Effects of repeated eccentric contractions with different loads on blood circulation and collagen fiber orientation in the human Achilles tendon

Tomonobu Ishigaki, Toshihiro Ikebukuro and Keitaro Kubo*

Department of Life Science, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan

Abstract The purpose of this study was to compare changes in blood circulation and collagen fiber orientation in the Achilles tendons after repeated eccentric contractions (ECC) with different loads. Thirteen healthy male subjects performed two ECC protocols with different loads and then remained relaxed for 40 min after ECC. Each leg was randomly allocated to low-load protocol (180 repetitions at 50% of one repetition maximum [1RM]) and high-load protocol (75 repetitions at 120% of 1RM). Before and after ECC, blood volume and oxygen saturation in the Achilles tendons were measured using a laser oxygenation monitor. Tendon collagen fiber orientation was also estimated from the coefficient of variation (CV) of echogenicity on transverse ultrasonic images of the Achilles tendon. In the low- and high-load protocols, blood volume in the tendon was significantly higher than the resting level until the end of the recovery period, although oxygen saturation returned to the resting level at the 20-min point of recovery. The CV of echogenicity was significantly lower than the resting level until the end of the recovery period. No differences in the changes in these variables were observed between the two protocols. These results suggest that blood volume and collagen fiber orientation in tendons are changed to the same degree by repeated eccentric contractions with the same amount of work regardless of the magnitude of the load.

Keywords: blood volume, oxygen saturation, ultrasonography, echogenicity

Introduction

Recent studies have reported good clinical results with eccentric training for patients with chronic tendinopathy1-4). However, the mechanisms for the effectiveness of eccentric training in the management of tendinopathy currently remain unknown. Furthermore, previous studies showing good clinical results with eccentric training did not describe how the authors decided load and number of repetitions of the eccentric training. Blood supply into tendons has been suggested to contribute to the healing of tendon injuries5-8). Indeed, recent studies reported that blood volume and oxygen saturation of the tendons significantly increased after repeated eccentric contractions9,10). However, we recently demonstrated that these variables in the patellar tendon did not change after 12 weeks of eccentric knee extension training11). In these studies9,11), eccentric contractions with higher intensities (maximal isokinetic contractions for Kubo9 and Yin et al.10, 80% of one repetition maximum for Kubo and Yata11) were adopted. On the other hand, a protocol with a low load and high repetition was used in previous studies showing good clinical results with eccentric training for tendinopathy5-10). Therefore, changes in blood circulation in tendons may be more prominent after a low-load and high-repetition of eccentric contractions such as the Alfredson protocol (e.g. Alfredson et al.15). To establish the evidence of eccentric training as a treatment for tendinopathy, we need to compare the effects of eccentric contraction with both a low-load and high-repetition protocol and high-load and low-repetition protocol.

Previous histopathological studies revealed the disordered arrangement of collagen fibers in symptomatic tendons12,13). We recently introduced a non-invasive technique to quantify collagen fiber orientation in the human Achilles tendon by measuring the coefficient of variation (CV) of echogenicity in transverse ultrasonic images of tendons14). On ultrasound images, the echogenicity of tendons changes with alteration of the angle of incidence of the ultrasound beam (i.e. acoustic anisotropy).15) When arrangement of collagen fibers within tendons was aligned parallel to the tendon axis, the grayscale distribution would be small based on acoustic anisotropy. Therefore, in this technique, a decrease in CV of echogenicity implies the alignment of collagen fibers within tendons. An in vitro study using a polarizer light method revealed that tendon collagen fibers aligned after the application of a pre-conditioning load to the tendons16). Therefore, collagen fibers in tendons appear to be aligned with repeated tendon stretching during repeated eccentric contractions.
Furthermore, it may be possible to develop more effective protocols with eccentric contractions for the alignment of collagen fibers within injured tendons.

The purpose of the present study was to compare the effects of repeated eccentric contractions with different loads (i.e., low-load and high-load) on blood circulation and collagen fiber orientation in the Achilles tendon. Based on previous findings, we hypothesized that an increase in blood circulation and alignment of collagen fibers in tendons may be more prominent after a low-load protocol than a high-load protocol because good clinical results for tendinopathy were obtained using a protocol with high-repetition and low-load of eccentric contractions (e.g. Alfredson et al.19).

Materials and methods

Subjects. Thirteen healthy males (age: 26.6 ± 5.0 yrs, height: 175.1 ± 4.6 cm, body mass: 71.5 ± 13.5 kg, mean ± SD) volunteered for this study. Exclusion criteria included a history of injuries and/or surgery on the Achilles tendon, systematic diseases related to collagen metabolism, and cardiovascular diseases related to blood circulation. This study was approved by the Ethical Committee on Human Experimentation, Department of Life Science (Sports Sciences), The University of Tokyo (Issue Number: 438). Prior to participating in this study, all subjects were fully informed of the procedures to be utilized as well as its purpose. Written informed consent was obtained from all subjects.

Repeated eccentric contractions. At least one week before the first exercise intervention, the unilateral one repetition maximum (1RM) of the calf muscle of each leg was measured in all subjects according to a previously described procedure17. In recent years, previous studies have reported that a low-load (20–50% 1RM) resistance training combined with restricted venous blood flow can result in muscle hypertrophy18,19. On the other hand, previous researchers adopted a high-load (>120% 1RM) to investigate the effects of eccentric contractions on neuromuscular properties20,21. In the present study, therefore, we adopted 50% 1RM as low-load and 120% 1RM as high-load. Each leg of the subjects was randomly allocated to a low-load protocol (at 50% of 1RM) and high-load protocol (at 120% of 1RM). Each subject performed two tests on 2 separate days, with at least 2 weeks between sessions. The order of the two experimental conditions was randomized for each subject. Subjects lay in the supine position on the backrest of a leg press machine (VR-4100, Cybex Corp.). The ankle joint was fully plantar flexed and the sole of the forefoot was grounded on the footboard in the starting position. In order to perform repeated eccentric contractions (ECC), subjects were instructed to move their ankle joints from the starting position to the fully dorsiflexed position at an approximately constant velocity within 3 seconds (s). Movement velocity was controlled using a metronome (60 bpm). While returning to the starting position, an investigator pulled up the backrest of the leg press machine. In the low-load protocol, one set consisted of 15 repetitions with the knee straight and 15 repetitions with the knee slightly bent, and subjects performed 6 sets of this protocol (i.e., a total of 180 repetitions) with a 2-min rest between sets. This procedure was similar to that described by Alfredson et al. in the points of low-load and high-repetition protocol13. In the high-load protocol, one set consisted of 5 repetitions with the knee straight and 5 repetitions with the knee slightly bent. In order to ensure that the amount of work performed during the high-load protocol was the same as that during the low-load protocol, subjects performed 7 sets of the high-load protocol and 5 repetitions with the knee straight (i.e. a total of 75 repetitions) with a 2-min rest between sets. In both high- and low-load protocols, subjects were instructed to keep a given knee joint angle [about 20 degrees (deg); full extension 0 deg] during the ECC with the knee slightly bent. An investigator attentively watched the angle of the knee joints, and verbally instructed a correction to the knee angle whenever a subject’s knee bent too much.

Maximum voluntary contraction and muscle thickness.

In order to confirm there was no significant difference in the extent of fatigue between the two protocols, maximum voluntary contraction (MVC) and the muscle thickness of the plantar flexor muscles were measured before and after the exercises. MVC was measured after the end of the recovery period (40 min) because the variables of blood circulation and echogenicity tested (see below) were affected by exerted contractions during the recovery period. The subject lay prone on a test bench and the waist and shoulders were secured using adjustable lap belts and held in position. The ankle joint was set at 90° (anatomical position) with knee full extension, and the foot was securely strapped to a footplate connected to a specially designed dynamometer (Applied Office, Tokyo, Japan). After a standardized warm-up, subjects were asked to perform two 3-s MVC of the plantar flexor muscles. The peak torque for each 3-s contraction was recorded, and the mean of the two contractions was used as the performance measure. Transvers ultrasonic images of the plantar flexor muscles, i.e. the medial gastrocnemius muscle (MG), lateral gastrocnemius muscle (LG), and soleus muscle (SOL), were obtained using a B-mode ultrasound apparatus (SSD-4000, Aloka, Japan) and 7.5 MHz linear transducer (UST-5410, Aloka, Japan). Transverse ultrasonic images were obtained at proximal levels of 40% of the lower leg length for MG and LG and 50% of the lower leg length for Sol. The muscle thickness of each muscle was measured using open-source image analysis software (Image J, NIH, Bethesda, MD). The mean value for MG, LG, and SOL thicknesses was adopted as the
muscle size of the plantar flexor muscles. In our preliminary study, the repeatability of measurements of these muscle thicknesses was examined on 2 separate days with 12 young male subjects. The intraclass correlation coefficients (ICC(1,2)) for MG, LG and Sol were 0.973, 0.847, and 0.995, respectively.

Blood circulation in the Achilles tendon. Before and after ECC, we measured blood circulation (oxyhemoglobin: Oxy, deoxyhemoglobin: Deoxy, total hemoglobin: THb, oxygen saturation: StO2) of the Achilles tendons. A red laser light (BOM-L1TRSF; Omega Wave, Tokyo, Japan) and a probe (SF-DS; Omega Wave, Tokyo, Japan) were used to measure blood circulation in the Achilles tendon (e.g. Kubo9)). According to the previous findings22), Achilles tendinopathy commonly occurred in the region more than 20 mm proximal to the calcaneus. Therefore, the probe was placed on the center of the Achilles tendon 20 mm proximal to the calcaneus (Fig. 1). The probe was secured with elastic tape to prevent any movement during the exercises. Measurements of blood circulation in the tendon were performed when subjects lay in the prone position on a treatment table. This method used three red laser lights (635, 650, and 690 nm) to calculate the relative tissue levels of Oxy, Deoxy, and THb. The probe used in this study allowed blood circulation in the Achilles tendon to be measured at a depth of 3-5 mm from the skin23). Although the variables measured in the present study were not actual physiological volumes, the units of Oxy, Deoxy, and THb were expressed in μmol/l. StO2 was calculated as a relative value of Oxy to THb using the following formula.

\[
\text{StO}_2 (\%) = \frac{\text{Oxy}}{\text{THb}} \times 100
\]

Data were recorded using a personal computer through A/D transducer (Power Lab, AD Instruments, Australia) with a sampling rate of 1000 Hz. The mean of the measured variables, during 10 min of rest in the prone position for at least 20 min after the completion of the initial MVC measurement, was used as the resting value. After ECC, each subject immediately returned (within 2 min) to the prone position on the treatment table from the leg press machine. Measurements of the recovery period were performed over 40 min, and the mean of the measured variables was calculated every 10 min using analytical software (Chart ver. 7.3.7, AD Instruments, Australia). As reported in our previous study (e.g. Kubo9)), these data were expressed as the amount of change from pre-exercise values. In our previous studies23,24), the repeatability of measurements in tendon blood circulation was confirmed.

Cross-sectional area and collagen fiber orientation in the Achilles tendon. During the measurements of cross-sectional area (CSA) and collagen fiber orientation in the Achilles tendon, the posture of each subject was the same as that for the measurement of tendon blood circulation, as described above. A real-time ultrasonic apparatus was used to obtain transverse ultrasonic images of the Achilles tendon 40 mm proximal to the calcaneus (Fig. 1). Ultrasonic images were taken before, immediately after ECC, and every 10 min over the recovery period. During the measurements, subjects were requested to relax completely and not offer any voluntary resistance with their feet hanging off the end of the treatment table. Unfortunately, we did not measure ankle joint angle during the measurements. In the present study, the ankle joint angle may change to a slightly plantarflexed position after ECC, since the elbow joint angle during rest changed to a flexed position (around 10°) after repeated eccentric contractions25). However, we previously reported that a change in collagen fiber orientation in the Achilles tendon was greater at more dorsiflexed positions14). Therefore, we considered that this point did not affect the main results in the present study. Settings (gain, focus, and time gain compensation) were maintained throughout the experimental period. Ultrasonic images were recorded on videotape and analyzed using ImageJ software (NIH, Bethesda, MD). When obtaining ultrasound images, we payed attention to putting the ultrasound probe perpendicularly to the Achilles tendon. Moreover, ultrasound images were printed out; and an investigator confirmed that appearances of skin and tendon were similar to the images obtained before ECC. The CSA of the Achilles tendon was measured by manually tracing an outline of the Achilles tendon. In order to quantify tendon collagen fiber orientation, the coefficient of variation (CV) of echogenicity was calculated using the procedure previously described by
Ishigaki et al.14). The region of interest (ROI), which included as much of the Achilles tendon as possible without the surrounding tissue, was selected on ultrasonic images. The gray scale of each pixel in the ROI was represented as a gray scale histogram. Mean echogenicity and the standard deviation (SD) were calculated from the gray scale histogram. The CV of echogenicity was presented as a percentage of the SD relative to mean echogenicity (CV of echogenicity = SD/mean echogenicity*100). All measurements were repeated three times in each image, and the mean of the three measurements was used in the statistical analysis. The repeatabilities of the measurements of tendon CSA, mean echogenicity, and the CV of echogenicity on two separate days were confirmed in our previous studies14,26).

### Statistical analysis

Descriptive data are presented as mean ± SD. A paired t-test was used to compare differences in MVC and muscle thickness values between before and after exercises. In addition, the paired t-test was used to compare significant differences in relative changes in MVC and muscle thickness between the two protocols. A two-way ANOVA (load × time) with repeated measures was used to identify significant differences in each measured variable from the resting level. When the ANOVA revealed a significant main effect of load and time, a one-way ANOVA with repeated measures and Bonferroni’s post hoc test were conducted to identify any significant changes from the resting level. If a significant interaction was detected between load and time, a multiple comparison test for simple effects was performed as a post hoc analysis. A p value of less than 0.05 was considered significant for all analyses. As a result of calculation of the effect size with more than 80% of statistical power using G*Power3.1.9.2 (http://www.gpower.hhu.de/), effect size in this study was 0.359. According to Cohen27), the effect size of this study was classified to medium.

### Results

In both protocols, MVC significantly decreased (114.2 Nm ± 23.5 Nm to 94.9 Nm ± 23.2 Nm in low-load protocol, p = 0.003, 114.2 Nm ± 23.6 Nm to 101.1 Nm ± 26.1 Nm in high-load protocol, p < 0.001; Fig. 2A), and muscle thickness significantly increased after the exercises (19.5 mm ± 3.3 mm to 20.6 mm ± 3.4 mm in low-load protocol, 18.7 mm ± 3.2 mm to 19.4 mm ± 3.2 mm in high-load protocol, both protocols p < 0.001; Fig. 2B). However, no differences in the relative changes in MVC (-16.6% ± 11.9% in low-load protocol, -11.6% ± 11.4% in high-load protocol) or muscle thickness (6.4% ± 2.6% in low-load protocol, 3.9% ± 2.8% in high-load protocol) were found between the two protocols (Fig. 2A and B).

The effect of time was significant for all measured variables for blood circulation in the Achilles tendon (p < 0.001 for Oxy, Deoxy, and THb, p = 0.001 for StO2), whereas the effects of load (p = 0.420 for Oxy, p = 0.506 for Deoxy, p = 0.423 for THb, and p = 0.645 for StO2) and the interaction between time and load (p = 0.344 for Oxy, p = 0.773 for Deoxy, p = 0.393 for THb, and p = 0.914 for StO2) were not significant (Table 1, Fig. 3). The post hoc test to compare each time point with the resting level revealed that Oxy and THb remained higher than the resting level, except for at the 40-min point of recovery for Oxy, whereas StO2 returned to the resting level at the 20-min point of recovery.

The effect of time was significant for all measured variables in transverse ultrasonic images of the Achilles tendon (p < 0.001 for all), whereas the effects of load (p = 0.529 for CSA, and p = 0.529 for mean echogenicity, p = 0.141 for CV of echogenicity) and the interaction between time and load (p = 0.811 for CSA, p = 0.700 for mean echogenicity, and p = 0.166 for CV of echogenicity) were not (Table 2, Fig. 4). The results of the post hoc test to compare each time point with the resting level showed that CSA significantly decreased immediately after the exercises and returned to the resting level at the 40-min point of recovery. Mean echogenicity significantly increased and the CV of echogenicity significantly decreased, and these values remained unchanged during the recovery period.
Discussion

The results of the present study showed an increase in blood circulation and the alignment of collagen fibers in the Achilles tendons after repeated eccentric contractions. However, no significant differences in changes of blood circulation and collagen fiber orientation in the Achilles tendons were observed between the low- and high-load protocols.

Previous studies reported that the THb of tendons increased immediately after maximal repeated eccentric contractions\(^9,10\), whereas that of the patellar tendons did not change after 12 weeks of high-load (80% of 1RM) eccentric knee extension training\(^11\). Based on previous studies showing good clinical results with a low-load and high-repetition protocol (i.e., Alfredson protocol) for patients with tendinopathy (e.g. Alfredson et al.\(^1\)), we hypothesized that changes in blood circulation in the Achilles tendon may be more prominent for a low-load (and high-repetition) protocol than a high-load (and low-repetition) protocol. However, this hypothesis was rejected in the present study. According to our previous findings, a significant increase in the THb of tendons was found after 50 repetitions of isometric contractions at 70% of MVC\(^23\), whereas the THb of tendons did not change after 50 repetitions of isometric contractions at 50% of MVC\(^24\). In the present study, the amount of work performed was equal between the low- and high-load protocols. Therefore, no significant differences were expected in the effects of repeated eccentric contractions on blood circulation in the tendons between the low- and high-load protocols if the amount of work performed during the exercises was the same regardless of the magnitude of the load.

To the best of our knowledge, this is the first study to investigate changes in collagen fiber orientation in the

### Table 1. Oxyhemoglobin, deoxyhemoglobin, total hemoglobin and oxygen saturation

|                     | Rest  | 10min | 20min | 30min | 40min |
|---------------------|-------|-------|-------|-------|-------|
| **Oxyhemoglobin (μmol/l)** |       |       |       |       |       |
| Low-load protocol   | 8.1 ± 1.5 | 10.1 ± 2.0 | 9.5 ± 2.1 | 9.2 ± 2.3 | 8.9 ± 2.3 |
| High-load protocol  | 8.5 ± 1.1 | 11.3 ± 1.9 | 10.4 ± 1.6 | 9.9 ± 1.5 | 9.6 ± 1.3 |
| **Deoxyhemoglobin (μmol/l)** |       |       |       |       |       |
| Low-load protocol   | 2.9 ± 0.5 | 3.3 ± 0.7 | 3.2 ± 0.7 | 3.2 ± 0.6 | 3.2 ± 0.7 |
| High-load protocol  | 3.2 ± 0.4 | 3.7 ± 1.0 | 3.7 ± 0.9 | 3.6 ± 0.9 | 3.6 ± 0.9 |
| **Total hemoglobin (μmol/l)** |       |       |       |       |       |
| Low-load protocol   | 11.0 ± 1.9 | 13.4 ± 2.4 | 12.7 ± 2.7 | 12.4 ± 2.9 | 12.2 ± 2.8 |
| High-load protocol  | 11.7 ± 1.3 | 15.1 ± 2.6 | 14.1 ± 2.1 | 13.6 ± 2.0 | 13.2 ± 1.8 |
| **Oxygen saturation (%)** |       |       |       |       |       |
| Low-load protocol   | 73.3 ± 2.4 | 75.3 ± 3.5 | 74.5 ± 2.8 | 73.9 ± 2.5 | 73.2 ± 2.9 |
| High-load protocol  | 72.6 ± 3.0 | 75.4 ± 4.1 | 74.1 ± 4.5 | 73.3 ± 4.1 | 72.8 ± 4.7 |

Fig. 3 Time course changes in oxyhemoglobin (Oxy; A), deoxyhemoglobin (Deoxy; B), total hemoglobin (THb; C), and oxygen saturation (StO2; D) in the Achilles tendon for low-load (closed) and high-load (open) protocols. * significantly different from the resting level (* p < 0.05)
Achilles tendons after repeated eccentric contractions in vivo. According to our recent study\(^1\), a decline in the CV of echogenicity after ECC implied the alignment of collagen fibers within the Achilles tendons. Furthermore, in both protocols, the CV of echogenicity was significantly lower than the resting level until the end of the recovery period (Fig. 3C). A previous in vitro study revealed that tendon collagen fibers were aligned by cyclic tendon stretching\(^16\). Furthermore, Hooley et al.\(^28\) described that the crimping of collagen fibers in tendons was straightened out with structural changes during static creep. Therefore, the results for the CV of echogenicity in the present study suggest that collagen fibers within the tendons were aligned by cyclic stretching during ECC; this state remained during the recovery period.

Previous studies reported a decrease in tendon thickness measured by ultrasonography after eccentric exercise\(^29,30\). The results for tendon CSA in the present study (Fig. 3A) was consistent with the previous findings. Wearing et al.\(^31\) reported that 90 repetitions of a squat exercise resulted in an immediate decrease in the thickness of the patellar tendon and were accompanied by an increase in the mean echogenicity of the tendon. They stated that not only a decrease in tendon thickness, but also an increase in mean echogenicity resulted from load-induced interstitial fluid movement. Therefore, the decrease in tendon CSA and increase in mean echogenicity immediately after ECC in the present study may reflect interstitial fluid movement within tendons. However, the increase observed in mean echogenicity remained during the recovery period, whereas tendon CSA gradually returned to the resting level (Fig. 3A and B). A previous in vitro study reported that the mean echogenicity of tendons was non-linearly related to the applied tendon stress\(^32\). Nevertheless, the mean echogenicity of tendons increased during the relaxation period of a stress-relaxation test\(^33\). This unexpected finding was suggested to be affected by (1) the alignment of collagen fibers, (2) alterations in acoustic reflection intensity, and (3) local collagen density after interstitial fluid move-
ment. Collagen density within tendons is unlikely to increase immediately after ECC. Unfortunately, it currently remains whether acoustic reflection intensity changes after ECC. Therefore, changes in mean echogenicity during the recovery period may be affected by other factors besides interstitial fluid movement within tendons.

In the present study, we attempted to clarify a suitable protocol with eccentric exercises through observations of acute changes in blood circulation and collagen fiber orientation in the tendons with repeated eccentric contractions. As described above, we expected increases in blood circulation and the alignment of collagen fibers in tendons to be more prominent after the low-load (and high-repetition) protocol, which is similar to Alfredson’s protocol\(^1\), than the high-load (and low-repetition) protocol. However, the present results showed no significant differences in the measured variables between the two protocols. Overall and higher strain of tendon are believed to be risk factors of tendon injuries\(^{38,39}\). Therefore, it is dangerous to perform rehabilitative exercises using higher loads for patients with chronic tendinopathy. Taking the safety of patients into account together with the present results, we recommend a low-load (and high-repetition) protocol such as Alfredson’s protocol for the treatment of patients in clinical practice. However, it is an advantage of the high-load and low-repetition protocol to complete an exercise routine in a relatively short period compared to a low-load and high-repetition protocol. Therefore, the intensity of load and number of repetitions should be controlled depending on the symptoms of patients with tendinopathy.

There were some limitations in the present study. Firstly, the measurement site of blood circulation and ultrasonic images was in a limited region of tendons (20 mm proximal for blood circulation and 40 mm proximal to the calcaneus for ultrasonic images). Responses to ECC may differ depending on the measurement site in tendons. However, the results appear to have important clinical implications for the conservative treatment of tendinopathy, because the location at which Achilles tendinopathy commonly occurs (15 to 70 mm proximal to the calcaneus\(^{30}\)) included the measurement site in the present study. Secondly, changes in blood circulation, tendon CSA, and tendon collagen fiber orientation were only measured 40 min after ECC. If measurements during the recovery period continue for longer than 40 min, differences may be observed in the measured variables between the two protocols. However, it is thought that this point would not affect the main results for blood circulation and the CV of echogenicity because the pattern of changes in the measured variables during the recovery period (40 min) was similar for both protocols (Fig. 2 and 3). Thirdly, our results for healthy subjects may differ from those for injured tendons. A previous study reported differences in the morphological and mechanical responses of tendons to exercises between tendinopathy and healthy subjects\(^{37}\).

In future studies, we need to investigate the effects of ECC for the treatment of actual injured tendons. Fourthly, the range of motion of the ankle joint during repeated eccentric exercises would differ among subjects, since there was inter-subject variability of maximum range of motion of the ankle joint. In the present study, however, we intended to compare the measured variables between the two protocols. Therefore, this point is considered to have an insignificant effect on the results.

In conclusion, repeated eccentric calf muscle contractions produced an increase in blood circulation and the alignment of collagen fiber in the Achilles tendons. However, no significant differences in changes in the measured variables were observed between the low- and high-load protocols. Although the high-load and low-repetition protocol has the advantage of a shorter training period, in consideration of the safety of patients, the present results suggest that eccentric exercises with low-load and high-repetition are more suitable for the treatment of patients with symptomatic tendinopathy.

**Conflict of Interests**

The authors declare that they have no conflict of interests.

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