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Adaptational responses of the human Achilles tendon by modulation of the applied cyclic strain magnitude

Adamantios Arampatzis*, Kiros Karamanidis and Kirsten Albracht
German Sport University of Cologne, Institute of Biomechanics and Orthopaedics, Carl-Diem-Weg 6, 50933 Cologne, Germany
*Author for correspondence (e-mail: Arampatzis@dshs-koeln.de)

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Summary

Tendons are able to remodel their mechanical and morphological properties in response to mechanical loading. However, there is little information about the effects of controlled modulation in cyclic strain magnitude applied to the tendon on the adaptation of tendon's properties *in vivo*. The present study investigated whether the magnitude of the mechanical load induced as cyclic strain applied to the Achilles tendon may have a threshold in order to trigger adaptation effects on tendon mechanical and morphological properties. Twenty-one adults (experimental group, *N*=11; control group, *N*=10) participated in the study. The participants of the experimental group exercised one leg at low-magnitude tendon strain (2.85±0.99%) and the other leg at high-magnitude tendon strain (4.55±1.38%) of similar frequency and volume. After 14 weeks of exercise intervention we found a decrease in strain at a given tendon force, an increase in tendon-aponeurosis stiffness and tendon elastic modulus and a region-specific hypertrophy of the Achilles tendon only in the leg exercised at high strain magnitude. These findings provide evidence of the existence of a threshold or set-point at the applied strain magnitude at which the transduction of the mechanical stimulus may influence the tensional homeostasis of the tendons. The results further show that the mechanical load exerted on the Achilles tendon during the low-strain-magnitude exercise is not a sufficient stimulus for triggering further adaptation effects on the Achilles tendon than the stimulus provided by the mechanical load applied during daily activities.

Key words: MRI, ultrasonography, tendon plasticity, *in vivo*, exercise, strain.

Introduction

Mechanical load induced as cyclic strain, imposed externally to fibrous connective tissues such as tendons, may induce several signals at the extracellular matrix. This happens through mechanotransduction pathways affecting the anabolic as well as the catabolic responses (Brown et al., 1998; Hsieh et al., 2000; Zeichen et al., 2000). It has been reported that the cells sense the applied strain (Chiquet, 1999; Chiquet et al., 2003) and regulate the synthesis of matrix proteins (Arnoczky et al., 2002; Skutek et al., 2003; Miller et al., 2005), the gene expression of proteoglycans and collagen (Robbins and Vogel, 1994; Kim et al., 2002; Hsieh et al., 2000), the alignment and density of the collagenous matrix (Pins et al., 1997; Wang et al., 2001; Wang et al., 2003a) as well as the expression of several growth factors (Skutek et al., 2001; Yang et al., 2004; Olesen et al., 2006). Studies examining the influence of mechanical loading on the regulation of the biosynthesis of connective soft tissues demonstrated that the strain magnitude, strain frequency, strain rate and strain duration of cells influence the cellular biochemical responses (Skutek et al., 2003; Arnoczky et al., 2002; Yang et al., 2004) and the mechanical properties of collagen fascicles (Yamamoto et al., 2002; Yamamoto et al., 2003; Yamamoto et al., 2005). Furthermore, the above studies illustrate the highly plastic nature of fibrous connective tissues and provide evidence that strain of tendon cells is an important regulator for the homeostasis of connective tissues.

More than two decades ago, Woo et al. (Woo et al., 1982) formulated the hypothesis that the homeostatic responses of soft tissues subjected to mechanical loads may be represented by a non-linear curve. Immobilisation causes a rapid decline in the mechanical properties whereas long-term exercise initiates a slight increase in mechanical properties compared with normal daily activities (Woo et al., 1982). More recently, it has been suggested that the applied strain on the connective tissues may have a threshold or set-point to create a homeostatic perturbation in the collagenous matrix that regulates the catabolic and anabolic responses of the cells (Brown et al., 1998; Lavagnino and Arnoczky, 2005). An external mechanical loading of the tissue above the upper limit at which the endogenous contraction of the fibroblasts may maintain their tensional homeostasis should stimulate cells for remodelling, whereas a reduction of the mechanical loading below the lower limit will lead to tissue destruction (Lavagnino and Arnoczky, 2005; Lavagnino et al., 2006).

In agreement with both the hypothesis formulated by Woo et al. (Woo et al., 1982) and the concept of the 'homeostatic calibration point' (Lavagnino and Arnoczky, 2005; Lavagnino et al., 2006), which supports the existence of an upper and a lower

limit determining a homeostatic perturbation, we did not find a graded response between exercise intensity and mechanical properties of the human triceps surae tendon and aponeurosis by comparing sprinters, endurance runners and subjects not being active in sports (Arampatzis et al., 2007a). Only the sprinters group showed a higher stiffness at the triceps surae tendon and aponeurosis compared with the other two groups examined (Arampatzis et al., 2007a). Further, we suggested (Arampatzis et al., 2007a) that the mechanical properties of the human triceps surae tendon and aponeurosis remain at control level in a wide range of applied strains and that the strain magnitude, strain frequency and strain rate should exceed a given threshold in order to trigger additional adaptation effects. Although numerous important studies have previously demonstrated the plasticity of human tendons in response to resistance exercise (Kubo et al., 2001a; Kubo et al., 2001b; Kubo et al., 2002; Reeves et al., 2003a; Reeves et al., 2003b; Reeves et al., 2005), and even though it is well accepted that tendons are able to remodel their mechanical and morphological properties in response to mechanical loading, there is little information about the effects of controlled modulation in cyclic strain magnitude, frequency or rate applied to the tendon on the adaptation of the mechanical and morphological properties of tendons *in vivo*. Thus, it may be concluded that the tendon responses to different cyclic strain magnitudes *in vivo* remain a fundamental unanswered question. Knowledge of tendon plasticity in response to the magnitude of the mechanical load induced as cyclic strain applied to the tendon may help improve the intervention process of tendon adaptation and tendon healing.

The purpose of this study was to examine the effect of two different exercise interventions of cyclic strain applied to the Achilles tendon on the adaptation of its mechanical and morphological properties. Both interventions were performed at the same frequency and volume but at different magnitudes of tendon strain (2.85±0.99% *vs* 4.55±1.38% strain). Based on reports about human tendon plasticity (Kubo et al., 2001a; Kubo et al., 2001b; Kubo et al., 2002; Reeves et al., 2003a; Reeves et al., 2003b; Reeves et al., 2005), the concept of the homeostatic calibration point (Lavagnino and Arnoczky, 2005; Lavagnino et al., 2006) and the non-graded response of the mechanical properties of the human triceps surae tendon and aponeurosis in an intensity-dependent manner of sport activity (Arampatzis et al., 2007a), we expected an adaptation effect on the Achilles tendon only after the high-strain-magnitude exercise intervention, demonstrating a threshold in strain magnitude for further adaptational effects *in vivo*.

Materials and methods

Participants

Twenty-one healthy, not strength-trained, adults (23–42 years old) from the university population participated in the study after giving informed consent to the experimental procedure, complying with the rules of the local scientific board. Eleven of them (eight females and three males; 64.1±5.0 kg body mass, 172±5 cm body height, 29.5±5.0 years old; means ± s.d.) were recruited for the experimental group (exercise intervention). The remaining 10 participants (six females and four males; 70.4±4.5 kg body mass, 172±4 cm body height, 28.6±4.5 years old) formed the control group (no exercise intervention).

Exercise protocol

The intervention lasted 14 weeks. Four times per week the experimental group performed five sets of repetitive (3 s loading, 3 s relaxation), isometric plantar flexion contractions (ankle angle at 85° dorsal flexion, knee angle fully extended at 180° and the hip flexed at 140°). Repetitive isometric plantar flexion contractions were used to induce cyclic strains on the triceps surae tendon and aponeurosis. The participants exercised one leg at low-magnitude tendon–aponeurosis strain (low-strain-magnitude exercise) and the other leg at high-magnitude tendon–aponeurosis strain (high-strain-magnitude exercise). The assignment of low and high strain exercise to each leg was random. Based on earlier experience (Arampatzis et al., 2005a; Mademli et al., 2006), we predicted that a plantar flexion moment at 55% of the achieved maximum moment during a maximum voluntary contraction (MVC) should induce a tendon–aponeurosis strain between 2.5 and 3.0% whereas a plantar flexion moment at 90% of the MVC should induce between 4.5 and 5.0% strain. At each set, the leg exercised at high strain magnitude performed four repetitions (3 s loading, 3 s relaxation) at 90% of the MVC whereas the other leg (low strain magnitude) performed seven repetitions at 55% of the MVC (Fig. 1). This way (four *vs* seven repetitions per set for the high- and the low-strain-magnitude exercise, respectively), both legs were trained at the same exercise volume (integral of the plantar flexion moment over time). The above experimental design provided an intervention of similar frequency and volume but different magnitude of applied cyclic strain to the triceps surae tendon and aponeurosis of each trained leg.

The exercise intervention was performed on a dynamometer (Biodex-System 3; Biodex Medical Systems, Inc., Shirley, NY, USA). During the 14 weeks of intervention and at each exercise set, the participants had to match the target moment (55 or 90% of MVC; 3 s loading, 3 s relaxation) displayed on a screen (Fig. 1). Before and after the intervention we examined the maximum plantar flexion moment during a MVC, the voluntary activation (VA) during the MVC and the elongation–force relationship of the triceps surae tendon and aponeurosis from all 21 participants. For the exercise group, we additionally measured the cross-sectional area (CSA) of the Achilles tendon of both legs. None of the examined subjects (exercise and control group) participated in any other organised exercise activity during the 14 weeks.

Measurement of plantar flexion moment and voluntary activation

The subjects were seated on a dynamometer (Biodex-System3) with the ankle angle in a dorsal flexed position at 85° (tibia perpendicular to the sole, corresponding to 90° ankle angle), the knee fully extended at 180° and the hip flexed at 140°. In this position, the subjects performed maximal isometric plantar flexion contractions. After a warm-up period, consisting of 2–3 min submaximal isometric contractions, and three MVCs the participants were instructed to produce a maximal isometric force ramp with the highest possible rate of force generation. We used the three MVCs to exclude the preconditioning effect on the tendon strain–force relationship. The three MVCs at the beginning of the intervention should not have any substantial training effect. The twitch interpolation technique (Merton,

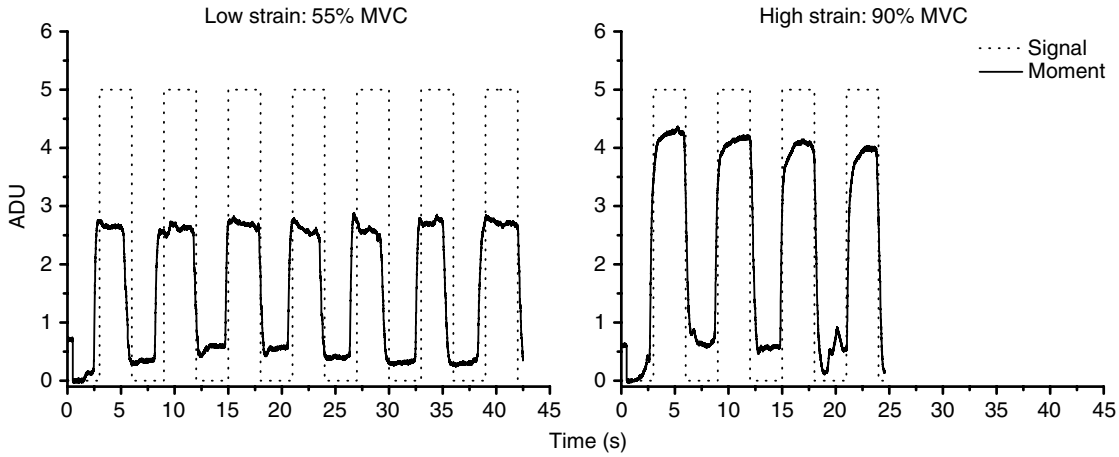

Fig. 1. Each training day of the intervention protocol consisted of five sets of repetitive (3 s loading, 3 s relaxation) isometric plantar flexion contractions to induce cyclic strain on the triceps surae tendon and aponeurosis. One leg exercised at low-magnitude tendon–aponeurosis strain [55% of the maximum voluntary contraction (MVC)] whereas the other one exercised at high-magnitude tendon–aponeurosis (90% MVC). The total exercise volume (integral of the plantar flexion moment over time) was identical for both legs. Signal: signal displayed on a computer monitor (3 s loading, 3 s relaxation) for controlling the exercise loading. Moment: plantar flexion moment generated during an exercise set.

1954) was used to determine the VA of the plantar flexor muscles during the contraction. We evoked a superimposed twitch (three 500 μs square-wave pulses separated by 5 ms) at the plateau of the MVC and three supramaximal twitches after the MVC when the plantar flexor muscles were relaxed (Fig. 2) using a stimulator (Model DS7A digitimer; Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). The voluntary activation was calculated by normalising the evoked interpolated twitch torque (ITT) to the mean of the three resting twitch torques (RTT): VA=[1–ITT/RTT)×100].

The resultant moments at the ankle joint were calculated through inverse dynamics. The method for calculating the resultant joint moments has been previously described (Arampatzis et al., 2005b). Kinematic data were recorded using the Vicon 624 system (Vicon Motion Systems, Oxford, United Kingdom) with eight cameras operating at 120 Hz. To calculate the lever arm of the ankle joint during ankle plantar flexion, the centre of pressure under the foot was determined by means of a flexible pressure distribution insole (Pedar-System, Novel GmbH, Munich, Germany) operating at 99 Hz (Arampatzis et al., 2005b). The compensation of moments due to gravitational forces was determined for all subjects before each ankle plantar flexion contraction. The antagonistic moment of the tibialis anterior (TA) during MVC was estimated by establishing a relationship between electromyographic (EMG) activity and exerted moment for the TA, while working as agonist (Mademli et al., 2004). This was established by measuring EMG and moment during relaxation and during two submaximal ankle dorsiflexion contractions (Mademli et al., 2004).

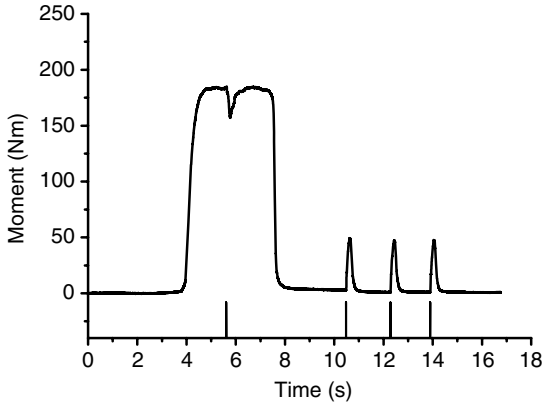

Fig. 2. Plantar flexion moment–time history during a maximum voluntary contraction (MVC) from one participant with superimposed twitches. A superimposed twitch was evoked through a triplet electrostimulation (three 500 μs square-wave pulses separated by 5 ms) at the plateau of the MVC. Three more twitches were evoked after the MVC, when the triceps surae muscles were relaxed. The vertical lines indicate the instant of the electrostimulation.

Measurement of tendinous tissue elongation

After the MVC with the superimposed twitches, the participants were instructed to produce another maximal isometric force ramp, gradually increasing the plantar flexion effort over 3 s (loading), and to hold the achieved moment for about 2–3 s. A 7.5 MHz linear array ultrasound probe (Aloka SSD 4000; Tokyo, Japan; 43 Hz) was used to visualise the distal tendon and aponeurosis of the gastrocnemius medialis (GM) during the MVC. The ultrasound images were recorded on video tapes for further analysis. On the video images, a clear visible cross-point (intersecting point between the distal aponeurosis and a fascicle of the GM muscle) was identified and its displacement was measured in relation to a skin marker (Fig. 3). The exact protocol for analysing the tendinous tissue elongation during ankle plantar flexion is described in detail elsewhere (Arampatzis et al., 2005a). Briefly, the ultrasound probe was placed above the muscle belly at about 50% of its length. For the analysis of the video tapes every single frame was digitised using video analysis software (Simi Motion 5.0; SIMI Reality Motion System GmbH, Unterschleißheim, Germany). The effect of inevitable joint angular displacement on the observed elongation of the tendon and aponeurosis during the MVC was

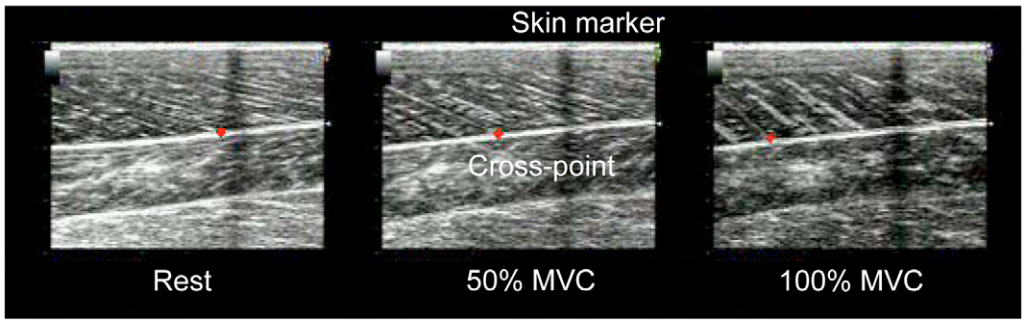

Fig. 3. Ultrasound images of the gastrocnemius medialis (GM) at rest, at 50% of the maximum voluntary contraction (MVC) and at the plateau of the MVC. The elongation of the tendon and aponeurosis was examined at the GM muscle belly at about 50% of its length. The displacement of the analysed cross-point in relation to the skin marker was defined as measured elongation of the tendon and aponeurosis.

taken into account by capturing the motion of the tendons and aponeuroses from the GM during a passive (inactive) motion of the ankle joint (Muramatsu et al., 2001). The passive motion of the ankle joint has been analysed during the plantar flexion because the angular rotation at the ankle joint during the 'isometric' MVC was also a plantar flexion. The error of this method on the strain value is ~0.3% and, thus, has a negligible effect on the examined *in vivo* strain of the tendon and aponeurosis (Arampatzis et al., 2007b). The analysed cross-point at the aponeurosis was digitised during the inactive condition at the same ankle angle changes as observed during the MVC (Arampatzis et al., 2005a). The elongation of the GM tendon and aponeurosis was calculated as the difference between the measured and the passive (due to joint rotation) displacement of the analysed point at the aponeurosis (Fig. 4).

In order to estimate the resting length of the GM tendon and aponeurosis, the subjects were seated on the dynamometer with the knee at 180° and the ankle at 110°. We used this specific position because De Monte et al. (De Monte et al., 2006) reported the existence of slackness in the inactive GM muscle–tendon unit between 121° and 107° ankle angle and 180° knee angle and that the 110° ankle angle is a suitable position to examine the resting length of the GM tendon and aponeurosis. The length of the curved path from the tuberositas calcanei (defined as the origin of the Achilles tendon) to the skin marker (Fig. 3) was measured along the skin using flexible measuring tape. Thus, the resting length of the GM tendon and aponeurosis was defined as the length of the path between the tuberositas calcanei and the analysed cross-points identified on the ultrasound images. The tendon force was calculated by dividing the plantar flexion moment by the tendon moment arm. The moment arm of the Achilles tendon was calculated using the data provided by Maganaris et al. (Maganaris et al., 1998). The elongation and strain of the tendon and aponeurosis during the MVC was identified and analysed at the maximum calculated tendon force and at every 100 N. The stiffness of the triceps surae tendon and aponeurosis has been calculated as the slope of the calculated tendon force *vs* tendon–aponeurosis elongation between 50% and 100% of the maximum tendon force by means of linear regressions.

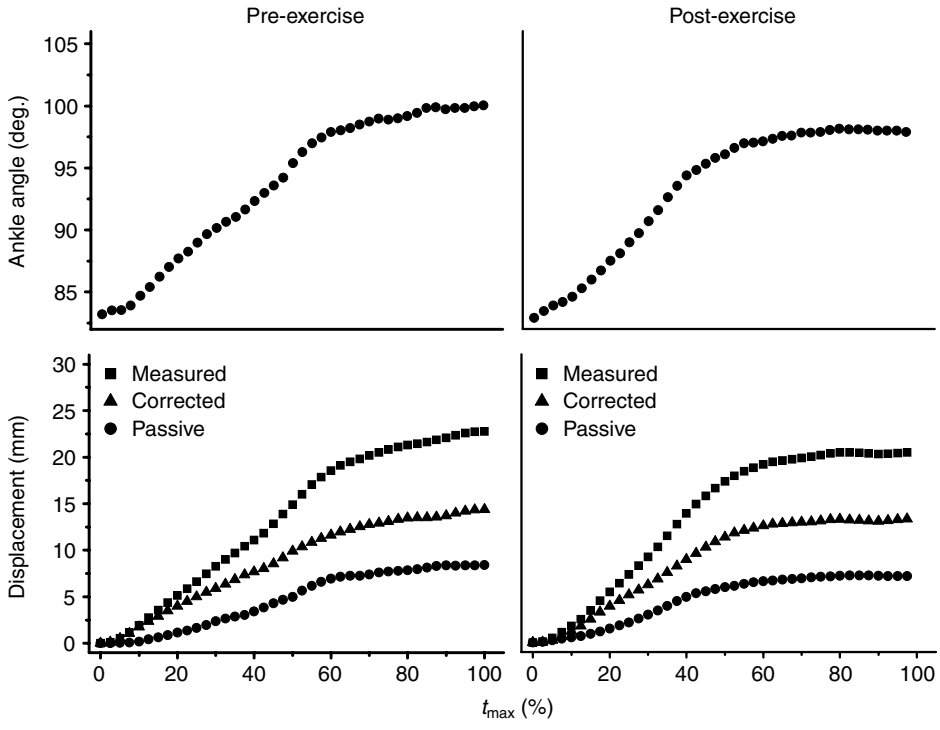

Fig. 4. Mean curves (N=11) of the ankle angles and tendon–aponeurosis displacement during the maximum voluntary contraction (MVC) at the high-strain-magnitude exercised leg before (pre-exercise) and after (post-exercise) the intervention. The elongation of the tendon and aponeurosis (corrected) was calculated as the difference of measured displacement (measured) and the passive displacement due to ankle joint rotation (passive) of the analysed cross-point at the aponeurosis of the gastrocnemius medialis. t_{max}: time to achieve maximum tendon force.

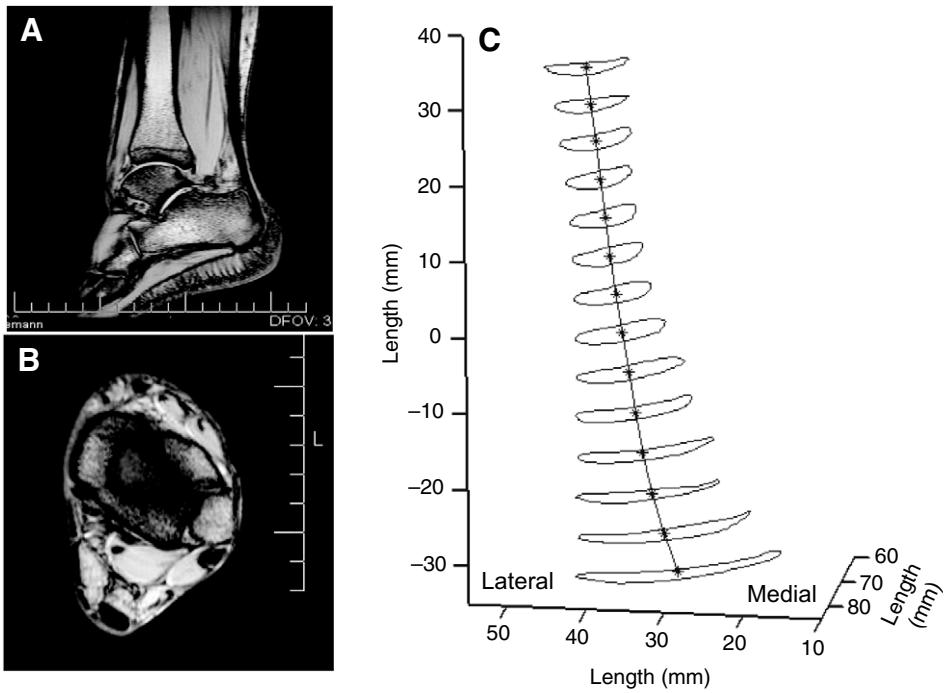

Fig. 5. Sagittal (A) and transversal (B) magnetic resonance images as well as the digitised Achilles tendon boundaries (C). The sagittal images served to obtain the location of the most proximal aspect of the tuberositas calcanei and the most distal aspect of the soleus muscle. On each transversal image, the boundaries of the Achilles tendon were outlined manually. The length of the Achilles tendon was calculated as the curved path passing through the centroids of the cross sections (C).

Measurement of the CSA of the Achilles tendon

In order to determine the CSA of the Achilles tendon along its length, transversal and sagittal T1 weighted magnet resonance (MR) images (Fig. 5) were recorded using a scanner (Magnetom Symphony; Siemens, Erlangen, Germany) with a magnetic strength field of 1.5 T and an image frequency of 64 MHz. For the transversal images, the scanning parameters were TR/TE 590/11, FOV 29.9×29.9 cm, pixel size 0.58594×0.58594 mm, slice thickness 4 mm, spacing between slices 0.8 mm. For the sagittal images, the parameters were TR/TE 665/11, FOV 29.9×29.9 cm, pixel size 0.58594×0.58594 mm, slice thickness 3 mm, spacing between slices 0.6 mm. Throughout the scan process the subjects laid unloaded in a supine position. No muscle contraction was apparent during the measurements.

To standardise the levels of the transversal images, two landmarks, the most proximal aspect of the tuberositas calcanei and the most distal aspect of the soleus muscle, were utilised. The sagittal images served to obtain the locations of both points. On each transversal image, the boundaries of the Achilles tendon were outlined manually using the software 3D Doctor (Able Software Corp., Lexington, MA, USA). The tendon boundaries and the coordinates of the two landmarks were exported and processed using Matlab (The Mathworks, Natick, MA, USA). For each of the subsequent cross-sections, the area and the location of the centroid were calculated. The length of the Achilles tendon was calculated as the curved path through the centroids of the cross-sections between the two landmarks. The CSA of the Achilles tendon was identified and analysed at every 10% of tendon length. To examine the elastic modulus of the Achilles tendon we calculated the relationship between tendon stress and tendon–aponeurosis strain from 50% to 100% of the maximum tendon stress by means of linear regressions. To calculate the tendon stress (tendon force/tendon CSA) we used the average value of the CSA of the Achilles tendon from 10% to 100% of the tendon length.

Statistics

A *T*-test for two dependent samples was used to check the intervention-related differences in the examined parameters (maximum plantar flexion moment, voluntary activation, tendon–aponeurosis strain at every 100 N and CSA of the Achilles tendon at every 10% of the tendon length) in each group. Further, to check the ratios (post- to pre-exercise values) of the examined parameters we used a one-way analysis of variance (ANOVA) and Bonferroni *post-hoc* comparisons between the three groups (control group, no exercise intervention; experimental group 1, low-strain-magnitude exercise intervention; experimental group 2, high-strain-magnitude exercise intervention). The level of significance for all comparisons was set to α=0.05. In all figures, the data are presented as means ± standard error of mean (s.e.m.), whereas in the text and tables they are expressed as means ± standard deviation (s.d.).

Results

The body mass of neither the experimental nor the control group changed after the 14 weeks of intervention (experimental: 64.1±5.0 kg pre-exercise, 64.8±4.8 kg post-exercise; control: 70.4±4.5 kg pre, 70.2±4.3 kg post). The mean values of the applied tendon–aponeurosis strain during the exercise intervention were 2.85±0.99% and 4.55±1.38% for the 55% and 90% MVC-exercised legs, respectively. After the 14 weeks of intervention, the maximum plantar flexion moment and the maximum calculated tendon force showed a statistically significant increase (P<0.05) in both exercise protocols (Table 1). However, statistically significant changes in the maximum elongation and strain before and after the exercise were found only in the low-strain-exercise intervention. Both

Table 1. *Comparison of the maximum values of the examined parameters during the maximal voluntary contraction before (pre-exercise) and after (post-exercise) the intervention*

	Low strain (*N*=11)		High strain (*N*=11)		Control (*N*=10)	
Parameter	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre	Post
Moment (Nm)	114.4±12.7	137.7±15.8*	109.5±15.2	144.1±22.7*	135.0±38.3	141.2±39.2
Force (N)	2093±325	2688±350*	2084±447	2992±444*	2702±896	2869±735
Activation (%)	96.7±3.3	98.8±0.9	98.2±1.7	98.9±1.3	98.1±1.2	97.3±1.8
Elongation (mm)	12.4±3.7	14.4±3.4*	13.8±4.3	13.6±2.8	15.1±2.6	16.2±3.4
Strain (%)	4.6±1.5	5.4±1.3*	4.8±1.6	4.8±0.9	5.1±0.9	5.5±1.2
Rest length (mm)	275.0±36.2	272.4±38.9	288.6±20.6	282.8±14.8	294.3±20.8	293.9±24.5
Stiffness (N mm^{-1})	186.7±38.3	201.4±41.2	167.7±36.8	228.1±39.7*	180.2±42.5	184.1±39.7

For the Control group, 'pre' corresponds to the values at the start of the experiment and 'post' corresponds to the values at the end of the experiment. Moment is the maximum plantar flexion moment, force is the maximum calculated tendon force, activation is the voluntary activation, elongation is the maximum elongation of the tendon and aponeurosis, strain is the maximum strain of the tendon and aponeurosis, rest length is the length of the curved path from tuberositas calcanei to the examined cross-point on the ultrasound images, and stiffness is the tendon–aponeurosis stiffness of the triceps surae. Low strain is the low-strain-magnitude exercise intervention, high strain is the high-strain-magnitude exercise intervention, and control is the group without any specific exercise during the 14 weeks of the intervention.

*Statistically significant differences between pre- and post-exercised values (P<0.05).

the maximum elongation and maximum strain were higher after the intervention in the low-strain-exercised leg (Table 1). Tendon–aponeurosis stiffness increased significantly (P<0.05) only in the high-strain-exercised leg (Table 1). The control group did not show any statistically significant (P>0.05) differences in the above-reported parameters (maximum moment and strain and tendon–aponeurosis stiffness) before and after 14 weeks (Table 1). The voluntary activation during the maximal voluntary plantar flexion efforts were, on average, 97–99% and were similar (no statistically significant changes, P>0.05) before and after the intervention for both experimental groups and the control group (Table 1).

The ratios (post- to pre-exercise values) of the maximum plantar flexion moment of the two exercised legs were significantly higher than those of the control group, and the moment ratio of the high-strain-exercised leg was significantly (P<0.05) higher than that of the low-strain-exercised leg (Fig. 6). These results indicate a higher increase in the maximum plantar flexion moment for the leg exercised at 90% of the MVC. The maximum strain ratios (post- to pre-exercise) of the control and the high-strain-exercised leg did not show any statistically significant differences (P>0.05), whereas the strain ratio for the low-strain-exercised leg was higher than those of the other two groups (Fig. 6).

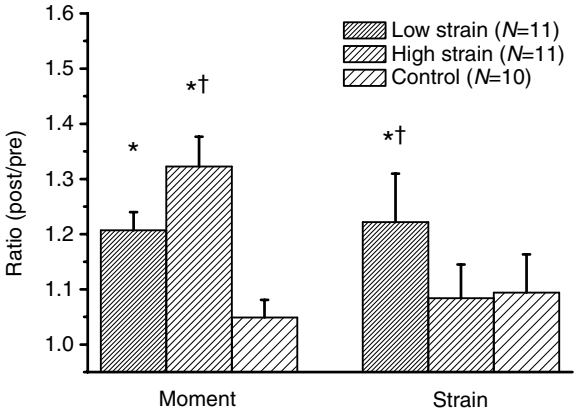

Fig. 6. Ratio (post- to pre-exercise values) of the maximum plantar flexion moment and strain of the triceps surae tendon and aponeurosis during the maximum voluntary contraction (MVC). *Statistically significant differences to the control group (P<0.05). †Statistically significant differences between low- and high-strain-magnitude exercised legs (P<0.05).

After the 14 weeks intervention applying cyclic loading to the Achilles tendon, the tendon–aponeurosis strain for a given tendon force (every 100 N) did not show any statistically significant (P>0.05) changes in the low-strain-exercised leg (Fig. 7), indicating no alteration in the strain–force relationship of the tendon and aponeurosis due to the intervention. By contrast, after the 14 weeks intervention at high strain magnitude, the strain values for a given tendon force (every 100 N) up to 600 N were significantly (P<0.05) lower than before (Fig. 7), demonstrating a higher gradient in the force–strain curve as compared with the pre-exercise curve. As expected, the control group did not show any differences in the strain–force relationship before and after the 14-week period (Fig. 7). Up to 1200 N tendon force, the ratios of the strain values (post- to pre-exercise) were significantly (P<0.05) lower for the leg loaded with the high strain magnitude than for the low-strain-exercised leg and the control group (Fig. 7).

The rest length of the Achilles tendon (from tuberositas calcanei to soleus muscle) did not alter (P>0.05) after the 14-week intervention in either the low-strain- or the high-strain-exercised legs (low strain: 60.4±15.4 mm pre-exercise, 61.3±15.9 mm post-exercise; high strain: 59.8±11.1 mm pre-exercise, 60.9±11.2 mm post-exercise). The CSA of the Achilles tendon is greater in its distal portion than it is in its proximal part (Fig. 8). Following 14 weeks of exercise at low strain magnitude, the CSA of the Achilles tendon at every 10% of the tendon length did not show any statistically significant (P>0.05) differences from the pre-exercise values (Fig. 8). Although in most cases we did not find any statistically significant (P>0.05) differences in the CSA before and after the

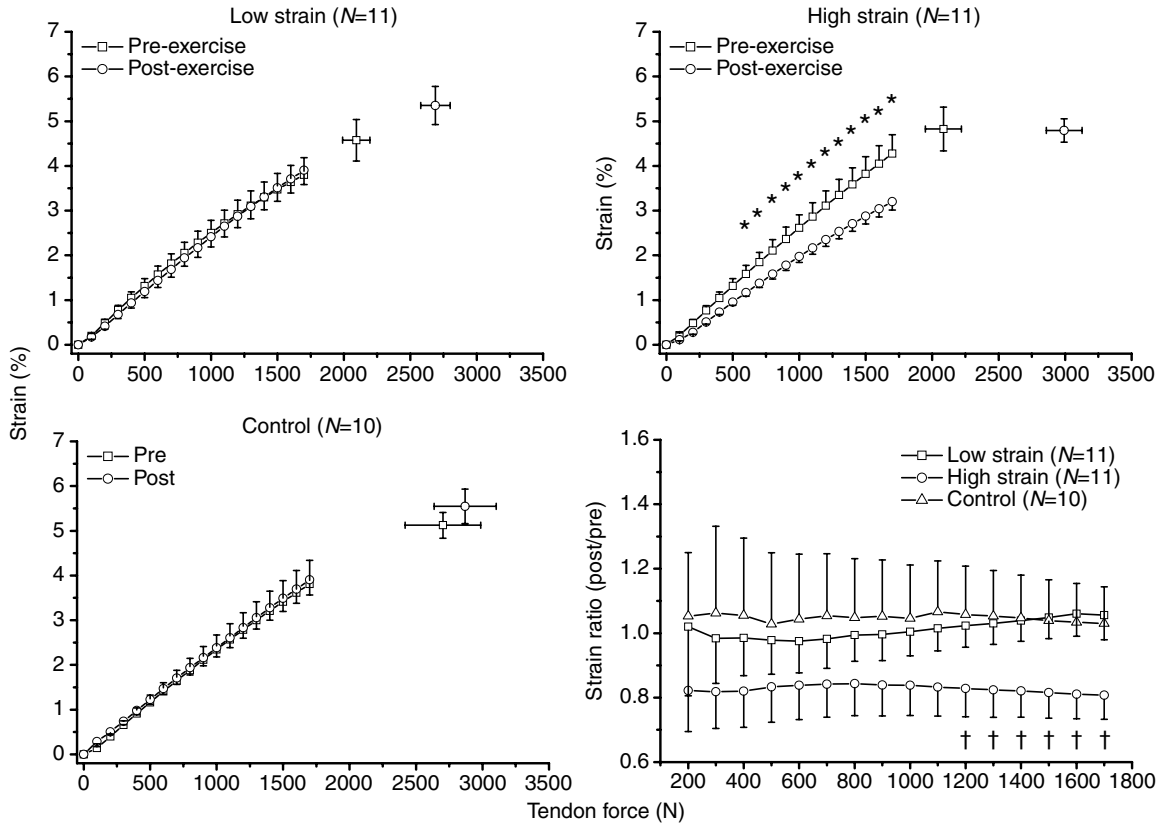

Fig. 7. Strain values and strain ratio (post- to pre-exercise) at every 100 N calculated tendon force of the triceps surae tendon and aponeurosis during the maximum voluntary contraction (MVC). *Statistically significant differences between pre- and post-exercise values (P<0.05). †Statistically significant differences between high-strain-magnitude intervention and the other two groups (P<0.05).

intervention along the tendon length of the leg exercised at high strain magnitude either, the CSA at 60 and 70% of the tendon length displayed greater values after the intervention than before (Fig. 8), demonstrating a region-specific hypertrophy of the Achilles tendon. In the same manner, the ratios of the CSA (post- to pre-exercise) showed higher values at the 60 and 70% of the Achilles tendon length for the high-strain- compared with the low-strain-exercised leg (Fig. 9). The Achilles tendon elastic modulus showed a statistically significant (P<0.05) increase after the intervention in the high-strain-exercised leg. However, the maximal stress values during the MVC increased significantly (P<0.05) in both legs (Fig. 10).

Discussion

The present study investigated whether the magnitude of the mechanical load induced as cyclic strain applied to the Achilles

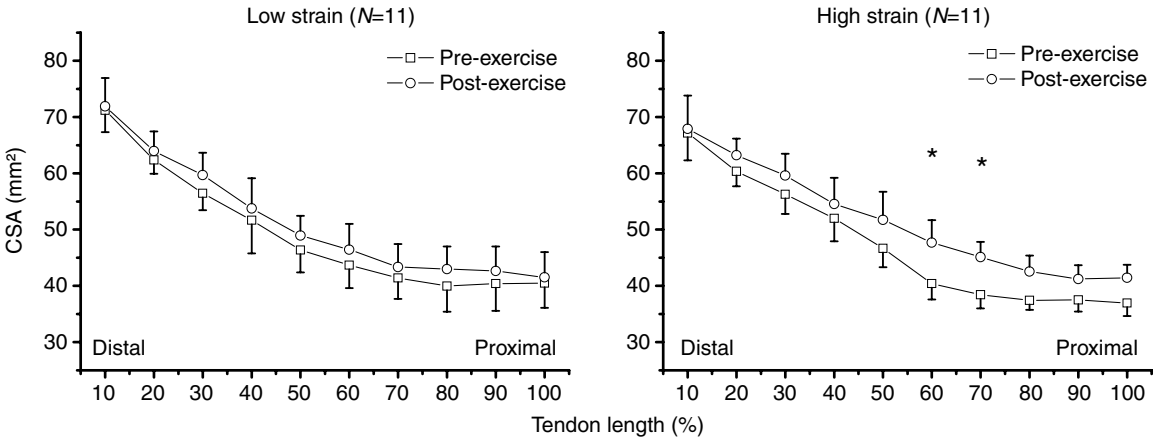

Fig. 8. Cross-sectional area (CSA) values of the Achilles tendon before (pre-exercise) and after (post-exercise) the exercise intervention at every 10% of the tendon length. *Statistically significant differences between pre- and post-exercise values (P<0.05).

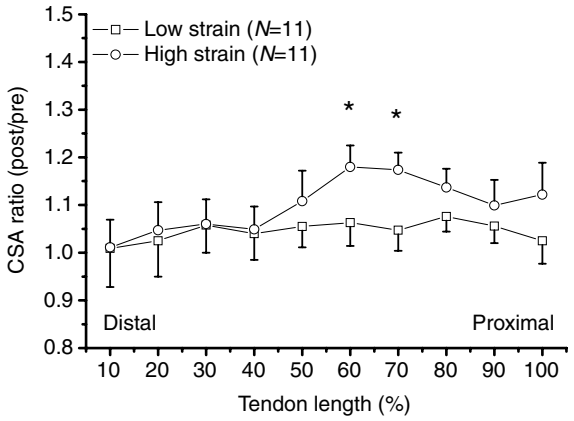

Fig. 9. Ratio (post- to pre-exercise values) of the cross sectional area (CSA) of the Achilles tendon at every 10% of the tendon length. *Statistically significant differences between low- and high-strain-magnitude exercised legs (P<0.05).

tendon may have a threshold in order to trigger adaptation effects on tendon mechanical and morphological properties. Therefore, we examined the effects of two different exercise interventions of cyclic mechanical load of similar frequency and volume but different magnitudes of strain (2.85±0.99% *vs* 4.55±1.38% strain) on the strain–force relationship and hypertrophy of the Achilles tendon. After 14 weeks of exercise intervention, we found a decrease in strain at a given tendon force, an increase in tendon–aponeurosis stiffness and tendon elastic modulus and a region-specific hypertrophy of the Achilles tendon only in the leg exercised at high strain magnitude. These findings provide evidence for the existence of a threshold or set-point at the applied strain magnitude at which the transduction of the mechanical stimulus may influence the tensional homeostasis of the tendon's extracellular matrix and, consequently, the regulation of the anabolic responses of the tendon cells (Wang, 2006; Wang and Thampatty, 2006; Lavagnino et al., 2006).

In the literature, it is well accepted that mechanical load induced as cyclic strain on connective soft tissues such as tendons is an important regulator of the fibroblast's metabolic activity as well as a regulator of the maintenance of the tendon matrix (Arnoczky et al., 2002; Barkhausen et al., 2003; Screen et al., 2005; Webb et al., 2006). Furthermore, the modulation of the mechanical stimuli affects several physiological parameters of human fibroblasts and coordinates the amount of proliferation, apoptosis and expression of proteins (Zeichen et al., 2000; Skutek et al., 2001; Skutek et al., 2003; Barkhausen et al., 2003). For example, loading of tendon cells causes a downregulation of catabolic gene expression and an upregulation of anabolic gene expression (Lavagnino and Arnoczky, 2005; Lavagnino et al., 2006) whereas immobilisation promotes catabolic responses (i.e. degeneration of the extracellular matrix) imposed by an upregulation of matrix metalloproteinases (Amiel et al., 1982; Hannafin et al., 1995; Brown et al., 1998; Arnoczky et al., 2004). Although there is little information in the literature about the effects of controlled tendon strain magnitudes on the homeostatic perturbation and the induced adaptational responses of tendons *in vivo*, *in vitro* studies have demonstrated the existence of a threshold in tendon strain magnitude for triggering fibroblast proliferation (Yang et al., 2004), stimulation of the gene expression of inflammatory mediators such as interstitial collagenase (Lavagnino et al., 2003) or prostaglandin E_2 (Wang et al., 2003b) and changes in the elastic modulus and tensile strength of cultured collagen fascicles after loading (Yamamoto et al., 2003). Recently, Kubo et al. (Kubo et al., 2006) reported an increase in human vastus lateralis tendon–aponeurosis stiffness after high-load isokinetic training of the knee extensor muscles (80% of the isokinetic MVC) but no changes in tendon–aponeurosis stiffness after low-load isokinetic knee extension training (20% of the isokinetic MVC), which is in agreement with our results.

The homeostatic perturbation in the connective tissues induced by mechanical loading affects several biochemical cellular responses (Robbins and Vogel, 1994; Hsieh et al., 2000; Kim et al., 2002). The concept of homeostatic calibration point (Lavagnino and Arnoczky, 2005; Lavagnino et al., 2006) predicts that mechanical loading of the tendon above the homeostatic calibration point (upper limit) will trigger anabolic responses whereas a reduction of tendon loading below the homeostatic level (lower limit) will lead to catabolic cell responses. The findings of the present study showing adaptational effects on the Achilles tendon only at the leg

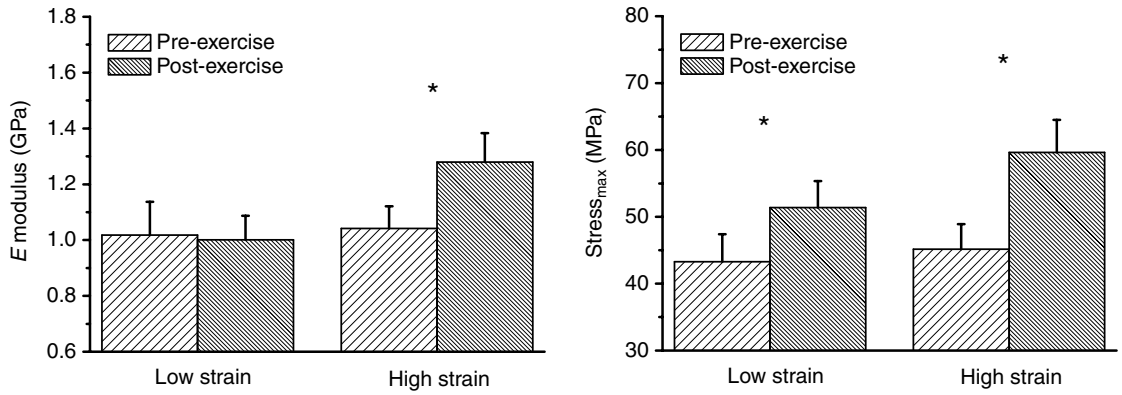

Fig. 10. Elastic modulus and maximal stress values of the Achilles tendon during the maximum voluntary contraction (MVC) before (pre-exercise) and after (post-exercise) the exercise intervention. *Statistically significant differences between pre- and post-exercise values (P<0.05).

exercised at a high strain magnitude indicate that the mechanical load applied to the leg exercised at a low tendon strain magnitude did not influence the existing internal tensional homeostasis of the tendon cells regulating the anabolic or catabolic responses. The results further show that the mechanical load exerted on the Achilles tendon during the low-strain-magnitude exercise is no more a sufficient stimulus for triggering further adaptation effects on the Achilles tendon than the stimulus provided by the mechanical load applied during daily activities. Furthermore, our findings indicate that the 4.55% strain applied during the high-strain-magnitude intervention was above the homeostatic calibration point and thus was sufficient to elicit a homeostatic perturbation at the Achilles tendon that triggered anabolic cell responses, causing the changes observed at the tendon–aponeurosis strain–force relationship and the region-specific hypertrophy of the tendon. In the present study, we controlled the strain magnitude, strain frequency and the exercise volume but not the strain rate during the interventions. The participants achieved the target moment as fast as possible and, therefore, the strain rate should not be very different between the two examined interventions. However, based on our experimental design it is not possible to investigate a potential effect of the strain rate on the tendon adaptational responses that we discovered.

In the present study, we found that 14 weeks exercise at high strain magnitude had a clear influence on the strain–force relationship of the tendon–aponeurosis unit and led to an increase of the CSA of the Achilles tendon at 60 and 70% of its length. The region-specific hypertrophy of the Achilles tendon may partly explain the changes in the strain–force relationship of the tendon–aponeurosis unit but not the increase in tendon elastic modulus after the intervention. Besides tendon hypertrophy, there are some other adaptation possibilities that may affect the tendon stress–strain relationship. The organisation of the extracellular matrix components includes mechanisms transmitting tensile forces along the interfibrilar matrix. Several studies have reported that cells have the ability to produce a better organised collagen matrix modulated by cyclic load (Steinmeyer and Knue, 1997; Wang and Grood, 2000; Wang et al., 2003a; Webb et al., 2006) and this way achieve an increase in tissue stiffness (Brown et al., 1998; Lo et al., 2000). The methods used in the present study do not permit examination of such adaptation possibilities at the Achilles tendon; nevertheless, the clear changes in the tendon–aponeurosis strain–force relationship, the only region-specific tendon hypertrophy, the increase of the tendon elastic modulus, as well as reports of other studies demonstrating an increase in human tendon stiffness and elastic modulus with no changes in the tendon's CSA (Kubo et al., 2002; Reeves et al., 2003a) provide evidence for the plasticity of the organisation of the tendon's extracellular matrix *in vivo* (i.e. density of matrix proteins, cell orientation, proteoglycan content and composition).

The maximum plantar flexion moment increased after the intervention in both exercised legs (on average 20 and 33% for the low- and high-strain-magnitude exercised legs, respectively). This is in agreement with other studies reporting an increase in muscle strength after low- and high-intensity resistance training (Kaneko et al., 1983; Takarada et al., 2000; Moore et al., 2004). The voluntary activation of the plantar flexor muscles during the MVC were quite high (97–99%) and did not show any differences before and after both exercise interventions. This indicates that the increase in muscle strength observed after the 14-week intervention was not due to neuronal factors. In the leg exercised at high strain magnitude the maximum tendon–aponeurosis strain during the MVC did not differ before and after the intervention. These findings, namely an increase in muscle strength with no changes in maximum tendon–aponeurosis strain, suggest a coordinated muscle–tendon unit adaptation at the high-strain-magnitude intervention. Recently, Miller et al. (Miller et al., 2005) reported similar changes in the time course of tendon collagen and myofibrillar protein synthesis rates after non-damaging exercise, supporting a coordinated musculotendinous adaptation. However, the results of the leg exercised at low strain magnitude (i.e. increase in muscle strength with no changes in the tendon–aponeurosis strain–force relationship) do not show any coordinated muscle–tendon unit adaptation. This indicates that the threshold of mechanical loading necessary to trigger adaptational effects is higher for the tendon than for the muscle.

We found an increase in tendon–aponeurosis stiffness, an increase in tendon elastic modulus and a region-specific hypertrophy of the Achilles tendon after the high-strain-magnitude exercise (i.e. 90% MVC). The reported maximal plantar flexion moment values calculated by inverse dynamic during daily activities such as walking are about 120–130 Nm (Winter, 1984). This means that the resultant maximal ankle plantar flexion joint moment while walking is similar or even higher than the applied plantar flexion moment at the high-strain-magnitude intervention. Therefore, it can be argued that the mechanical load on the Achilles tendon at the high-strain-magnitude intervention was not higher compared with normal walking and, thus, the applied mechanical stimulus does not explain the adaptational effects. However, it is difficult to compare the loading on the Achilles tendon induced by walking with the loading induced during the examined strength training. The maximal ankle plantar flexion joint moments while walking do not last for long (instantaneous values). The mean ankle plantar flexion joint moments while walking are about 50–55 Nm (Karamanidis and Arampatzis, 2007). The duration of the loading is also different between walking (~600 ms) and the exercise intervention used in the present study (3 s). Further, the resultant joint moments during daily activities calculated by inverse dynamics are not only compensated by muscles. Passive structures as well as contact forces between bones also absorb parts of these moments. For example, the maximal ankle plantar flexion joint moment while walking occurs at a joint position of 15–20° dorsiflexion (Winter, 1984). At this ankle joint angle, the passive joint moment can achieve values between 20 and 30 Nm (Riener and Edrich, 1999; Mullaney et al., 2006). Moreover, due to the viscoelastic behaviour of the connective tissues, in a dorsiflexed position the passive ankle joint moments may be higher in a dynamic condition (Gajdosik et al., 2005) such as walking. Studies examining the EMG activity of the triceps surae muscles while walking reported values between 19 and 42% of the maximal isometric EMG value (Ericson et al., 1986). Given that the muscle force depends at least on the force potential due to the force–length–velocity relationship and the

activation level (Winters, 1990), submaximal EMG activity suggests submaximal muscle forces.

In conclusion, our results demonstrate a decrease in tendon–aponeurosis strain at a given tendon force and a region-specific hypertrophy of the Achilles tendon after 14 weeks of high-strain-magnitude exercise (~4.6% tendon–aponeurosis strain) and no changes in tendon properties after the same period of low-strain-magnitude exercise (~2.9% tendon–aponeurosis strain) of similar frequency and volume. The contractile capacity of the plantar flexor muscles increased in both levels of exercised legs but the increase was higher at the high-strain-magnitude than at the low-strain-magnitude exercise. The results further show that the strain magnitude applied to the human Achilles tendon should exceed a given threshold to trigger adaptational effects on the mechanical and morphological properties of the tendon and that applied strains with low magnitude (2.5–3.0%) are not a sufficient stimulus to trigger adaptation effects on the Achilles tendon beyond those triggered by the mechanical load applied during daily activities.

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