Effect of habitual running on human Achilles tendon load-deformation properties and cross-sectional area

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Hansen, P., P. Aagaard, M. Kjaer, B. Larsson, and S. P. Magnusson. Effect of habitual running on human Achilles tendon load-deformation properties and cross-sectional area. J Appl Physiol 95: 2375–2380, 2003.—Whether the cross-sectional area (CSA) and mechanical properties of the human Achilles tendon change in response to habitual exercise remains largely unexplored. The present study evaluated the CSA and contraction-induced displacement of the aponeurosis-tendon complex of the triceps surae in 11 untrained subjects before (tests 1 and 2) and after (test 3) ~9 mo of regular running (~78 training sessions). Displacement of the tendon-aponeurosis complex obtained by ultrasonography; electromyography of the gastrocnemius, soleus, and dorsiflexor muscles; and joint angular rotation were recorded during graded isometric plantarflexion ramps. Tendon CSA and moment arm were measured by using MRI, and tendon force was calculated from joint moments and tendon moment arm. A treadmill test was used to determine submaximal oxygen consumption (V\textsubscript{O2}) at a given speed and maximal V\textsubscript{O2}. The total running duration was ~43 h, distributed over 34 wk. Maximal V\textsubscript{O2} increased 8.6% (P < 0.01), and submaximal V\textsubscript{O2} decreased 6.2% (P < 0.05). Tendon-aponeurosis displacement during maximal voluntary contraction was unchanged (tests 1–3, 5.2 ± 0.6, 5.2 ± 0.5, and 5.3 ± 0.4 mm, respectively) and yielded a structural stiffness of 365 ± 50, 358 ± 40, and 384 ± 52 N/mm for tests 1–3, respectively (P > 0.05). Tendon CSA also remained unchanged (tests 1–3, 34.2 ± 2.2, 33.9 ± 2.2, and 33.8 ± 2.1 mm\textsuperscript{2}, respectively). In conclusion, a total training stimulus of ~9 mo of running in previously untrained subjects was adequate to induce significant cardiovascular improvements, although it did not result in any changes in the mechanical properties of the triceps surae tendon-aponeurosis complex or in the dimensions of Achilles tendon.

stiffness; mechanical properties; tendon hypertrophy; collagen

PHYSICAL ACTIVITY IS ASSOCIATED with numerous health benefits (2); however, it also frequently results in overuse injuries, which constitute a considerable proportion (30–50%) of all sports injuries (6). The Achilles tendon, in particular, is often associated with various pathologies related to loading history (6, 9), perhaps due to the substantial forces placed on the triceps surae complex and Achilles tendon during locomotion. However, the exact injury mechanism(s) of tendons and exactly how tendons are influenced by physical activity or lack of exercise are incompletely understood. Specifically, we do not know whether increased physical activity is associated with tendon hypertrophy and/or qualitative change of the human tendon.

Studies on the influence of exercise on tendon properties are scarce, inconclusive, and based on animal models. A 40-wk running regimen in rabbits resulted in a leftward shift for the load-deformation curve of the posterior tibialis tendon, but notably not for the Achilles tendon (23, 24). Others have shown that 12 mo of treadmill endurance training in swine markedly increased both tendon cross-sectional area (CSA) and load deformation, as well as stress-strain properties of the low-stress extensor tendons. However, it did not affect the high-stress flexor tendons that are primarily subjected to large loads during locomotion (27, 28). Interestingly, the shorter term exercise program (3 mo) actually resulted in a decrease in tendon CSA (27).

Eight to twelve weeks of treadmill endurance training in guinea fowls altered the structural and mechanical properties of the tendon, although it did not result in any tendon hypertrophy (3), whereas 16 wk of running at various intensities resulted in a marked increase in CSA (40%) of the rat Achilles tendon (21). Numerous factors, such as species, age, training duration, and testing procedures, may have contributed to the inconclusiveness.

Studies that address tendon adaptations in response to training in a human model are rare, and none has examined the effect of prolonged endurance training on tendon CSA and load-deformation properties. The stiffness of the vastus lateralis tendon-aponeurosis complex was reportedly greater in runners compared with untrained subjects (8). Additionally, it was recently shown that habitual runners have a greater CSA of the Achilles tendon compared with sedentary controls (20). Whereas these findings imply that human tendinous tissue may adapt to increased use by changes in the structural properties and tendon dimensions, the cross-sectional designs of the studies preclude any firm conclusions. To the best of our knowledge, there are presently no studies that have examined the effect of increased tendon loading on the mechanical and morphological properties of the human Achilles tendon in a...
longitudinal intervention study. Therefore, the purpose of the present study was to investigate the effect of initiation of habitual running in sedentary human subjects on the structural properties of the Achilles tendon-aponeurosis complex and the CSA of the Achilles tendon.

**MATERIALS AND METHODS**

**Subjects.** Sixteen untrained healthy subjects gave written consent to participate in the study, which was approved by the local ethics committee. Subjects were interviewed to ensure that they had no history of lower extremity or back injuries. The initial study sample consisted of 10 men and 6 women. However, three male and two female subjects failed to complete the training period, and, therefore, all results refer to the remaining subjects (n = 11). The male subjects had a mean (±SE) age, weight, height, percent body fat, and maximal oxygen consumption (VO₂max) of 29 ± 1 yr, 75.2 ± 2.5 kg, 178 ± 3 cm, 17 ± 1%, and 3.6 ± 0.2 l/min, respectively. The corresponding values for female subjects were 26 ± 1 yr, 66.0 ± 2.1 kg, 168 ± 3 cm, 30 ± 1%, and 2.6 ± 0.1 l/min, respectively. Several exclusion criteria were used to ensure that subjects were untrained and included 1) sporting activities with presumed large Achilles tendon loading, such as running, badminton, soccer, etc; 2) regular sports participation, defined as more than once per week for >3 consecutive mo within the last year; 3) regular running defined as more than two times per week for >3 consecutive mo within the last 2 yr and more than two times per week for >1 mo within the last year.

**Study design.** A crossover trial design was used. Load deformation properties of the Achilles tendon-aponeurosis complex and CSA of the Achilles tendon were examined on three occasions: test 1 and test 2 were conducted ~2 wk apart before the initiation of training to examine the stability of the measurements, and test 3 after completion of training to examine the effect of the intervention.

**Training regimen.** Subjects underwent a progressive training program that was designed to minimize the risk of injury, maximize compliance, and ensure “adequate” mechanical loading. The entire regimen included 70–80 training sessions over a 9-mo period. In the first 3 mo, running was gradually increased to an obligatory minimum of 30 min and a maximum of 50 min per training session. The duration interval was chosen to augment compliance and yet allow for a flexible training schedule. Because elevated running intensity is associated with an increased injury risk (9), subjects were specifically instructed to run at “low intensity,” defined as a pace at which the subjects were able to maintain a conversation, in the first 3 mo. Subsequently, subjects were gradually allowed to increase the training intensity. Subjects were instructed to keep a training diary and were contacted weekly by phone to maximize compliance. During the entire training period, subjects were instructed to refrain from participation in other sports to reduce the risk of sustaining an injury and to avoid confounding training influence.

**Oxygen consumption measurement.** Subjects performed a treadmill test to determine submaximal oxygen consumption (VO₂) and VO₂ max. During a 5-min warm-up, the treadmill was adjusted to a comfortable running speed. Subsequently, the submaximal VO₂ test was performed while this speed was held constant for 5 min. VO₂ max was determined ~5 min after the submaximal test. For the VO₂ max test, the treadmill speed was increased (1–2 km/h) to achieve exhaustion within 4–7 min. This pace remained constant throughout the test, and work level was increased by adjusting the slope of the treadmill to 2% uphill after the first 2 min, followed by 2% increments every 90 s until exhaustion. VO₂ and carbon dioxide production were monitored continuously (AMIS 2001 automated metabolic cart, INNOVISION, Odense, Denmark) and averaged each 15 s. The mean of the three highest 15-s values of VO₂ during the submaximal and maximal tests, respectively, were recorded as submaximal VO₂ and VO₂ max. Submaximal treadmill speed was the same before and after the training regimen. However, the treadmill speed was increased for the VO₂ max assessment for some subjects to reach exhaustion within 7 min after the training period. Heart rate was measured continuously by a heart rate monitor (Polar Sport Tester, Polar Electro OY, Kempele, Finland).

**Structural property.** The experimental procedure for determining the structural properties of the tendon aponeurosis has been described in detail previously (16, 20). Briefly, subjects were seated within a metal frame with the hip flexed to 90° and the knee fully extended. The left foot rested against a steel plate at 90° to the leg and with an axis of rotation corresponding to the lateral malleolus. Planatarflexion (3 mo) was measured by a strain-gauge load cell connected to the steel plate. After four to seven plantarflexion efforts that served as preconditioning (16), the subjects performed a slow-force ramp by applying gradually increased pressure on the steel plate over a period of 10 s. Simultaneously, tendon-aponeurosis displacement, force, electromyographic (EMG) activity, and ankle joint rotation were measured. The coefficient of variation (CV) for repeated measures was previously shown to be 11.3% (16).

In addition, a passive dorsiflexion was performed to obtain an individual correction factor for joint rotation (17). To monitor ankle joint rotation, a goniometer (Penny and Giles, Biometrics, Gwent, UK) was mounted on the lateral part of the fifth metatarsal and the distal fibula. The investigator moved the foot of the subject from ~7–10° of plantarflexion to 3–5° of dorsiflexion, while EMG and goniometer data were sampled with simultaneous ultrasonography (US) recording. Subjects were requested to relax completely during passive movement. Previous investigations have demonstrated that rotation about the transverse axis of the ankle joint results in significant tendon-aponeurosis displacement (16, 22). An individual correction factor (mm°) was established for each subject to account for any tendon-aponeurosis displacement due to joint rotation during the plantarflexion ramp contractions (17).

EMG activity of the lateral and medial gastrocnemius, soleus, and dorsiflexor muscles was recorded with bipolar Ag-AgCl surface electrodes (type QN-10-A, Medicotest, Øl- stykke, Denmark) placed with a 2.5-cm interelectrode distance. The EMG activity recorded during maximal isometric dorsiflexion (see below) was subsequently used to calculate the opposing force of dorsiflexor coactivation during the plantarflexion trial (16). Moreover, the EMG signals served to ensure that the passive movement maneuver occurred without any contractile activity. Custom-made amplifiers with a frequency response of 20 Hz to 10 kHz and 1:1 preamplifiers were used for the EMG signal measurements. During the ramp contractions and passive dorsiflexion, the EMG signal was full-wave rectified, integrated, and averaged by using a time constant of 200 ms (1).

Three dorsiflexion and ten voluntary contractions were performed. Each effort lasted ~4 s, with a 1-min rest period between contractions. Subjects retained the same position used in the plantarflexion ramp trial (90°). An inextensible strap was looped around the distal part of the metatarsals and connected to the strain-gauge load cell. The distance...
from the strap to the center of rotation (obtained from magnetic resonance images, see below) times the force produced during dorsiflexion yields the dorsiflexion moment (N·m). The force generated by dorsiflexor muscle coactivation during the plantarflexion efforts was estimated, assuming a linear relationship between EMG amplitude of the dorsiflexor muscles and muscle tension (14).

The goniometer, EMG, force, and ultrasound image signals were continuously recorded during the 10-s plantarflexion trial. The goniometer, EMG, and force signals were sampled at 50 Hz by using an analog-to-digital converter (DT 2801A, Data Translation). A trigger signal initiated goniometer and EMG sampling while producing a visual marker on the US recording, which allowed for subsequent synchronization.

The Achilles tendon divides proximally into the superficial and deep aponeuroses of the medial gastrocnemius muscle complex. The deep aponeurosis separates the gastrocnemius muscle from the underlying soleus. US of the medial gastrocnemius was performed by using a linear array B-mode transducer (Siemens Sonoline Sienna Apparatus, Erlangen, Germany) with a width and a depth resolution of 0.51 and 0.34 mm, respectively. The transducer was secured in a styrofoam block, which was oriented in the sagittal plane and taped on the skin overlying the medial gastrocnemius. The position of the styrofoam block was carefully noted to obtain the exact same position in subsequent trials. Ultrasonographically, the deep aponeurosis is seen as two parallel hyperechoic lines at an oblique angle to the surface. Muscle fascicles of the gastrocnemius attach to the aponeurosis with interfascicle connective tissue defining hyperechoic cross points at the insertion on the aponeurosis. Displacement of cross points during graded plantarflexion was considered to correspond to tendon-aponeurosis displacement. The S-VHS US output was sampled to a computer by a 50-Hz frame-grabber. An image tracking software package using a pyramidal implementation of a Lukas-Kanade feature tracking was recently developed at our laboratory that provides automatic tracking of US tendinous displacement (17). Reproducibility of the analysis has yielded $r^2 = 0.99$ with CV for repeated measures of 0.6–1.5% and an accuracy of 0.2 mm (<2%) (17).

Axial plane images of the Achilles tendon were obtained by MRI (General Electric Sigma Horizon LX, 1.5 T, T1-weighted spin echo; repetition time-to-echo time ratio, 400:15; field of view, 12 cm; matrix, 512 × 512; slice thickness, 6 mm; spacing, 6 mm). The images were obtained with the ankle joint at 90°. The subjects were instructed to refrain from exercise activities 1 day before the MRI. Achilles tendon CSA was obtained from axial images (Fig. 1a). It was recently shown that the CSA of the most distal portion of the Achilles tendon has a greater CSA than the more proximal portion of the free Achilles tendon (18), and, therefore, CSA was measured 48, 60, and 72 mm above the inferior margin of the calcaneus. To remove investigator bias, a macro was created that automatically outlined the Achilles tendon and calculated its CSA ($\text{mm}^2$) by using National Institutes of Health Image software (http://rsb.info.nih.gov/nih-image) (Fig. 1b).

A modified Reuleaux method was used to estimate the individual Achilles tendon moment arm based on sagittal plane magnetic resonance images obtained with the ankle in the neutral position (T2-weighted fast spin echo; repetition time-to-echo time ratio, 4,000:88; field of view, 12 cm; matrix, 256 × 192; slice thickness, 3 mm), as described previously (16). The tendon force was calculated by dividing the externally measured moment measured with the tendon moment arm while accounting for antagonist coactivation (16). Although the instantaneous moment arm may be influenced by joint rotation (15), it was not considered to have a substantial effect because the average joint rotation was <3°.

By combining strain-gauge, goniometer, dorsiflexor, and US tracker displacement data, a load-displacement relationship was obtained for each subject. As a measure of tendinous mechanical properties of the combined free tendon and aponeurosis, the displacement corresponding to 90% of the least common maximal isometric tendon force of the three plantar flexion ramps (test 1–3) was used to calculate stiffness (N/\text{mm}) (16).

**Data reduction and statistics.** Only values corrected for both ankle joint rotation and antagonist dorsiflexor coactivation are reported. Errors in data collection precluded complete analysis of the ramp contractions of one subject. The S-VHS video signal was analyzed at 25 Hz and expanded to 50 Hz by linear point-to-point interpolation, followed by low-pass filtering (4th order Butterworth filter, 2.5-Hz cutoff), whereas all other signals were analyzed at 50 Hz. $V_{O_2}$ and body composition measurements were analyzed with (two-tailed) paired student’s t-test. Two-tailed separate one-way ANOVA with repeated measures was used to address whether a change occurred over time on tendon structural properties and CSA. Post hoc Bonferroni-corrected Student’s t-test was applied when appropriate. Furthermore, CV for duplicate measures (%) were calculated for CSA, displacement, and stiffness from tests 1 and 2 as a measure of reliability. An $\alpha$-level of $P < 0.05$ was considered significant. Results are reported as means ± SE.
RESULTS

The entire training period was 34 ± 2 wk. It consisted of a total training duration of 43 ± 2 h, distributed over an average of 78 ± 2 training sessions, which corresponded to 2.4 ± 0.2 sessions/wk. There was a significant increase in $V\dot{O}_2$ max [change (Δ) 0.29 ± 0.06 l/min; $P < 0.01$; Fig. 2B] with a concomitant decline in submaximal $V\dot{O}_2$ (Δ 0.16 ± 0.06 l/min; $P < 0.05$; Fig. 2A) and submaximal heart rate (Δ 11.2 ± 2.3 beats/min; $P < 0.001$; Fig. 2C). Body mass remained unchanged, whereas percent body fat declined (Δ 1.4 ± 0.5%, $P < 0.05$).

Both dorsiflexion and plantarflexion moment remained unchanged. The least common maximal isometric tendon force for the three plantarflexion ramps (tests 1–3) was 1,432 ± 155 N. The corresponding tendon-aponeurosis displacement was unchanged (test 1, 5.2 ± 0.6 mm; test 2, 5.2 ± 0.5 mm; test 3, 5.3 ± 0.4 mm). The average difference calculated as a mean of tests 1 and 2 vs. test 3 was 0.15 ± 0.25 mm. The structural stiffness was 365 ± 50 N/mm for test 1, 358 ± 40 N/mm for test 2, and 384 ± 40 N/mm for test 3. The CV for repeated tests (tests 1 and 2) was 11.7% for stiffness and 8.9% for displacement.

The tendon CSA remained unchanged over time (Table 1). An average of the three levels yielded 34.2 ± 2.2 mm$^2$ for test 1, 33.9 ± 2.2 mm$^2$ for test 2, and 33.8 ± 2.1 mm$^2$ for test 3 (Fig. 3). The average difference calculated as a mean of tests 1 and 2 vs. test 3 was 0.27 ± 0.42 mm$^2$. The CV for repeated tests (tests 1 and 2) yielded 4.5% at 48 mm, 7.5% at 60 mm, and 7.2% at 72 mm.

DISCUSSION

In the present study, untrained subjects completed a training regimen that included a total of 43 h of running over a 34-wk period. The training resulted in measurable cardiovascular effects as evidenced by an elevated $V\dot{O}_2$ max and a reduced submaximal $V\dot{O}_2$ and heart rate at a given speed, indicating improved running economy. However, although the present training stimulus was adequate to induce significant cardiovascular changes, there were no measurable effects on the load-deformation properties of the tendon aponeurosis of the medial gastrocnemius muscle or any significant increases in the CSA of the Achilles tendon.

Information on the influence of exercise on the mechanical properties of tendon is inconclusive and almost exclusively based on animal models. It has been shown that a 40-wk running regimen in rabbits did not affect the load-deformation curve of the Achilles tendon (24). Similarly, others (27) have shown that 12 mo of running in swine did not affect the load deformation or stress-strain properties of the load-bearing, high-stress flexor tendons, whereas these properties were augmented in the low-stress extensor tendon, which suggests a tendon-specific adaptation. Others (3) have demonstrated that 8–12 wk of treadmill endurance training in guinea fowls altered the load-deformation curve of the high-stress gastrocnemius tendon and the mechanical properties (material composition), without Fig. 2. Mean ± SE values before (pre) and after (post) the running intervention. A: submaximal oxygen consumption. B: maximal oxygen consumption. C: submaximal heart rate. bpm, Beats/min. Significantly different from pre: *$P < 0.05$, **$P < 0.01$.
causing any tendon hypertrophy, which led the authors to conclude that the adaptation reflected a change in material properties. The present data show, for the first time, that initiation of a long-term running regimen in untrained subjects did not produce a measurable change in the load-deformation curve of the high-stress tendon-aponeurosis complex of the triceps surae, which is in accordance with previous animal studies (24, 27).

Whether human tendons adapt to increased physical activity by changing their CSA remains unknown. Animal studies have shown that the CSA of the tendon may either increase (21, 27, 28), decrease (27), or remain unchanged (3) in response to increased physical activity. In humans, the CSA did not differ between older athletes and controls measured with US (5). While using high-resolution MRI, it was recently shown that runners had markedly greater Achilles tendon CSA than that of the age-matched controls (20). However, the present data are the first to examine the effect of running training on human tendon morphology in a longitudinal experimental design. The data suggest that the CSA of the human Achilles tendon remained unchanged in response to the 9-mo training stimulus. The lack of increase in CSA further supports the unchanged load-deformation curve. It is noteworthy that some animal data suggest that tendon CSA is reduced initially followed by a subsequent hypertrophy (27).

Such a biphasic response would indicate that collagen net formation is preceded by a net breakdown of collagen. Albeit purely speculative, a transient reduction in net collagen formation would reduce tendon CSA, which would translate into an increased tendon stress and possible risk for injury. However, the present study cannot answer whether transient changes in CSA took place because of the lack of serial measurements.

It was recently demonstrated that human peritendinous tissue is metabolically more active in response to activity than previously thought (11, 12), and it has been shown that a 36-km run acutely increases markers for collagen (12). Furthermore, the chronic load of military-type training appears to result in a net synthesis of collagen type I in human tendinous tissue (10). Although the lack of increase in CSA in the present study does not directly support the notion of a net collagen synthesis, it cannot be ruled out that other adaptations, such as altered collagen fibril density and altered proteoglycan content, which play a role in fibrillar interaction (4), may have taken place. At the same time, the training intensity or duration of the training period was perhaps inadequate to produce a net synthesis of collagen that would have been detectable on the MRI. Previous cross-sectional observations of a greater tendon CSA in runners included subjects with a considerable loading history (~80 km/wk for 5 yr) (20), which far exceeds that of the present study. Yet the training of the present study is likely comparable to that of many recreational athletes. Alternatively, the inherent strength of tendon far exceeds the loads to which it is subjected during locomotion, and thus the need to increase in size may be marginal. It has been suggested that the safety factor, i.e., the stress during strenuous activity divided by the fracture stress, is about eight for a majority of tendons (7). However, the human Achilles tendon may have a safety factor (~2.4) that approaches its safety limit (16), and thus even minor changes may be important. It was recently shown that strength training in old age resulted in an unchanged patellar tendon CSA (19), which is in accordance with the present findings. However, in contrast to the unchanged stiffness in the present study, strength training in older people yielded an increased stiffness and Young's modulus of the patellar tendon, indicating a change in its composition. It remains to be identified whether such differences can be attributed to the training mode or an age effect.

There are inherent limitations associated with the present study. First, training compliance may be an issue because the individual training sessions of the subjects were not carried out in the laboratory. However, the principle investigator frequently contacted the participants by telephone to ensure that training compliance was maintained, and subjects were instructed to fill out a training diary. Furthermore, it is apparent that the training regimen of the present study affected certain cardiovascular parameters in a magnitude that was to be expected over the training period and training intensity (25, 26). The training produced a significant decrease in submaximal heart rate and $V_{O2}$ and an increase in $V_{O2, max}$, and these
increases indicate that the subjects complied with the training regimen. Second, it is appropriate to address the issue of statistical power because the tendon parameters remained unchanged. Previous studies have demonstrated a 20% difference in CSA (20, 27); however, exactly what represents a clinically relevant change is difficult to ascertain. If a 5% increase is deemed meaningful, the present CSA data (A 0.27 mm², or 0.8%) are associated with a power of 99%, whereas a 2.5% increase is associated with a lower power (66%). Similarly, if a presumed tendon hypertrophy were associated with a 10% reduction in tendon deformation at a given force, the present data would be associated with a power of 60%, whereas a smaller but yet meaningful change would be associated with a lesser power.

In summary, the present study examined the effect of ~9 mo of running on human tendinous tissue. The total training stimulus included running for ~43 h over 34 wk and was adequate to induce significant cardiovascular improvements. However, the stimulus did not result in any changes in the tendon CSA or load-deformation properties of the triceps surae tendon-aponeurosis complex.

REFERENCES