexion moment stagnated and did not increase further during the 1.5 year exercise intervention (see Fig. 5). Knowing that the AT biomechanical properties are associated with the TS muscle strength (Arampatzis et al., 2007b; Kubo et al., 2011; Stenroth et al., 2012), a plateau in the increment would also be expected for the AT properties, as the muscle strength was not altered further. When conducting a correlation analysis between TS muscle strength and tendon biomechanical properties, we found similar correlations as in previous literature in the current older female adults ( $r=0.512$ ,  $P=0.017$ ;  $r=0.484$ ,  $P=0.020$ ; and  $r=0.473$ ,  $P=0.028$  for mean AT CSA, AT stiffness and Young's modulus, respectively). Therefore 14 weeks of increased mechanical loading seems to be a sufficient time period to trigger adaptive changes in the mechanical, material and morphological AT properties in both younger and older adults, despite the potential general deterioration in structure and function



**Fig. 5. Ankle plantarflexion moment measurements throughout the exercise intervention.** The weekly maximal isometric ankle plantarflexion moment (means and s.d.) over 1.5 years of exercise intervention in the experimental group ( $N=12$ ).

of older tendons. Furthermore, it appears that in the elderly, adaptations in AT properties in response to loading are adjusted in a way that the maximal strain of the tendinous structures is kept relatively constant, as seen in younger adults (Arampatzis et al., 2007a). However, unlike in muscles, it has to be ensured that the exercise intervention implements tendon strains corresponding to high mechanical loading (intensities of at least 80–90% of MVC) to induce tendon adaptation (Arampatzis et al., 2007a; Grosset et al., 2014). Similar to adaptations to loading, a relatively fast deterioration in the tendon mechanical, material and morphological properties has been shown during bed rest and paralysis (Kubo et al., 2000; Maganaris et al., 2006; Reeves et al., 2005), as well as training–detraining studies (Kubo et al., 2012). Such fast adaptation caused by high loads and deterioration following disuse or detraining indicates that the homeostatic response to changes in mechanical loading is a relatively quick process, occurring within 12–14 weeks, in both younger and older adults. It is also important to note that in the control group we found no significant differences in tendon biomechanical properties between the measurement sessions which is an interesting finding as we were expecting to find an age-related reduction in AT stiffness after 1.5 years. However, we believe that the potential age-related changes in tendon properties in 1.5 years were obviously too small to detect *in vivo*.

In earlier studies with older adults by Grosset and colleagues (2014) and Reeves et al. (2003a), no change in PT CSA was detected following 12 weeks of resistance training. Based on our results (tendon hypertrophy along the whole tendon in response to an increased mechanical loading), this may not have resulted from the low number of scans. However, the 9–14% increase in quadriceps femoris muscle strength found in these studies was markedly lower than the increases in TS muscle strength in the current study. The differences of ~42% between GroupStrong and GroupWeak in the cross-sectional investigation and the increment of 22% (after 14 weeks of exercise) or 25% (after 1.5 years) may have been sufficient to cause dimensional differences in the AT.

When considering the cross-sectional approach, the stronger older adults possessed a higher tendon stiffness (~33% higher than weaker older adults) with similar contributions from the AT Young's modulus (~17% higher) and the mean free AT

CSA (~12% higher). However, when the AT stiffness was experimentally increased (~30%) over 1.5 years of exercise, the adaptive changes in the tendon structure (~23% higher Young's modulus versus ~5% higher AT CSA compared with baseline) seemed to be the main mechanism of tendon adaptation to the altered mechanical environment. Accordingly, the suggestions that changes in material properties in the early stages of training may be transient (Wiesinger et al., 2015) and that long-term increases in tendon stiffness are mainly accomplished via tendon hypertrophy (Couppé et al., 2008; Ker et al., 1988; Maganaris and Paul, 2002) might not be the case for tendons in old age. According to the current study, the long-term increase in tendon stiffness in older adults seems to be a combined result of changes in the tendon's material and dimensions, with the adaptation in material properties appearing to play a more decisive role in the change in tendon stiffness.

Concerning the limitations of the longitudinal part of our study, one might argue that we used a different number of subjects between 14 weeks and 1.5 years of exercise intervention ( $N=21$  versus  $N=12$ ). However, when analyzing the relative changes after 14 weeks of exercise in comparison to baseline between those subject groups, we did not find any significant differences in the analyzed parameters (TS muscle strength, muscle architecture, AT stiffness, Young's modulus, mean AT CSA) after the exercise period. A further potential limitation of the current study may be the position of the subjects in the MRI scanner, where the subjects' hips and knees were fully extended with the ankle joints fixed at 20 deg plantarflexion, which does not match the joint configuration used for the tendon biomechanical properties assessment (ankle and knee fixed at 90 deg angle). This specific ankle joint angle configuration during the MRI scans was chosen to provide the most comfortable position for the older adults, as we additionally scanned the entire calf musculature (data not presented in this study) as part of a 40 min MRI protocol. Although not previously investigated, it cannot be excluded that differences in the ankle joint angle would significantly affect AT CSA. If this was the case, this may potentially lead to errors in our Young's modulus calculations in absolute terms. However, because we used the same joint angle configurations for all of our MRI measurements, we believe that this drawback does not significantly affect our main finding with respect to the relative differences between subject groups or time points. Another drawback might be the positioning of the ultrasound probe for the tendon displacement measurements, where the MTJ of GM was analysed instead of the more distal MTJ of the m. soleus, as the tendon CSA was inspected in the free Achilles tendon. It has to be pointed out, that to detect and analyse the displacement of MTJ of the m. soleus in this group of older adults during contraction was particularly problematic and in some cases even impossible, which would have delivered inaccurate findings. Furthermore, while our average maximal ankle plantarflexion moment and maximal tendon strain at baseline were similar to previous studies (see Table 2), our calculated maximal AT force was higher and the maximal tendon elongation was slightly lower than in earlier studies. As a consequence, our higher tendon stiffness values may be associated with differences in the assessed Achilles tendon moment arms and tendon resting lengths, when compared with previous literature (see Table 2).

Not accounting for the moment contributions of other agonist and antagonist muscles is an additional limitation, potentially affecting the tendon stiffness and Young's modulus values calculated in absolute terms more than the validity of the corresponding comparative data. As a result of the knee being flexed at 90 deg during training and testing, it is possible that the SOL muscle contributed more than the gastrocnemius muscle to the increased TS muscle strength. However,



**Table 2. Biomechanical properties of the triceps surae muscle-tendon unit in young and older adults from different studies in comparison to the mean values of the current study (baseline values of all older adults)**

Study	Subjects (N, sex)	US probe placement	Max joint moment (Nm)	Max calculated ATF (N)	AT MA (mm)	AT resting length (mm)	Max $\Delta L$ (mm)	Max strain (%)	Tendon stiffness (N mm <sup>-1</sup> )
Young adults									
Magnusson et al. (2001)	5M	GM <sub>APO</sub>	161±11	3255±206	51±1	248±11	10.7±1.3	4.4±0.5	467
Maganaris and Paul (2002)	6M	GM <sub>APO</sub>	162±11	875±85	63±4	225±20	11.1±3.1	4.9±1.0	150±28
Lichtwark and Wilson (2005)	10M	GL <sub>MTJ</sub>	–	–	–	–	–	8.3±2.1	188±43
Kubo et al. (2007a)	10M	GM <sub>APO</sub>	116±23	–	–	–	13.7±2.3	–	129±36
Arampatzis et al. (2007a)	8M, 3F	GM <sub>APO</sub>	114±13	2093±325	–	275±36	12.4±3.7	4.6±1.5	187±38
Peltonen et al. (2010)	10M	GM <sub>MTJ</sub>	–	3500±600	49±4	212±17	–	6.2	430±200
Houghton et al. (2013)	7M	GM <sub>MTJ</sub>	–	5694±715	–	186±19	14.3±3.4	7.7±2.0	940±473
Bohm et al. (2015b)	36M	GM <sub>MTJ</sub>	234±38	4436±726	53±6	213±28	14.0±2.5	6.7±1.5	339±114
Older adults									
Morse et al. (2005)	9M	GL <sub>MTJ</sub>	81±14	–	–	–	–	7.6±1.2	–
Karamanidis and Arampatzis (2006)	20M	GM <sub>APO</sub>	92±20	1413±333	–	268±27	–	6.0±1.9	–
Kubo et al. (2007b)	17M	GM <sub>APO</sub>	64±21	–	–	–	9.8±2.7	3.8±1.0	–
Stenroth et al. (2015)	26M	GM <sub>MTJ</sub>	–	1021±237	–	–	–	–	160±33
Current study	34F	GM <sub>MTJ</sub>	117±26	2830±614	41±4	161±18	7.0±1.6	4.3±1.1	491±128

Values are means±s.d. M, males; F, females; GM<sub>APO</sub>, gastrocnemius medialis aponeurosis; GM<sub>MTJ</sub>, gastrocnemius medialis myotendinous junction; GL<sub>MTJ</sub>, gastrocnemius lateralis myotendinous junction; ATF, Achilles tendon force; AT, Achilles tendon; MA, moment arm;  $\Delta L$ , tendon elongation.

because of the relatively homogenous changes in SOL and GM muscle thickness (about 10% increment in both muscles), it seems that the observed exercise-related increment in the maximal voluntary isometric plantarflexion moment and Achilles tendon properties were not solely caused by a strength increment of the SOL muscle. Lastly, the results showed no significant increase of the analyzed parameters when comparing the results after 14 weeks and after 1.5 years of exercise intervention, which indicates that another mechanical stimulus may be required to trigger an increase the TS muscle strength after reaching the plateau after 11–12 weeks (see Fig. 5) to stimulate further adaptive changes in the Achilles tendon in older female adults. It should be stated, that post-menopausal elderly women have a diminished tendon modulation to exercise (Onambele-Pearson and Pearson, 2012). However, whether or not the identified stagnation in the muscle and tendon adaptation can be related to a possible sex-specific reduction in the ability to respond to the anabolic stimuli in the musculotendinous tissue (Hansen and Kjaer, 2014) cannot be answered based on the current study design. Therefore the role of sex-specific anabolic resistance on the stagnation of muscle adaptation deserves a systematic investigation.

In conclusion, the current work provides evidence that the human AT preserves its mechanosensitivity at older age and is capable of increasing its stiffness in response to long-term mechanical loading exercise by changing both its structure and dimensions, and may thereby tolerate higher mechanical loading. However, adaptations in AT material properties due to long-term mechanical loading appear to be the key factor in the increased tendon stiffness observed in older female adults. Moreover, our controlled AT cyclic loading exercise intervention over 1.5 years shows that a medium-term intervention

over 14 weeks is obviously sufficient to trigger AT hypertrophy. Continuing AT cyclic loading exercise seems to maintain rather than cause further structural and dimensional adaptive changes in older adult tendons, suggesting that the adaptive time–response relationship of tendons subjected to mechanical loading is nonlinear.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

G.E., G.-P.B. and K.K. conception and design of research; G.E., A.M., J.D., J.A.L. and K.K. performed experiments; G.E. and K.K. analyzed data; G.E., C.N.M., G.-P.B. and K.K. interpreted results of experiments; G.E. and K.K. prepared figures; G.E. and K.K. drafted manuscript; G.E., A.M., J.D., J.A.L., L.S., G.M.K., H.B., C.N.M., G.-P.B. and K.K. edited and revised manuscript; G.E., A.M., J.D., J.A.L., L.S., G.M.K., H.B., C.N.M., G.-P.B. and K.K. approved final version of manuscript.

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