Combination of hormone replacement therapy and high physical activity is associated with differences in Achilles tendon size in monozygotic female twin pairs

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Submitted 3 November 2008; accepted in final form 15 January 2009

Finni T, Kovanen V, Ronkainen PHA, Pöllänen E, Bashford GR, Kaprio J, Alén M, Kujala UM, Sipilä S. Combination of hormone replacement therapy and high physical activity is associated with differences in Achilles tendon size in monozygotic female twin pairs. J Appl Physiol 106: 1332–1337, 2009. First published January 22, 2009; doi:10.1152/japplphysiol.91439.2008.—Estrogen concentration has been suggested to play a role in tendon abnormalities and injury. In physically active postmenopausal women, hormone replacement therapy (HRT) has been suggested to decrease tendon diameter. We hypothesized that HRT use and physical activity are associated with Achilles tendon size and tissue structure. The study applied cotwin analysis of fourteen 54- to 62-yr-old identical female twin pairs with current discordance for HRT use for an average of 7 yr. Achilles tendon thickness and cross-sectional areas were determined by ultrasonography, and tendon structural organization was analyzed from the images using linear discriminant analysis (LDA). Maximal voluntary and twitch torques from plantar flexor muscles were measured. Serum levels of estradiol, estrone, testosterone, and sex hormone binding globulin were analyzed. Total daily metabolic equivalent score (MET-h/day) was calculated from physical activity questionnaires. Results showed that, in five physically active (MET > 4) pairs, the co-twins receiving HRT had greater estradiol level (P = 0.043) and smaller tendon cross-sectional area than their sisters (63 vs. 71 mm², P = 0.043). Among all pairs, Achilles tendon thickness and cross-sectional area did not significantly differ between HRT using and nonusing twin sisters. Intrapair correlation for Achilles tendon thickness was high, despite HRT use discordance (r = 0.84, P < 0.001). LDA distinguished different tendon structure only from two of six examined twin pairs who had a similar level of physical activity. In conclusion, the effect of HRT on Achilles tendon characteristics independent of genetic confounding may be present only in the presence of sufficient physical activity. In physically active twin pairs, the higher level of estrogen seems to be associated with smaller tendon size.

tendon structure; women; strength; calf; image analysis

TENDONS NEED STRENGTH FOR transferring forces from muscles to bones and resilience to do this task effectively. The most studied components of tendon strength are tendon thickness and cross-sectional area (CSA) (5, 13). A thicker tendon has to bear lower stress and is stiffer than a tendon with the same qualities but thinner or smaller CSA. The Achilles tendon (AT) can bear stresses near to the maximum failure stress of 100 MPa, and this may make AT susceptible for tears and ruptures (23).

Both repetitive loading (19) and intermittent high-load physical activity (13) have been shown to increase tendon CSA. Besides physical activity, pathological conditions and high cholesterol level may also increase tendon size (12). Furthermore, sex, probably as a result of hormonal status and, specifically, levels of estrogen, has been hypothesized to affect tendon diameter (5). In a cross-sectional study, Cook et al. (5) reported that physically active women (golf players) using hormone replacement therapy (HRT) had a lower AT diameter than those not using HRT. They concluded that HRT may ameliorate tendon health in active postmenopausal women, because the nonusers had a greater frequency of tendon disorders. Also, in other studies, the differences in hormonal status between the sexes have been suggested to have a role in the higher prevalence of connective tissue injuries in women than in men (16). This may be because the response of tendon to exercise may be reduced with high levels of estradiol (8). Recently, Hansen et al. (8) investigated the acute effects of exercise on synthesis of patellar tendon collagen using microdialysis method in young women with low or high levels of estradiol and found that the synthesis was greater in subjects with low estradiol levels. Since hereditary factors can be hypothesized to affect both physical activity and tendon properties, a genetically controlled study design with postmenopaual monozygotic (MZ) twin sisters discordant for HRT use provides a powerful tool to study the phenomena. The cotwin study design, in which HRT-exposed MZ twin sisters are compared with their unexposed co-twins, has advantages over traditional case-control and experimental designs. The cotwin design intrinsically adjusts for genetic factors and also for a number of shared environmental factors, starting from childhood.

Our study aims to investigate the effect of HRT use on tendon structure by comparing AT thickness, CSA, and tissue

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organization in MZ twins from pairs discordant for HRT use. In addition, the levels of estrogen, testosterone, sex hormone-binding globulin (SHBG), physical activity, muscle strength, and contractile capacity were determined to examine the primary determinants of tendon size. We hypothesized that the level of estrogen and physical activity are associated with AT size after menopause.

METHODS

Study subject’s recruitment and clinical assessment. This study is part of a larger study “Sarcopenia– Skeletal Muscle Adaptation to Postmenopausal Hypogonadism and Effects of Hormone Replacement Therapy and Physical Activity in Older Women: a Genetic and Molecular Biological Study on Estrogen-related Pathways” (SAWEs). The participants were recruited from The Finnish Twin Cohort (10, 11), which had 13,888 twin pairs of known zygosity at baseline in 1975. The target group of the present study contained all currently living female MZ twin pairs born between 1943 and 1952 (n = 537). Only those twin pairs in which one cotwin was a current HRT user and the other cotwin did not currently use HRT were asked to respond to the invitation. From all of the responders (n = 114 pairs), those twin pairs who were willing to participate in the laboratory measurements and of which one sister had never used HRT, while the other sister was a current user, were contacted (n = 21 pairs). Sixteen twin pairs had no contraindications for the present study and participated in all laboratory measurements. The verification of zygosity of the twins was done using DNA extracted from a venous blood sample with a battery of 10 highly polymorphic gene markers and showed one pair to be dizygotic. Finally, the study design included fifteen 54- to 62-yr-old MZ twin pairs discordant for HRT prescribed for the treatment of menopausal symptoms. Acceptable ultrasonography scans of AT were obtained from 14 MZ twin sisters. One pair was lost due to technical failure. The mean age of the 28 subjects was 57.2 ± 1.8 yr (range 54–62 yr) and mean body height was 163.8 ± 4.0 cm. The mean body mass was 69.3 ± 11.5 and 68.7 ± 8.8 kg for the HRT users and nonusers, respectively. The duration of HRT usage was on average 6.8 ± 4.2 yr (range, 2–16 yr). The mean duration of the treatment was 7.1 ± 3.9 yr (2–16 yr) for the women taking estradiol only (n = 5) or combined preparations including estradiol and progesterone (n = 5), and 6.0 ± 5.5 yr (2–14 yr, n = 4) for the tibolone users.

History of AT disorders was established with a personal interview whether any symptoms in the AT had occurred. Six subjects had experienced some symptoms more than 4 yr ago, and only one subject had current symptoms in the right tendon that were described as swelling and tenderness. She had had no medication to the problem but treated it with immobilization and rest. This was not considered to affect the results, since the measurements were taken from the left leg and there were no identified irregularities in the tendon of this particular subject.

The Ethics Committee of the Central Finland Health Care District approved the study, and it was conducted according to the guidelines in the Declaration of Helsinki. Written, informed consent was provided by the subjects before participating in the measurements. For the safety of the subjects, a study physician was present during all measurements.

AT measurements. Tendon thickness and CSA were determined from left leg using B-mode ultrasonography (Acuson CV70, Siemens, Erlangen, Germany). A 10-MHz, 4-cm linear probe was used. The images were stored on the hard drive and later analyzed using open-source software OsiriX (OsiriX Foundation, Geneva, Switzerland, www.osirix-viewer.com). The thickness was analyzed from mid-longitudinal images (Fig. 1) and the measurement distance from calcaneus was 1.51 cm (SD 0.16). The CSAs were measured from the same location as the thickness (CSAprox) and 1 cm proximal from this measurement site (CSAmid). The CSAs of the tendons were outlined manually. All the analysis were done twice in a separate session by the same analyzer with a typical error and intraclass correlation between the two measurements of 0.1 and 0.99 mm for thickness, 3 and 0.96 mm² for CSAprox, and 3 and 0.97 mm² for CSAprox, respectively. The tendon CSA was also scaled (9) to body mass to the power of 2/3, but absolute values are given in the results, since the interpretation of the results was unaffected by the scaling.

Linear discriminant analysis (LDA) on extracted image features was performed on the longitudinal AT images of six twin pairs. Due to variability in specific imaging parameters and the strict requirements of the algorithm, the analysis could not be completed from the entire subject group. The purpose was to find out whether HRT caused tendon structural organization to be different between the discordant twin sisters. The method has been described in detail previously when healthy and abnormal tendons were examined (2). Briefly, eight spatial frequency parameters were extracted from small (2 mm × 2 mm) regions of interest in the ultrasound image. The spatial frequency parameters indicate the dominant spatial frequency in the region of interest (by amplitude) and the quality of the dominant peak (by bandwidth). Subjects were assumed to fall into two groups, with each subject tested for agreement with the assigned HRT user or nonuser group. A leave-one-out analysis was performed in the following manner. For each subject, the remaining subjects were used as training data for a LDA algorithm. Each region of interest of the subject data was classified according to the training data.

Other measures. Planar flexor torque was measured in a seated position with fully extended knee. The right foot was placed onto a footplate, and ankle joint axis of rotation was aligned as well as possible with the rotational axis of the ergometer. The foot was secured to the plate with two straps over the foot at an angle of 90°. Planar flexion torque was measured with piezoelectric crystal transducer (Kistler, Winterthur, Switzerland), amplified and sampled at 1,000 Hz to a computer. After practice, three trials of maximal planar flexion were performed, and the highest torque was considered to be maximal voluntary torque.

In addition to voluntary plantar flexion torque, a twitch response to a supramaximal electrical stimulation to the tibial nerve was recorded. A recording bipolar electrode (Beckmann type) was placed to the soleus muscle after shaving, abrasion, and cleansing of the location. A 6.98-cm round self-adhering reusable carbon film anode coated with
A detailed assessment of leisure time physical activity volume over the previous 12 mo and over 7 consecutive days were done using a modified version of the Kuopio Ischemic Heart Disease Risk Factor Study Questionnaire (14, 22). The questionnaires included questions concerning leisure time physical activity, physical activities during journeys to and from work, and daily activities, such as gardening. The subjects marked down their type of activity and duration. The mean total daily metabolic equivalent (MET) scores (as MET-h/day) were calculated, and the pairs were categorized into groups based on MET levels: twin pairs having MET score < 4, twin pairs having MET score > 4, and twin pairs where one cotwin had MET score < 4 and the other had MET score > 4. Twins having daily MET score > 4 were classified as physically active (high activity). The score can be achieved with different types of activities, such as long duration of brisk walking or shorter bouts of intense activities. Also, the MET score of 4 was close to the subject median value. The 12-mo and 7-day MET scores correlated positively (r = 0.928, P = 0.0001). The daily MET scores from the 7-day recall are reported in RESULTS.

Fasting blood samples were taken after 15-min rest in the supine position between 0700 and 0900, before the other laboratory measurements. The sera were stored at -70°C after sampling. SHBG, insulin-like growth factor I (IGF-I), insulin-like growth factor binding protein-3, and high sensitivity C-reactive protein levels were measured by using commercial kits (Konelab mulite 1000, Diagnostic Products, Los Angeles, CA). Serum cholesterol values were measured by using Cholesterol CHOD-PAP (Boehringer Mannheim, Mannheim, Germany) and triglyceride assay CHOD-PAP (Boehringer Mannheim) as previously described (18). Estrone was determined as a dansyl derivative by liquid chromatography-tandem mass spectrometry on API 4000 mass spectrometer, as previously described (18). 17β-Estradiol levels were utilized together with SHBG in calculating the free E2 levels, according to a previously presented method (3). Testosterone was measured as previously described (21).

Means and standard deviations (SD) were calculated for the users, nonusers, and groups classified based on physical activity level. Normal distribution of the data was checked with the Shapiro-Wilk test. Paired samples t-test or Wilcoxon signed rank test was used to identify significant (P < 0.05) differences between cotwins. Wilcoxon’s test was used to determine the differences between the physically active subgroup. Intrapair differences (IPD%) were calculated as follows: (HRT user – nonuser)/nonuser * 100%. Pearson’s correlation coefficients between different variables were calculated with adjustment for the twin pairs.

RESULTS

Table 1 shows the results for the HRT users and nonusers. As expected, estradiol and estrone concentrations were significantly higher among the HRT users compared with the nonusers. There were no statistically significant differences in AT CSA (absolute or scaled) within the HRT user and nonuser sisters. The AT was, on average, 5% thicker in HRT users compared with the nonusers, although this difference was not statistically significant. The intrapair correlation for AT thickness was high, despite HRT use discordance (r = 0.84, P < 0.001, Fig. 2). The thinnest area measured was CSAprox in all groups. The thickness correlated positively with CSAmid (r = 0.71, P = 0.001) and CSAprox (r = 0.75, P = 0.001). Maximal voluntary torque or twitch torque did not correlate with tendon thickness or CSA.

The mean serum cholesterol was 5.46 ± 0.66 mmol/l with maximum value of 7.08 mmol/l for the entire subject group (Table 1). Of the hormones and related proteins analyzed, only SHBG correlated significantly with tendon size (thickness r = −0.41, P = 0.032; CSAmid r = −0.41, P = 0.033). Testosterone and free testosterone correlated with plantar flexor torque (r = 0.50, P = 0.009 and r = 0.63, P = 0.001, respectively).
with no HRT history ($P = 0.043$, Fig. 3, Table 3). IGF-binding protein-3 was higher in physically active HRT users than their sisters.

**DISCUSSION**

The present results show that HRT alone does not induce differences in tendon size within postmenopausal MZ twin pairs discordant for long-term use of HRT. Contrary to our hypothesis, neither the level of estrogen nor physical activity was directly associated with AT size. Instead, the genetic factors seem to play a major role, as observed by intrapair correlation of tendon thickness. However, in a subgroup of physically active women, a significantly smaller tendon CSA was found in HRT users compared with their cotwin not using HRT.

Previously, the effects of HRT and physical activity on AT size have been studied by Cook et al. (5). They reported maximum tendon diameter in postmenopausal women, but it was uncertain how and from what location along the tendon the diameter was actually measured. If the diameter was measured from cross-sectional images, it would be very different from the thickness measured in the present study from the longitudinal images. Indeed, the present thickness values of $\sim 0.5$ cm are lower than diameter values of $\sim 1$ cm by Cook et al. (5).

Importantly, Cook and coworkers (5) concluded that, in physically active women, HRT is beneficial because HRT users had a smaller tendon diameter than nonusers. Their conclusion was based on the observation that, in their cross-sectional study, one-half of the active population (golf players) had tendon abnormalities, which was interpreted as a reason for greater AT diameter. In contrast, the present study population did not have abnormalities in their measured tendons. Although one twin pair (nonuser and user having MET 9.7 and 3.6, respectively) had a considerably greater tendon thickness than others, they had normal cholesterol levels (5.7 and 6.1 mmol/l in nonuser and user, respectively), which excludes the possibility that the large tendon size was due to familial hypercholesterolemia (12). Besides visual inspection, our im-

<table>
<thead>
<tr>
<th>Pair</th>
<th>Nonuser</th>
<th>User</th>
<th>Absolute Difference</th>
<th>ΔMET (Nonuser − User)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>0.9</td>
<td>0.1</td>
<td>0.8</td>
<td>0.26 (highPA)</td>
</tr>
<tr>
<td>2*</td>
<td>0.98</td>
<td>0.19</td>
<td>0.79</td>
<td>−1.67 (diff PA)</td>
</tr>
<tr>
<td>3</td>
<td>0.48</td>
<td>0.09</td>
<td>0.39</td>
<td>4.52 (highPA)</td>
</tr>
<tr>
<td>4</td>
<td>0.65</td>
<td>0.32</td>
<td>0.33</td>
<td>−0.55 (highPA)</td>
</tr>
<tr>
<td>5</td>
<td>0.36</td>
<td>0.53</td>
<td>−0.17</td>
<td>0.31 (low PA)</td>
</tr>
<tr>
<td>6</td>
<td>0.8</td>
<td>0.94</td>
<td>−0.14</td>
<td>−4.56 (diff PA)</td>
</tr>
</tbody>
</table>

*Only pairs 1 and 2 were identified clearly to have differences in tendon structure. The analyzed pairs had daily MET scores below 4 [low physical activity (PA)], above 4 (high PA), or were slightly discordant for their daily physical activity (diff PA). There was no association between the difference in MET score (ΔMET) and absolute difference in kernels classified as the nonuser.
able that was directly related with tendon size was SHBG, and systemically differences in structural organization, as analyzed using LDA. The only hormonal or related protein variable that was directly related with tendon size was SHBG, and it correlated negatively with tendon thickness and CSA_mid. It may be possible that SHBG could act as a route for the effects of estradiol, but the complex mechanisms require future work. Since SHBG binds both male and female sex steroids, the combined effects of hormonal status may be the reason why SHBG shows a stronger association to tendon properties than a single hormone alone.

It has been suggested that the mechanism for estradiol action on the adaptation of collagenous tissues could be via IGF-I (8).

In the present study, serum IGFs did not correlate directly with any of the variables, and neither were the IPDs of the IGFs associated with IPDs for other variables. This, however, leaves open the possibility for local IGF-I to contribute tendon regulation. Also, because muscle force did not show positive correlation with tendon size in neither users nor nonusers, it could be speculated that, if serum concentrations reflect those in the tissue, estradiol may have a direct effect on tendon in conditions of elevated physical activity.

The tendon size affects tendon properties. Muraoka et al. (17) reported that AT mechanical properties between men and women seem to be correlated to the difference in muscle strength rather than sex. In the present study, maximal voluntary torque or twitch torque did not correlate with tendon thickness or CSA. However, in a recent study, AT strain during isometric voluntary contractions has been reported to be lower in women using oral contraceptives (4). Although the study did not report tendon size, the finding follows the logic that lower estrogen levels could allow enhanced collagen synthesis and thus increase tendon size, making it stronger, and, consequently, the strain at given force level would be lower in oral contraceptive users than nonusers.

Rosager et al. (19) reported greater AT CSA in endurance runners compared with the age-matched controls, but no significant differences in muscle force. Correlation between the variables was not reported. Similarly, Kongsgaard et al. (13) found greater normalized AT CSA from runners than from controls, while maximal AT force was not different. Interestingly, in the same study, AT CSA was reported to be best predicted by maximal anatomical CSA of the triceps surae muscle (13). If CSA of a given muscle predicts tendon CSA, it would be logical to suggest that also muscle strength would be related to tendon CSA. However, this link has not been explicitly reported. Reasons for the lack of correlation between muscle strength and tendon CSA in the present study may be related to the role of deep plantar flexor contribution in plantar flexor muscles (6, 7) and most likely a relatively homogeneous study population.

When the significance of the present findings is evaluated, it must be noted that there were only five twin pairs that were regarded as physically active. Furthermore, AT CSA was significantly different only at proximal measurement site, but

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### Table 3. Descriptive statistics of the measured variables in hormone replacement therapy-discordant monozygous twin pairs with mean total daily MET score > 4 in both twin sisters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonuser</th>
<th>User</th>
<th>IPD% (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torque, N</td>
<td>131.7±24.6</td>
<td>131.1±20.9</td>
<td>2.0 (−27.2-31.1)</td>
<td>0.72</td>
</tr>
<tr>
<td>Tendon thickness, cm</td>
<td>0.45±0.04</td>
<td>0.44±0.03</td>
<td>−1.3 (−12.1-9.6)</td>
<td>0.89</td>
</tr>
<tr>
<td>CSA_mid, cm^2</td>
<td>0.77±0.16</td>
<td>0.67±0.06</td>
<td>−10.4 (−29.1-8.3)</td>
<td>0.08</td>
</tr>
<tr>
<td>CSA_prox, cm^2</td>
<td>0.71±0.12</td>
<td>0.63±0.10</td>
<td>−11.2 (−18.6 to −3.9)</td>
<td>0.043</td>
</tr>
<tr>
<td>Daily MET score</td>
<td>9.0±3.1</td>
<td>15.1±14.0</td>
<td>57.3 (−55.9-170.5)</td>
<td>0.35</td>
</tr>
<tr>
<td>IGF-I, nmol/l</td>
<td>14.76±1.23</td>
<td>18.76±4.55</td>
<td>26.6 (−4.8-58.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>IGFFB-3, mg/l</td>
<td>4.06±0.35</td>
<td>4.52±0.27</td>
<td>11.8 (−2.4-21.1)</td>
<td>0.042</td>
</tr>
<tr>
<td>F-testosterone, pmol/l</td>
<td>742±409</td>
<td>694±298</td>
<td>3.3 (−32.2-38.8)</td>
<td>0.89</td>
</tr>
<tr>
<td>S-testosterone, pmol/l</td>
<td>9.9±5.0</td>
<td>10.8±5.6</td>
<td>12.7 (−23.5-48.8)</td>
<td>0.50</td>
</tr>
<tr>
<td>F-estradiol, pmol/l</td>
<td>26.4±8.3</td>
<td>83.2±51.1</td>
<td>209.5 (38.0-381.1)</td>
<td>0.043</td>
</tr>
<tr>
<td>E-stradiol, pmol/l</td>
<td>0.63±0.27</td>
<td>1.97±1.14</td>
<td>210.7 (72.3-349.0)</td>
<td>0.043</td>
</tr>
<tr>
<td>Estrone, pM</td>
<td>102±34</td>
<td>360±261</td>
<td>250.6 (47.2-454.0)</td>
<td>0.043</td>
</tr>
<tr>
<td>SHBG, nmol/l</td>
<td>53.6±23.1</td>
<td>45.9±14.9</td>
<td>3.1 (−60.7-67.7)</td>
<td>0.89</td>
</tr>
<tr>
<td>hsCRP, mg/dl</td>
<td>1.04±0.49</td>
<td>1.20±0.95</td>
<td>82.5 (−182.7-347.7)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Values are means ± SD. Data are from 5 twin pairs, and nonparametric tests have been applied.
the same tendency was found in CSA_{mid}. Also, the discrimination image analyses to find out whether nonusers had abnormalities in their tendon compared with HRT users were done only on six twin pairs due to technical reasons. While the number of subjects may be rather low, the strengths of this study are the long-term HRT use and genetically controlled twin study design.

Conclusion. The use of HRT alone does not induce differences in AT CSA, thickness, or structural organization between MZ twins from pairs discordant for HRT use, but otherwise well matched for both measured and unmeasured characteristics. However, in physically active twin pairs, the higher level of estrogen seems to be associated with smaller tendon size.

ACKNOWLEDGMENTS

We thank all of the twins for participating in the study. Kaisa-Leena Tulla, Erkki Helkala, Tuovi Nylänen, and Mervi Matero are acknowledged for technical assistance in the laboratory; Eila Voipio for assistance in the recruitment of twins from the Finnish Twin Cohort; Eeva-Maija Palonen and Kirsti Salo for taking care of our study subjects during the whole period of data collection; and the personnel at the Paternity Testing Laboratory, National Public Health Institute, Helsinki, Finland for carrying out the determination of the zygosity of the twins.

GRANTS

We thank the Academy of Finland, the Centre of Excellence in Complex Disease Genetics, and the Finnish Ministry of Education for funding.

REFERENCES


