Effects of eccentric training with different training frequencies on blood circulation, collagen fiber orientation, and mechanical properties of human Achilles tendons in vivo

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Abstract

Purpose The purpose of the present study was to compare the effects of eccentric training with different training frequencies on the blood circulation, collagen orientation, and mechanical properties of the human Achilles tendon in vivo.

Methods Ten healthy males completed 12 weeks of a unilateral eccentric training program {(15 repetitions with knee straight and 15 repetitions with knee slightly bent) × 6 sets in a single session} for the plantar flexor muscles. They performed training three times per week on one side (3TW) and six times per week on the other side (6TW). Before and after training, changes in blood volume, coefficient of variation (CV) of echogenicity (reflects collagen fiber orientation), and stiffness of the Achilles tendon were compared by two-way analysis of variance.

Results The tendon blood volume tended to increase after 3TW and 6TW \((p = 0.064)\). Tendon stiffness did not change after 3TW and 6TW, whereas the elongation of tendon structures at three force levels (50, 100, and 150 N) significantly decreased with 3TW, but not 6TW. The CV of echogenicity significantly decreased after 3TW and 6TW. However, no significant differences were observed in the relative changes in these measured variables between 3TW and 6TW.

Conclusion The present results demonstrated an increase in blood volume, the alignment of collagen fibers, and unchanged stiffness of the Achilles tendon after 12 weeks of eccentric training. Furthermore, the training frequency did not influence these training-induced changes in the tendon properties.

Keywords Blood volume · Ultrasonography · Coefficient of variation of echogenicity · Tendon stiffness

Abbreviations

ANOVA Analysis of variance
CSA Cross-sectional area
CV Coefficient of variation
ICC Interclass correlation coefficient
LG Lateral gastrocnemius muscle
MA Moment arm length
MG Medial gastrocnemius muscle
MVC Maximal voluntary contraction
1RM One repetition maximum
Oxy Oxyhemoglobin
\(\eta^2\) Partial eta-squared
ROI Region of interest
SD Standard deviation
6TW Six times per week
SOL Soleus muscle
StO2 Oxygen saturation
3TW Three times per week
TQ Torque values
THb Total hemoglobin

Introduction

Previous studies demonstrated that low-load and high-repetition eccentric training is an effective treatment for chronic mid-portion Achilles tendinopathy (Alfredson et al. 1998; Fahlstrom et al. 2003; Ohberg and Alfredson 2004). Patients with tendinopathy showed alterations in the blood circulation (De Jonge et al. 2014; Ohberg and Alfredson 2004), collagen fiber orientation (Khan et al. 1999), and mechanical properties (Arya and Kulig 2010;
effects of long-term training on the mechanical properties of tendons would differ between high- and low-frequency training.

The aim of the present study was to compare the effects of low-load and high-repetition eccentric training on the blood circulation, collagen fiber orientation, and mechanical properties of the human Achilles tendon between different training frequencies (3 or 6 times per week). We hypothesized that long-term eccentric training would induce an increase in blood circulation, collagen fiber alignment, and an increase in tendon stiffness. Moreover, we expected these changes in tendon properties to be more prominent after the low- compared with high-frequency training protocol. The results obtained may provide insight into a more appropriate eccentric training protocol as treatment for tendinopathy.

Methods

Subjects

The sample size was estimated using the data from previous studies (de Villarreal et al. 2008; Kim et al. 2010; Ochi et al. 2018) that determined the effects of training frequencies on the muscle strength after 7–12 weeks of training were determined. On the basis of an α level of 0.05 and a power (1 − β) of 0.8, it was considered that at least ten subjects were necessary for this study. Ten healthy males (age 20.9 ± 3.1 years, height 171.5 ± 4.7 cm, weight 61.9 ± 12.9 kg) participated in the present study. They did not train regularly before starting this experiment. Other exclusion criteria included having a history of Achilles tendon surgery, active Achilles tendon injury, systematic diseases affecting collagen metabolism, and cardiovascular diseases. All subjects were fully informed of the procedures and purpose of the present study before they provided written informed consent. The present study was approved by the Ethics Committee for Human Experiments, Department of Life Science (Sports Sciences), The University of Tokyo.

Eccentric training

According to a previous study (Alfredson et al. 1998), one set of eccentric plantar flexor muscle exercise consisted of 15 repetitions with the knee straight and 15 repetitions with the knee slightly bent. Each subject performed 6 sets of the exercise (i.e., a total of 180 repetitions) in a single session. The training regimen (knee posture, number of repetitions for one set, and total number of sets) in the laboratory was consistent with that in the home, although the training in the home was not exactly the same as that in the laboratory (described later). In order to compare the effects of differences in the training frequency, subjects performed...
unilateral eccentric plantar flexion training three times per week on one side (3TW) and six times per week on the other side (6TW). In each subject, the right and left legs were randomly allocated to the training protocols.

Regarding the training in the laboratory, subjects lay in a supine position against the backrest of a leg press machine (VR-4100, Cybex Corp.). The ankle joint was fully planter flexed and the sole of the forefoot was grounded on the footboard in the starting position. Subjects were instructed to move their ankle joints against the resistance from the starting position to the fully dorsiflexed position with controls within 3 s. They repeated this movement at an approximately constant rate using a metronome (60 bpm). In order to avoid the effect of any other contraction modes, an investigator pulled up the backrest of the leg press machine while returning it to the starting position. The load of eccentric training in the laboratory (3 times per week) was defined as 50% of the one repetition maximum (1RM) according to a previously described procedure (Jenkins et al. 2015). At least 1 week before starting the present study, all subjects visited the laboratory in order to measure unilateral concentric 1RM of the right and left legs. Furthermore, each subject performed eccentric training using their body weight another three times per week in the home for 6TW. They were instructed on how to perform eccentric training at home by an experienced physical therapist (T.I.). They stood on a 15 cm-high box with all their body weight loaded on the forefoot and then performed eccentric contractions by lowering the heel beneath the level of the box at an approximately constant velocity within 3 s. In order to prevent concentric contractions, they were instructed to use the other leg and their arms to return to the starting position. When returning to the starting position, they were advised to use their arms as much as possible to minimize the effects of concentric contractions on the other leg assigned to 3TW.

Before starting the training period, subjects were instructed to complete weekly training on weekdays (Monday–Friday), and rest on Sunday. All subjects reported their implementation status by e-mail. Average compliance with the home exercise was 93.9% (88.3–97.2%) through 12 weeks of the training period. The measurement of 1RM was performed every 4 weeks to adjust the load of eccentric training performed in the training room. After the training periods, 1RM significantly increased by 9.7 ± 5.9% with 3TW and 11.8 ± 4.4% with 6TW, and no significant difference was observed in relative increases in 1RM between the different training frequencies (p = 0.124).

Maximal voluntary contraction

Subjects lay prone on a test bench with the knee fully extended and the ankle at 90° (anatomical position), and their foot was securely strapped to a footplate connected to a custom-designed dynamometer (Applied Office, Tokyo, Japan). Adjustable lap belts were fastened over the waist and shoulders to hold this position. After a standardized warm-up, subjects performed several submaximal planter flexor isometric contractions to familiarize themselves with the test procedures. Subjects then performed two to three maximal voluntary contractions (MVC) that lasted for 3 s, with a 1-min rest period between each trial. The peak torque value within all trials was recorded as the MVC value. In our recent study (unpublished data), the repeatability of MVC measurement was investigated on three separate days (7–10th day among the tests) with 11 young males. The average coefficient of variation of MVC was 3.7%.

Muscle thickness and tendon cross-sectional area

An ultrasonic apparatus (SSD-4000, Aloka, Tokyo, Japan) with an electronic linear array probe (7.5-MHz wave frequency, UST-5410, Aloka, Tokyo, Japan) was used to measure the muscle thickness and tendon cross-sectional area (CSA). Subjects were in a prone position on a test bench with the knee fully extended and ankle at 90°, and were instructed to relax completely during all measurement procedures. Muscle transverse images were obtained at proximal levels of 30% of the lower leg length for the medial gastrocnemius (MG) and lateral gastrocnemius (LG), and 50% of the lower leg length for the soleus muscle (SOL). Two transverse images of the Achilles tendon were taken 3 and 5 cm proximal to the tendon insertion on the calcaneus to measure the tendon CSA. The muscle thickness and tendon CSA were measured in the same manner as in our previous study (Ishigaki et al. 2018) using an open-source image processing program (ImageJ, NIH, Bethesda, MD). The averages of three muscle thickness and two tendon CSA values (3 and 5 cm proximal to the tendon insertion) were taken to represent the plantar flexor muscle thickness and tendon CSA, respectively. In a preliminary study, the repeatability of muscle thickness and tendon CSA measurements was investigated on two separate days (5–10 days between the test and retest) in the 10 young males. The test–retest correlation coefficient and coefficient of variation were 0.973 and 3.1% for MG, 0.847 and 6.3% for LG, 0.995 and 2.0% for SOL, and 0.944 and 2.5% for tendon CSA, respectively.

Blood circulation of the tendon

In the measurement of tendon blood circulation (total hemoglobin: THb, oxygen saturation: StO2) using a laser tissue oxygenation monitor (BOM-L1TRSF, Omega wave, Tokyo, Japan), a probe (SF-DS, Omega wave, Tokyo, Japan) was placed on the center of the Achilles tendon 30 mm proximal to its insertion on the calcaneus (Kubo 2015). This instrument uses three red laser lights (635, 650, and 690 nm) to
calculate relative tissue levels of THb (corresponding to the blood volume). The probe used in the present study enables measurement of blood circulation in the Achilles tendon substance (measurement depth from the skin: 3–5 mm) according to the Beer–Lambert law. Although this method was not able to measure actual physiological volumes of THb, the unit of THb was expressed in µmol/l. StO2 was calculated as oxyhemoglobin (Oxy) relative to THb using the following formula:

$$\text{StO}_2 \% = \frac{\text{Oxy}}{\text{THb}} \times 100.$$

These data were recorded on a personal computer at a sampling rate of 1000 Hz via an A/D transducer (Power Lab, AD Instruments, Australia). During the measurement of blood circulation, subjects were asked to maintain the same posture as that aforementioned for measurement of the muscle thickness and tendon CSA. Average values over a given duration (approximately 10 min) after at least 20 min of rest in a prone position were calculated using LabChart ver. 7.3.7. (AD Instruments, Australia). Before data recording, we confirmed that there was no fluctuation in the real-time data. In a preliminary study, the repeatability of THb and StO2 measurements was investigated on two separate days (7–14 days between the test and retest) in 10 young males. The test–retest correlation coefficient and the coefficient of variation were 0.910 and 5.1% for THb and 0.750 and 2.0% for StO2, respectively.

**Collagen fiber orientation of the tendon**

In order to quantify the collagen fiber orientation of the Achilles tendon, the coefficient of variation (CV) of echogenicity was calculated from the same transverse ultrasonic images of the tendon for measurement of the tendon CSA. The procedures used to calculate the CV of echogenicity were according to our previous study (Ishigaki et al. 2016). The region of interest (ROI) was selected in each image to include as much of the Achilles tendon as possible without the surrounding tissue. All pixels inside the ROI were expressed as a grayscale value between 0 (black) and 255 (white). The mean echogenicity and standard deviation (SD) were calculated from the grayscale histogram using ImageJ software (NIH, Bethesda, MD, USA). The CV of echogenicity was calculated as follows:

$$\text{CV of echogenicity (\%)} = \frac{\text{SD}}{\text{mean echogenicity}} \times 100.$$

These variables (i.e., mean echogenicity and CV of echogenicity) were measured in two images taken 3 and 5 cm proximal to the tendon insertion on the calcaneus, and the mean of each variable was used in statistical analyses. In our previous study (Ishigaki et al. 2016), the repeatability of the mean echogenicity and CV of echogenicity measurements was investigated on two separate days (5–8 days between the test and retest) with 12 young males. The test–retest correlation coefficient and coefficient of variation were 0.86 and 2.6% for mean echogenicity and 0.891 and 2.6% for CV of echogenicity, respectively.

**Mechanical properties of the tendon**

The posture of subjects and the dynamometer were the same for the measurement of MVC, as described above. Subjects were asked to gradually produce isometric plantar flexion torque from a relaxed state to MVC within 5 s. After several familiarization trials, at least two trials were performed by each subject with a 2-min rest period between each trial. Torque signals were recorded on a computer using an A/D transducer (PowerLab/16SP, AD Instruments, Australia) at a sampling rate of 1000 Hz.

In order to evaluate the elongation of tendon structures (outer tendon and aponeurosis) during isometric contractions, longitudinal MG ultrasonic images were obtained at a proximal level of 30% of the lower leg length using a B-mode ultrasonic apparatus (Kubo et al. 2012). The probe placed at the measurement site was securely fixed with adhesive tape to prevent any movement during the contractions. Ultrasonic images during isometric contractions were recorded on a videotape at 30 Hz, and synchronized with torque signals using a clock timer trigger device for subsequent analyses. The displacement of the cross-point of one fascicle and the aponeurosis during an isometric contraction was considered to indicate the elongation of tendon structures. The displacement of the cross-point was measured using ImageJ software (NIH, Bethesda, MD).

The displacement of the cross-point is caused by angular joint rotation and contraction torque, because angular joint rotation occurs in the direction of plantarflexion during isometric plantarflexion contractions. Therefore, during isometric plantarflexion contractions, we measured ankle angular rotation using an electrogoniometer (Penny and Giles, Biometrics Ltd., Gwent, UK) placed on the lateral aspect of the ankle. The ratio was calculated between angular joint rotation and displacement of the cross-point of one fascicle and the aponeurosis caused by a 9° passive ankle plantarflexion from 90° of the ankle joint. This allowed corrections of the elongation of the tendon structure due to angular joint rotation for isometric plantarflexion trials. Thus, the elongation of the tendon structure was corrected for angular joint rotation alone, and then used in further analyses.

Exerted torque values (TQ) during isometric plantarflexion contractions were converted to the muscle force of MG ($F_m$) as follows:

$$F_m = k \times TQ \times MA^{-1},$$

where $k$ represents the relative contribution of MG based on its physiological CSA within the entire plantar flexor.
muscles, and MA is the moment arm length of the plantar flexor muscles at 90° of the ankle joint, which was estimated from the lower leg length of each subject (Kubo et al. 2012). According to previous study (Kubo et al. 2012), the force–elongation relationship of the tendon structure greater than 50% of MVC was fitted with a linear function, and the slope of the linear function was adopted as the tendon stiffness. In the present study, the average coefficient of variation of the tendon stiffness between the two trials in each subject was 5.9%. In our previous study (Kubo et al. 2012), the repeatability of tendon stiffness measurement was investigated on two separate days (7–12 days between the test and retest) with eight young males. The test–retest correlation coefficient and coefficient of variation were 0.89 and 5.6%, respectively.

**Statistical analysis**

All data are reported as the mean ± SD. After confirming the statistical normality of the distribution using the Kolmogorov–Smirnov test, a two-way analysis of variance (ANOVA) with repeated measures and a post hoc analysis were used to examine the effects of time (before and after training) and training frequency (3TW and 6TW) on the measured variables. The F ratios for main effects and interactions were considered to be significant. Significant differences among means were detected using a Bonferroni post hoc test. Moreover, significant differences in tendon elongation between before and after training at the same force levels were analyzed by a paired t test. The level of significance was set at \( p < 0.05 \) for all tests. The effect size was measured using partial eta-squared (\( \eta^2 \)). As a result of power analysis using G*Power3.1.9.2 (http://www.gpower.hhu.de/), statistical power was more than 80% with a medium effect size in the present study.

**Table 1** Morphological and mechanical properties of the muscle and tendon

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal voluntary contraction (N m)</td>
<td>114.9 ± 17.9</td>
<td>122.7 ± 16.9*</td>
<td>114.8 ± 19.6 121.1 ± 19.9*</td>
</tr>
<tr>
<td>Muscle thickness (mm)</td>
<td>18.7 ± 2.7</td>
<td>19.3 ± 2.6*</td>
<td>19.0 ± 2.0 19.6 ± 2.2*</td>
</tr>
<tr>
<td>Tendon cross-sectional area (mm²)</td>
<td>52.7 ± 10.0</td>
<td>51.6 ± 9.4</td>
<td>52.0 ± 12.4 52.1 ± 9.3</td>
</tr>
<tr>
<td>Tendon stiffness (N mm⁻¹)</td>
<td>28.6 ± 7.1</td>
<td>29.0 ± 5.9</td>
<td>26.5 ± 8.1 29.6 ± 8.4</td>
</tr>
</tbody>
</table>

*Significantly different from before training

**Results**

MVC significantly increased by 7.3 ± 7.7% with 3TW and 5.7 ± 7.9% with 6TW (\( p < 0.05, \eta^2 = 0.481 \)); however, no significant differences were observed between the different training frequencies (effect of frequency: \( p = 0.824, \eta^2 = 0.006 \); interaction: \( p = 0.644, \eta^2 = 0.025 \)). Similarly, the muscle thickness significantly increased after training by 3.0 ± 2.9% with 3TW and 3.4 ± 1.8% with 6TW (effect of time: \( p < 0.001, \eta^2 = 0.037 \); effect of frequency: \( p = 0.569, \eta^2 = 0.916 \); interaction: \( p = 0.688, \eta^2 = 0.019 \)) (Table 1). In contrast, no significant changes in tendon CSA were found for either protocol (effect of time: \( p = 0.622, \eta^2 = 0.028 \); effect of frequency: \( p = 0.964, \eta^2 = 0.000 \); interaction: \( p = 0.589, \eta^2 = 0.034 \)) (Table 1).

Despite the training frequency, THb tended to increase after training (effect of time: \( p = 0.064, \eta^2 = 0.332 \); effect of frequency: \( p = 0.424, \eta^2 = 0.072 \); interaction: \( p = 0.379, \eta^2 = 0.089 \)) (Fig. 1a), whereas no change was noted in StO2 with either protocol (effect of time: \( p = 0.875, \eta^2 = 0.003 \); effect of frequency: \( p = 0.131, \eta^2 = 0.235 \); interaction: \( p = 0.196, \eta^2 = 0.178 \)) (Fig. 1b).

Mean echogenicity significantly increased \( (p < 0.001, \eta^2 = 0.768) \) (Fig. 2a) and the CV of echogenicity significantly decreased \( (p < 0.01, \eta^2 = 0.660) \) (Fig. 2b) after training. However, no significant differences were observed in the relative changes in these variables between the training frequencies (effect of the frequency for mean echogenicity: \( p = 0.860, \eta^2 = 0.004 \); for the CV of echogenicity: \( p = 0.418, \eta^2 = 0.074 \); interaction for mean echogenicity: \( p = 0.523, \eta^2 = 0.047 \); for the CV of echogenicity: \( p = 0.158, \eta^2 = 0.208 \)).

The relationships between force and the elongation of tendon structures are shown in Fig. 3. The elongation of tendon structures at three force levels (50, 100, and 150 N) significantly decreased after 3TW, but not 6TW. The stiffness of tendon structures did not change after 3TW or 6TW (effect of time: \( p = 0.216, \eta^2 = 0.081 \); effect of frequency: \( p = 0.723, \eta^2 = 0.187 \); interaction: \( p = 0.509, \eta^2 = 0.042 \)) (Table 1).
Discussion

The present study demonstrated that low-load and high-repetition eccentric training showed a tendency to increase the blood volume despite the training frequency. The THb and StO₂ values in our study were in line with those reported previously (Ishigaki et al. 2018; Kubo et al. 2012). However, our recent study showed that the blood volume of the patellar tendon did not change after 12 weeks of high-load and low-repetition eccentric training (80% 1RM, 10 repetitions × 5 sets) (Kubo and Yata 2017b). Based on these findings, a low-load and high-repetition protocol, such as the Alfredson protocol (Alfredson et al. 1998), may be a more appropriate eccentric training protocol for increasing the tendon blood volume than a high-load and low-repetition protocol.

A previous study suggested that blood supply to the tendon was important for the healing of an injured tendon (e.g., Lin et al. 2004). Therefore, as a clinical relevance, an increase in blood supply to the tendon after eccentric training with a low-load and high-repetition protocol would contribute to tendon healing in patients with Achilles tendinopathy. Furthermore, since the ingrowth of neovascularization was time dependent (Wang et al. 2003), we hypothesized that an increase in blood circulation is more prominent at low- compared with high-frequency training. However, our hypothesis was rejected based on the results of the present study. Thus, the present results indicate that differences in training frequencies do not affect changes in blood circulation within the tendon induced by low-load and high-repetition eccentric training.

To the best of our knowledge, this is the first study to demonstrate a change in the tendon collagen fiber orientation after a training period of several months. The mean echogenicity and CV of echogenicity values in our study were in line with those reported previously (Ishigaki et al. 2016, 2018). We recently reported an acute decrease in the CV of echogenicity over 40 min immediately after repeated eccentric contractions (Ishigaki et al. 2018). The present results may be attributed to the accumulation of the acute effects of repeated eccentric contractions on the tendon collagen fiber orientation. The change observed in the tendon collagen fiber orientation may be associated with the mechanism responsible for the efficacy of eccentric training as a treatment for Achilles tendinopathy. However, in spite of improvements in symptoms, a previous study reported that the tendon structure (collagen density and collagen fiber orientation) did not return to normal values after eccentric
training in patients with Achilles tendinopathy (de Vos et al. 2012). The discrepancy between the present and previous findings may be related to differences in the characteristics of subjects (healthy young males in the present study, patients with tendinopathy in de Vos et al. 2012) and the method of measurement of the collagen fiber orientation [the CV of echogenicity in the present study, ultrasound tissue characterization (UTC) in de Vos et al. 2012]. The UTC technique classified echogenicity in tendon transverse ultrasonic images into four echo types (de Vos et al. 2012). Consequently, slight changes in the angle of incidence of the ultrasound beam to collagen fibers did not affect the results of the UTC technique (van Schie et al. 2010). On the other hand, our quantitative method of the tendon collagen orientation using the CV of echogenicity has been developed based on changes in echogenicity that are dependent on the degree of inclination of collagen fibers within tendons with respect to the ultrasound beam (Ishigaki et al. 2016). Therefore, the present results may be due to slight changes in the collagen fiber orientation within tendons after 12 weeks of eccentric training. Regardless, future studies using our quantitative method are needed in order to clarify the relationship between clinical outcomes and changes in the tendon collagen fiber orientation after eccentric training in patients with tendinopathy.

Regarding the relationship between force and the elongation of tendon structures, tendon elongation significantly decreased after 3TW but not 6TW (Fig. 3). Figure 4 shows changes in tendon elongation in each subject with 3TW and 6TW at the three exerted force levels. The elongation of tendon structures for most subjects decreased after training with 3TW. In contrast, marked intersubject variability was observed in changes in tendon elongation after 6TW. Previous studies showed that the mean extension of tendons increased slowly during the fatigue test but much faster just before rupture (e.g., Wang et al. 1995). Wang et al. (1995) suggested that this failure resulted from the accumulation of damage. Furthermore, Zamora and Marini (1988) suggested that the tendon went through a “remodeling” process similar to muscle hypertrophy after resistance training. Therefore, changes in tendon elongation after training may be associated with individual differences in the required time periods to recover from tendon fatigue.

In the present study, the stiffness of tendon structures remained unchanged after eccentric training with 3TW and 6TW. Over the past 15 years, many studies have investigated the effects of resistance training on tendon mechanical properties (e.g., Kubo et al. 2012); however, few have examined the effects of eccentric training on such properties (Foure et al. 2013; Kubo and Yata 2017; Malliaras et al. 2013). In these studies, the increase induced in tendon stiffness by eccentric training tended to be lower than that by other contraction modes. The present results on tendon stiffness were consistent with these findings. We previously reported that the blood volume of the tendon significantly increased after dynamic training (tendon stiffness slightly increased),
although it did not change after static training (tendon stiffness markedly increased) (Kubo et al. 2009). In the present study, THb tended to increase after 3TW and 6TW. Considering these findings, unchanged tendon stiffness after eccentric training may be related to an increasing in blood volume of the tendon. However, these discussions are speculative, and additional data are required for clarification. On the other hand, Verrall et al. (2017) stated that an increase in tendon extensibility would enhance the ability to withstand applied force during tendon lengthening. In other words, a decrease in tendon extensibility may reduce the ability to withstand force applied to the muscle–tendon complex during exercise (e.g., walking and running), and this may increase the risk of tendon injuries. Therefore, although the reason for unchanged tendon stiffness after eccentric training currently remains unclear, the lower efficacy of eccentric training for increasing tendon stiffness would contribute to the treatment for tendinopathy.

Regarding our hypotheses, we expected changes in each measured variable to be more prominent with 3TW than with 6TW. However, the different training frequencies in the present study did not affect changes in any measured variables, except for the tendon elongation. Frohm et al. (2007) reported similar clinical outcomes of eccentric training performed two and seven times per week (every day) as a treatment for tendinopathy. On the other hand, many studies have shown good clinical outcomes using low-load and high-repetition eccentric training performed every day in order to treat patients with tendinopathy (e.g., Alfredson et al. 1998). Taking the results of the present study into consideration, eccentric training three times per week may be sufficient for the effective treatment of tendinopathy. Therefore, the present results suggest that eccentric training three times per week may reduce the patient burden more than conventional eccentric training such as the Alfredson protocol.

In the present study, the protocol (repetition per set and number of sets) of training in the home was the same as that in the laboratory. However, we should have adopted exactly the same training protocol (in the laboratory) for six times per week in order to investigate the effect of “training frequency” on the measured variables. Unfortunately, it is very difficult to ask the subjects (university students) to visit the laboratory six times per week due to scheduling limitations. In the present study, therefore, the leg for 6TW was trained three times per week in the laboratory and three times per week in the home. In an additional experiment \((n = 6)\), we confirmed that there was no difference in the imposed load (the vertical component of the ground reaction force in the starting position) between the laboratory training and home training. In addition, we compared the degree of acute fatigue (decline in MVC and increase in muscle thickness) by the two training protocols (laboratory training and home training). As a result, there were no differences in the relative changes in maximal voluntary contraction \((-13.9\pm7.8\%\text{ for laboratory and }-13.1\pm6.3\%\text{ for home}) (p=0.709)\) and muscle thickness (mean of MG, LG, and SOL) \((+4.5\pm2.3\%\text{ for laboratory and }+4.7\pm2.8\%\text{ for home}) (p=0.672)\). Based on these findings, we considered that training in the home is almost the same as training in the laboratory.

In the present study, there were some limitations with the methodology followed. First, we could not exclude the possibility that tendon properties were affected by concentric contractions during home training for 6TW, although the subjects were advised to use their arms as much as possible to minimize the effects of concentric contractions on the other leg assigned to 3TW. However, our previous study showed that the blood volume of the tendon did not increase after repeated concentric contractions (Kubo 2015). Therefore, although the effect of concentric contraction on the collagen fiber orientation and mechanical properties of tendons remained unclear, repeated concentric contractions would have smaller effects on tendon blood circulation. Second, we cannot deny the possibility that a cross-transfer effect on the measured variables (especially tendon blood circulation) in the present study. To our knowledge, no study has investigated the cross-transfer effect of eccentric training on tendon blood circulation. On the other hand, we previously reported that changes in resting tendon blood circulation were present not only on the treated side but also on the non-treated side after acupuncture and heating (Kubo et al. 2011). Therefore, we cannot ignore the cross-transfer effect of unilateral eccentric training. However, previous studies showed no marked changes in muscle blood flow in the untrained limb during rest after unilateral resistance training with a hand ergometer for 6 weeks (Yasuda and Miyamura 1983). Furthermore, a cross-transfer effect of resistance training is likely to occur mainly as neural adaptation (Starbuck and Eston 2012). Therefore, cross-transfer effects of unilateral eccentric training would have little impact on tendon blood circulation. Third, the tendon CSA was measured using ultrasonography. Recent studies indicated an inaccuracy regarding the measurement of tendon CSA using ultrasonography (e.g., Bohm et al. 2016). On the other hand, there are conflicting findings on the effect of resistance training on the tendon CSA measured by magnetic resonance imaging. Some studies reported no significant changes in tendon CSA after training (e.g., Kubo et al. 2012), whereas other studies found significant increases (e.g., Arampatzis et al. 2007). In the present study, we paid attention to the training-induced changes in blood circulation, collagen fiber orientation, and mechanical properties of the tendon (stated in the title of this manuscript). Therefore, we considered that this point did not affect the main results of the study.

An important methodological issue in the present study was whether measured CV of echogenicity truly reflected...
tendon collagen orientation. Unfortunately, the validity of this methodology has not been demonstrated yet and may be not commonly accepted to estimate the tendon collagen fiber orientation. Since increased tendon collagen volume and density may cause decreases in spaces among the collagen fibers, changes in collagen volume and density would also affect the results of CV of echogenicity. In the present study, an increase in mean echogenicity was found without changes in tendon CSA. Some previous studies used echo intensity (i.e., mean echogenicity) for measurement of the collagen volume (Kubo et al. 2012) and density (Pardes et al. 2017). In our recent studies (Ishigaki et al. 2016, 2018), however, we found declines in CV echogenicity during passive stretching, isometric contraction, and repeated eccentric contractions, which may have revealed the alignment of collagen fibers within the tendons. Furthermore, a previous study reported that the influence of ultrasound anisotropy decreased in a symptomatic tendon (i.e., tendinopathy) compared with a healthy tendon (Lehtinen et al. 1994). This finding would result from increases in dispersion of tendon collagen fiber orientation in the symptomatic tendon. Considering these findings, we considered that quantification of the influence of ultrasound anisotropy using CV of echogenicity would be closely related to collagen fiber orientation. In a future study, we need to evaluate the validity of this methodology using histological techniques.

Conclusion

The present study revealed an increase in the tendon blood volume, the alignment of tendon collagen fibers, and unchanged tendon stiffness after 12 weeks of low-load and high-repetition eccentric training. These results may be associated with the mechanism responsible for the efficacy of eccentric training as a treatment for tendinopathy. Furthermore, the present study demonstrated that the training frequency did not affect changes in these measured variables. These results suggest that eccentric training three times per week may induce sufficient training effects as a treatment for tendinopathy. Future studies are required to investigate the treatment effects of eccentric training three times per week on patients with tendinopathy.

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