Effects of estrogen on the mechanical behavior of the human Achilles tendon in vivo

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Submitted 4 December 2007; accepted in final form 16 June 2008

Bryant AL, Clark RA, Bartold S, Murphy A, Bennell KL, Hohmann E, Marshall-Gradisnik S, Payne C, Crossley KM. Effects of estrogen on the mechanical behavior of the human Achilles tendon in vivo. J Appl Physiol 105: 1035–1043, 2008. First published June 19, 2008; doi:10.1152/japplphysiol.01281.2007.—The purpose of this study was to elucidate the effect of normal fluctuating [non-monophasic oral contraceptive pill (MOCP) users] and low, consistent (MOCP users) endogenous plasma estrogen levels on the strain behavior of the Achilles tendon in vivo. Twenty women (age 28.0 ± 4.2 yr, height 1.67 ± 0.07 m, mass 61.6 ± 6.8 kg) who had been using the MOCP for at least 12 mo together with 20 matched women who were non-MOCP users (age 31.9 ± 7.3 yr, height 1.63 ± 0.05 m, mass 62.5 ± 5.9 kg) participated in this study. Non-MOCP users were tested at the time of lowest (menstruation) and highest (ovulation) estrogen, whereas MOCP users, who exhibited constant and attenuated endogenous estrogen levels, were tested at day 1 and day 14 of their cycle. At each test session, maximal isometric plantarflexion efforts were performed on a calf-raise apparatus while synchronous real-time ultrasonography of the triceps surae aponeurosis was recorded. Achilles tendon strain (%) was calculated by dividing tendon displacement during plantarflexion by resting tendon length. Repeated-measures ANOVA revealed a significant (P < 0.05) main effect of subject group with significantly lower Achilles strain (25.5%) in the MOCP users compared with the non-MOCP users. In conclusion, acute fluctuations in plasma estrogen across the menstrual cycle in non-MOCP users did not alter the strain behavior of the Achilles tendon. Conversely, long-term exposure to attenuated estrogen in MOCP users resulted in a decrease in Achilles tendon strain, which is thought to be attributed to the effects of endogenous estrogen on collagen synthesis. These findings have a number of important functional and clinical implications.

HUMAN TENDON contains collagenous connective tissues that are elastic and, as such, have been depicted as springs in rheological models of skeletal muscle (22, 60). During locomotor activities, these tendinous structures are stretched and, analogous to a steel spring, store elastic strain energy, which is subsequently converted into kinetic energy on release (1, 27). Tendon compliance is therefore a predominant factor in the capacity of the musculotendinous unit to harness elastic strain energy during stretch-induced work (61). Furthermore, it is thought that decreased tendon and combined musculotendinous compliance will increase the stabilizing capacity of joints and ligaments and thereby decrease the likelihood of musculoskeletal injury (18, 19, 40). In general, factors that increase tendon collagen deposition, such as physical activity (24, 49), will alter the nonlinear springlike characteristics of the tendon, making it less compliant.

Conversely, recent evidence suggests that estrogen may decrease tendon collagen density by attenuating fibroblast activity (32), prompting speculation that estrogen may increase tendon compliance. Indeed, a sex-based study (43) has implicated higher endogenous estrogen levels in females with lower tendon collagen fractional synthesis rate, a finding that may explain smaller tendon cross-sectional area (50) and higher musculotendinous compliance (18, 19) in females compared with males. Apart from sex-related differences, the link between estrogen and tendon compliance also has important physiological implications for both eumenorrheic women and those with constant and attenuated estrogen levels, that is, women using the monophasic oral contraceptive pill (MOCP). Currently, no previous research has attempted to elucidate whether low levels of endogenous estrogen induced by MOCP use alters the compliance of female tendon in vivo, despite the reported effect of MOCP use on the intrinsic mechanical properties of other soft tissues, including the anterior cruciate ligament (ACL) (41), and the suggested prophylactic potential of the MOCP on musculoskeletal injury (36, 41).

The triceps surae muscle-tendon complex represents an ideal model for the study of the effects of estrogen on the strain behavior of human tendon in vivo for two reasons. First, the triceps surae plays an important absorptive and propulsive role during stretch-shorten cycle movements involving the lower limb, and, as such, potential fluctuations in Achilles tendon compliance may alter human performance and the propensity for bony and soft tissue injury. Second, advances in real-time ultrasonography have enabled the determination of fascicle movement during muscle contraction, which has provided a viable, noninvasive method for studying human triceps surae aponeurosis and Achilles tendon tissue behavior during isometric muscle contraction (16, 23, 39). Therefore, the aims of this study were to determine whether the in vivo strain behavior of the Achilles tendon is affected by J) acute fluctuations in

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endogenous plasma estrogen levels across a typical menstrual cycle (non-MOCP users), and 2) long-term reductions in endogenous plasma estrogen levels (MOCP users) compared with normal physiological endogenous plasma estrogen levels (non-MOCP users). It was hypothesized that while acute fluctuations in endogenous estrogen would have no effect of on Achilles tendon strain (H1), chronic attenuation of endogenous plasma estrogen would have no effect on Achilles MOCP users). It was hypothesized that while acute fluctuations in endogenous estrogen would have no effect on Achilles tendon strain (H1), chronic attenuation of endogenous plasma estrogen induced by MOCP use would be associated with comparatively lower Achilles tendon strain (H2).

MATERIALS AND METHODS

Subjects

Forty athletic women volunteered to participate in this study. Current running volumes, running history, and lower limb injury history were derived by interviewing each of the subjects. All subjects were currently running more than 20 km/wk, had been running these distances for at least 2 yr prior, and had not sustained a lower limb injury that had restricted their ability to exercise for more than 1 wk in the previous 12 mo. Twenty subjects had been taking an oral MOCP (e.g., Organon, Femodene) containing synthetic estrogen (30 μg ethinyl estradiol) and progestogen (either 150 μg levonorgestrel or 75 μg gestodene) for a minimum of 12 mo before recruitment. The types of MOCPs used by subjects in the present study are taken daily for 21 days, with subsequent courses taken following a 7-day interval (12). The remaining 20 subjects were not using any form of oral contraceptive pill (nor had they been for at least 12 mo prior) and demonstrated normal menstrual cycle function [i.e., cycle length between 24 and 35 days (13), no anovulation, and consistent flow between cycles (25)]. The protocol was approved by the University of Melbourne Human Ethics Committee, and all subjects read and signed an approved consent document before participating in the study.

Experimental Protocol

Determination of test days. Non-MOCP users were monitored for two complete cycles before testing using an online software package, Fertility Friend. Using this software, non-MOCP users recorded the timing of menstruation together with their basal body temperature (BBT), which was measured orally each morning on waking. In addition, ovulation prediction kits (OPK; Ambermex) were used daily for ~17 days (e.g., start on day 11 of a typically 28-day cycle) in the second cycle to confirm the timing of ovulation. Subjects were instructed to use the OPKs at the same time of day (between 2:00 PM and 10:00 PM) to ensure that the surge in luteinizing hormone was detected. The timing of positive test results was recorded. A research assistant was given access to the Fertility Friend accounts of each of the non-MOCP users, and using the data from the two cycles, average cycle duration and the timing of ovulation were estimated for each subject. The non-MOCP users were then contacted and allocated two test days, reflecting the timing of lowest (i.e., first day of menstruation) and highest (i.e., 24 h preceding ovulation) estrogen in the subsequent cycle. Non-MOCP users continued to record daily BBT and use the OPK within the test cycle to ensure accurate prediction of highest estrogen (=ovulation). If BBT or OPK results deviated dramatically from the preceding two cycles, subjects were instructed to contact the research assistant who examined their Fertility Friend account. Where necessary, the second test session was rescheduled to earlier or later in the cycle. To reflect the approximate timing of menstruation and =ovulation in the non-MOCP users, MOCP users were tested at day 1 and day 14 of their cycles. To control for daily fluctuations in endogenous estrogen concentrations and estrogen receptor β sensitivity, which are influenced by circadian rhythms (8), all testing sessions were performed within a 3-h window (i.e., 4:00 PM to 7:00 PM), and each subject was tested at ±1 h of their previous test (11). Testing late afternoon/early evening also increased the likelihood that serum concentrations of ethinyl estradiol, which are subject to considerable fluctuations in hours following MOCp consumption (56), had reached a steady state. Collection of experimental data was performed by two additional research assistants who were blinded as to whether subjects were non-MOCP or MOCP users.

Warm-up. At the start of each session, non-MOCP and MOCP subjects completed a standardized warm-up consisting of 6 min of treadmill running with 0° inclination at 10 km/h.

Truncated foot length. Following the warm-up, subjects were instructed to remove their shoes and stand with their feet shoulder width apart. A mark was placed on the skin overlying the midpoint of the right metatarsophalangeal joint. Truncated foot length (mm) was determined by measuring the distance from the posterior aspect of the calcaneus to the metatarsophalangeal joint center using a custom-designed slide ruler aligned with the medial border of the foot. Reliability testing in our laboratory on 10 healthy male subjects demonstrated intraclass correlation coefficient (ICC) values of 0.98 for truncated foot length (9).

Achilles tendon moment arm length and Achilles tendon thickness. As magnetic resonance imaging was not available, Achilles tendon moment arm length (i.e., the distance between the approximate joint center of the malleolus and the midpoint of the Achilles tendon directly posterior to the joint center) was assessed using a combination of ultrasonography (Mindray DP-6600) and anthropometry. Reliability testing demonstrated ICC values of 0.97 for Achilles tendon moment arm length (9).

Subjects were seated (knee and ankle at 90° flexion), and a set of anthropometrical calipers (0.5-mm resolution) was used to determine the width of the medial malleolus. The ankle joint center was then determined by marking the midpoint of the medial malleolus. A position directly horizontal to this midpoint (as identified using a spirit level) was marked on the posterior surface of the skin covering the Achilles tendon. The distance from the medial malleolus joint center to the mark on the posterior surface of the skin was measured and recorded. A sagittal plane ultrasound scan of the Achilles tendon was then performed at the level of the skin mark using a 10-MHz linear array B-mode ultrasound probe. This position also produced an image of the Achilles tendon insertion point on the posterior calcaneus, which was used in the assessment of tendon length.

Isometric plantarflexion strength and Achilles tendon strain testing. The strain behavior of the Achilles tendon of the right leg was assessed as subjects performed an isometric plantarflexion strength test in a custom-made, seated calf-raising apparatus. Reliability testing demonstrated ICC values of 0.95 and 0.98 for maximal isometric plantarflexion force and Achilles tendon strain, respectively (9). Although several studies [e.g., Magnusson et al. (39), Murakoa et al. (48)] have used similar methods to that described below for the determination of Achilles tendon strain in vivo, no data exist as to the validity of this method. From a practical perspective, this would require simultaneous recordings of Achilles tendon strain from strain gauges implanted in the tendon and, for this reason, is not ethically feasible. Nevertheless, the mechanical behavior of lower limb musculotendinous structures, as determined using noninvasive techniques, has been found to be closely associated with the restitution of elastic strain energy, muscle potentiation, and dynamic muscular function (57).

Before being seated, an electrogoniometer (Biometrics) was positioned on the lateral side of the subject’s foot, and a line was marked on the skin overlying the metatarsophalangeal joint. A depiction of a subject seated in the testing position is provided in Fig. 1.

The subjects were positioned so that a point along the anterior-posterior midline of their right foot, directly perpendicular to their metatarsophalangeal joint, was immediately superior to the center of the load cell (Celtron). This reduced the potential for leverage of force transfer to affect the results of the load cell. Once the foot was in place, the subject’s lower limb was positioned in a way that aligned the tibia at a slightly acute angle to the foot. This position was assigned for two reasons: 1) to result in the tibia shifting to an

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approximately perpendicular position to the load cell during the maximal plantarflexion, due to the small increase in ankle extension angle; and 2) to align the ultrasound transducer directly vertical to the Achilles tendon moment arm.

The subject’s upper leg was positioned so that the minimally padded lever was in contact with the lower thigh, which was directly in line with both the lever and the platform supporting the right foot. To minimize movement during the contraction, and therefore create an isometric protocol, the lever was adjusted using a locking screw to a point where the subject was able to exert only minimal changes in the ankle during plantarflexion.

With the subject positioned in the calf-raise apparatus, the insertion point of the Achilles tendon at the posterior aspect of the calcaneus was identified using images from the ultrasound probe previously described. The myotendinous junction was also determined by recording ultrasound images immediately inferior to the bulk of the medial gastrocnemius muscle. The probe was then repositioned until the myotendinous junction was clearly visible in the inferior third of the image with the probe perpendicular to the ground. Once this position was determined and marked using a nonpermanent pen, a rigid plastic brace moulded to the shape of the head of the ultrasound probe was secured to the skin using thick adhesive foam. This set-up was secured to the skin using thick adhesive foam. This set-up was in line with both the lever and the platform supporting the right foot.

Subjects then performed an isometric force ramp by gradually increasing plantarflexion force over 2 s, followed by a 3-s maximal voluntary contraction (MVC). During testing, the ultrasound probe was lightly held in place by the investigator to ensure that the head of the probe remained in contact with the moulded brace and to reduce the chance of a parallax error, which would occur if the angle of the transducer was altered. A 1-min rest interval was allocated between trials, with a total of three trials performed. During each test, ultrasound image signals, plantarflexion force, and electrogoniometer data were recorded. Raw force and electrogoniometer signals were amplified and then relayed to a data-collection computer running Spike 2 (version 4.10) software (CED, Cambridge, UK) via flexible cables. Analog-to-digital conversion was performed at 2,000 Hz. The ultrasound footage was synchronized with the kinetic data by overlaying the force trace and time data onto the ultrasound video using an image overlaying device. This combined footage was then recorded using a digital video camera (Sony) recording at 25 frames/s at a resolution of 720 × 576 pixels.

It is important to note that while coactivation of dorsiflexors during maximal plantarflexion occurs, the underestimation of peak tendon force due to this coactivation has been estimated to range from 2.6 to 5% (39, 53). Nevertheless, Magnusson et al. (39) corrected for antagonistic force given that even a small underestimation can significantly alter the calculated aponeurosis and Achilles tendon stiffness. However, this was deemed unnecessary in the present study given that Achilles tendon strain was the main outcome measure, and it is unlikely that antagonist coactivation would change dramatically and alter plantarflexion force between test sessions and/or subject groups. Hence, as in Muraoka et al. (48), electromyographic (EMG) activity of the leg muscles during maximal plantarflexion was not recorded.

Venous blood collection. At the end of each test session, 10 ml of venous blood was drawn from an antecubital vein (into a lithium heparin-coated tube) of each subject by a trained phlebotomist. Within 1 h, each blood sample was delivered to the pathology department of the university hospital for analysis.

Data Analysis

Achilles tendon moment arm length and Achilles tendon thickness. The ultrasound footage recorded at the Achilles tendon insertion point was converted into individual frames using image dubbing software (VirtualDub V.1.6.16). The image that represented the optimal representation of the Achilles tendon was then imported into an image analysis software program (ImageJ V.1.36B). Using this program, Achilles tendon thickness (i.e., distance from the anterior margin to the posterior margin of the tendon) together with the distance from the midpoint of the Achilles tendon (i.e., midpoint of the tendon located directly horizontal to the medial malleolus) to the region on the image representing the surface of the skin were measured. This value was then deducted from the distance from the medial malleolus to the posterior surface of the skin to create an estimated resting moment arm. Although this form of measurement cannot be considered precise in comparison with moment arm calculations performed using magnetic resonance imaging, it did provide an estimation of the resting moment arm for each subject. By controlling for this variable, along with force production, it could be ensured that any differences in tendon strain between groups were not influenced by beneficial mechanical leverage.

Isometric plantarflexion strength. Peak force was determined by subtracting the resting force level, with the subject in position, from the peak level obtained. This removed the force applied to the load cell by both the mass of the limb and the lever arm locking mechanism. Filtering consisted of a 10-point moving average applied to the data before analysis, and any trials that either did not result in a plateaued force peak or possessed an impact spike of >15% of the peak force plateau were discarded.

Achilles tendon strain. To accurately determine the change in tendon length, a correction for the displacement of the tendon insertion point was also performed based on the change in angle at the ankle, measured using an electrogoniometer. This process is described in Fig. 2.

The distance between the scanning positions at the Achilles tendon insertion and myotendinous junction was used to quantify Achilles tendon length at rest and during maximal plantarflexion. Video images derived from both scanning positions were used to enhance the

Fig. 1. Subject seated in the calf-raise apparatus. MTP, metatarsophalangeal joint; EG, electrogoniometer; US, ultrasound; •, approximate joint center; ↔, Achilles tendon moment arm.
accuracy of this measure by locating the exact pixel position of the anatomic landmarks.

The ultrasound footage during the MVC was converted into individual frames as previously described. A frame corresponding to the myotendinous junction at rest was then analyzed using the image analysis software, with the pixel position at which the junction occurred recorded. The pixel position was then averaged over three frames, each separated by 500 ms, which corresponded to the median 1 s of MVC plateaued peak force production. The calculation used for determining the change in tendon length incorporated trigonometry to determine the effect of any changes in vertical or horizontal tendon junction position in each of the MVC images. Examples of the pixel position at rest and during MVC, along with a diagram of the tendon length change assessment, are provided in Fig. 3.

Hormonal levels. Venous blood samples were allowed to clot and then centrifuged at 70 g for 10 min at room temperature to obtain plasma that was immediately stored at −80°C for later hormonal analysis. Total concentrations of 17β-estradiol and progesterone were assayed using commercially available ADIVIA Centaur estradiol-6 and ADIVIA Centaur Progesterone kits (Bayer Diagnostics). All samples were measured by immunochemiluminometrics using the ADIVIA Centaur immunoassay analyzer (Bayer Diagnostics Division).

While plasma estrogen levels were important for within- and between-subject group contrasts, plasma estradiol and progesterone levels were also essential to confirm the predicted date of peak estrogen (~ovulation) in the non-MOCP users. Where the hormone concentrations were not within the documented range [estradiol:
551–1,940 pg/ml (4); progesterone: 0.5–4.5 pg/ml (7)], it was assumed that either the test date was miscalculated or that the cycle was anovulatory. In both cases, non-MOCP users were instructed to continue recording daily BBT and using the OPKs and were retested in the subsequent cycle.

Statistical Analysis

Descriptive statistics (means ± SD) for subject physical characteristics together with the plasma estrogen levels and dependent variables derived from the maximal isometric plantarflexion tests at test occasions 1 and 2 were calculated for each subject group. After confirming normality (Kolmogorov-Smirnov test with Lilliefors’s correction) and equal variance (Levene median test), the following statistical tests were implemented.

Physical characteristics of the subjects. Independent samples t-tests were used to compare the age, height, mass, training status, training history, truncated foot length, Achilles tendon moment arm length, and Achilles tendon thickness of the non-MOCP and MOCP users.

Estrogen levels, isometric plantarflexion strength and Achilles tendon strain. A series of repeated-measures ANOVA were used to compare plasma estrogen levels, maximal plantarflexion force, and Achilles tendon strain of the non-MOCP and MOCP groups across the two test sessions. Therefore, each ANOVA design included one within factor [test occasion: test 1 (menstruation/day 1) and test 2 (= ovulation/day 14)] and one between factor [subject group: non-MOCP users and MOCP users]. In the event of a significant (P < 0.05) main effect or interaction following ANOVA contrasts, post hoc comparisons of the means were conducted using the least significant difference test to delineate differences among test occasion or subject groups. All analyses were conducted using Statistical Package for Social Sciences (SPSS, version 14.0;1; Chicago, IL) for Windows.

RESULTS

Descriptive data (means ± SD) pertaining to the physical characteristics and training status of the non-MOCP and MOCP users together with P values for each of the t-test contrasts are presented in Table 1. Statistical analysis revealed no significant differences between subject groups for the variables of age, height, mass, training status, training history, truncated foot length, Achilles tendon moment arm length, or Achilles tendon thickness.

Mean (± SD) plasma estrogen levels, maximal plantarflexion force, and Achilles tendon strain for the non-MOCP and MOCP users at test occasions 1 and 2 are presented in Table 2. For plasma estrogen levels, a significant interaction of subject group × test occasion (F = 19.388; P < 0.001) was identified. Between-subject group post hoc contrasts revealed significantly higher plasma estrogen levels for the non-MOCP group compared with the MOCP group at both test 1 (P < 0.001; %Diff = 41.7) and test 2 (P < 0.001; %Diff = 90.8). Within-subject group post hoc contrasts indicated that plasma estrogen levels were significantly higher (P < 0.001; %Diff = 84.2) at test 2 (=ovulation) compared with test 1 (menstruation) for the non-MOCP group. There were no significant differences between test 1 (day 1) and test 2 (day 14) for the MOCP users (P = 1.000; %Diff = 0.0). For maximal isometric plantarflexion force, there were no significant main effects or interactions between subject groups or test occasions. In contrast, a significant main effect of subject group was found for Achilles tendon strain (F = 4.791; P = 0.035) with significantly higher strain (25.5%) in the non-MOCP users compared with the MOCP users. Given the near significant between-subject group difference for age, this variable was included as a covariate in separate ANOVA contrasts comparing Achilles tendon strain and plantarflexion force across subject groups and test occasions. The results for Achilles tendon strain and plantarflexion force did not, however, change when age was included as a covariate.

DISCUSSION

While the physical characteristics, training status, and training history of the non-MOCP and MOCP users in the present study were similar, MOCP administration resulted in a dramatic downregulation of endogenous estrogen. Moreover, ingestion of the MOC produced plasma estrogen levels that were consistent at days 1 and 14 of the cycle. In contrast, endogenous estrogen levels in non-MOCP users were considerably higher and increased, on average, by almost 670 pg/ml from menstruation to =ovulation. Comparison of these hormonal data with that reported in previous studies [e.g., Elliott et al. (12), Eiling et al. (11)] indicates that subjects in the present investigation represented typical cross sections of both eumenorrheic women and women using the MOCP.

As the serum concentration of active sex steroids is directly related to the oral contraceptive dosage administered (6, 15), it is also important to consider circulating levels of exogenous estrogen (i.e., ethinyl estradiol) in MOC users. In a relatively recent paper, van den Heuvel et al. (56) found that daily consumption of an oral contraceptive containing 30 μg of ethinyl estradiol by women of similar age and physiological characteristics to those included in the present study resulted in average serum ethinyl estradiol concentrations of 43.5 ± 5.66 pg/ml across a 25-day cycle. Thus, given that average endogenous estradiol levels of <73 pg/ml were found in response to 30 μg of ethinyl estradiol, the total average estrogen levels of MOC users in the present study would be unlikely to exceed 117 pg/ml and thus would be, on average, ~77% lower than that of the non-MOCP users (average estradiol at menstruation and =ovulation = 433 pg/ml). It is also imperative to consider the acute effects of MOC use on ethinyl estradiol levels given that following an initial serum peak (~2 h after consumption), a second peak, attributed to the effect of enterohepatic circulation, is typically observed (55). Given that all subjects in the present study were tested late afternoon/early evening and MOCP administration occurred shortly after waking, it is...
and MOCP users at test occasions 1 and 2

muscle in a physiologically less advantageous position compared with the straight-leg posture incorporated in many of the related studies (39, 48). Furthermore, the majority of earlier studies only included male subjects, and women demonstrate less Achilles tendon strain during maximal plantarflexion compared with their male counterparts due to lower muscle strength (47). In addition, it has been argued that in vivo measurement of Achilles tendon strain during isometric voluntary contraction using ultrasonography leads to an overestimation of actual values since even small amounts of ankle joint rotation will contribute to tendon movement and thereby incorrectly add to the actual tendon displacement attributed to muscle tensile loading (38, 39, 46, 51, 53). Although it is extremely difficult to completely prevent any joint angular rotation during a forceful muscular contraction with external strap fixation, the present measurement method, like that of Magnusson et al. (39), attempted to account for ankle joint motion during maximal plantarflexion. In doing so, the Achilles tendon strain values recorded in the present study were comparable to average values estimated during real-life movements, including slow walking (11%; Fukunaga et al. (17), Kawakami et al. (26) and running [5.5%; Lichtwark et al. (34)], where motion analysis in combination with ultrasonography is thought to have avoided an overestimation of tendon displacement. Notably, average predicted triceps surae muscle/Achilles tendon forces during these locomotor activities were similar to that generated isometrically by non-MOCP and MOCP users.

Martineau et al. (41) found that MOCP use yielded statistically significant decreases in knee ligament laxity compared with non-MOCP users. Similarly, long-term exposure to attenuated endogenous estrogen levels in the present study was also associated with an increase in soft tissue compliance; that is, MOCP users demonstrated lower Achilles tendon strain in response to similar muscle force compared with non-MOCP users. Although skeletal muscle is responsive to estrogen fluctuations (11) and intrinsic muscle properties will influence Achilles tendon strain results given the nature of the test used in the present study, there is increasing evidence to suggest that tendon is particularly responsive to estrogen given the presence of hormone-specific receptors (20, 42). Hence, although the exact mechanism underpinning this between-subject group difference is unknown, it is thought that decreased Achilles tendon strain in MOCP users is a result of the receptor-mediated effects of estrogen on collagen fractional synthesis rate. It is also important to note that while the non-MOCP users were, on average, almost 4 yr younger than the MOCP users, there was no subject group × age interaction for Achilles tendon strain even though age-related decreases in Achilles tendon strain have been observed (30, 31). Hence, while Alnaqueeb and Goldspink (2) found an association between increasing age and increases in

Table 2. Mean ± SD estrogen levels, maximal plantarflexion force, and Achilles tendon strain for the non-MOCP and MOCP users at test occasions 1 and 2

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Non-MOCP Users</th>
<th>MOCP Users</th>
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</thead>
<tbody>
<tr>
<td>Test 1 (menstruation)</td>
<td>Test 2 (=ovulation)</td>
<td></td>
</tr>
<tr>
<td>Test 1 (day 1)</td>
<td>Test 2 (day 14)</td>
<td></td>
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<tr>
<td>Estrogen, pg/ml*</td>
<td>124.5±49.0</td>
<td>741.7±661.5</td>
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<tr>
<td>Plantarflexion force, N</td>
<td>648.6±118.4</td>
<td>682.2±146.2</td>
</tr>
<tr>
<td>Achilles tendon strain (ΔL), %†</td>
<td>4.7±2.2</td>
<td>4.9±2.7</td>
</tr>
<tr>
<td></td>
<td>2.2±0.6</td>
<td>2.1±0.6</td>
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</tbody>
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ΔL, change in tendon length. *Significant subject group × test session interaction (P < 0.05). †Significant main effect of subject group (P < 0.05).
connective tissue and collagen cross-linking, it is suggested that 4 years does not constitute an adequate period to detect potential age-related decreases in tendon extensibility.

Lee et al. (32) reported that, in an estrogen-free environment (i.e., in vitro tissue culture model), mechanical loading of ACL fibroblasts produced an increase in type I collagen mRNA; however, the increase in type I collagen mRNA in response to loading was lessened at increasing estrogen concentrations (33). Consequently, estrogen attenuated ACL collagen synthesis in response to cyclic tensile loading, a finding supported, in part, by clinical studies that have found an association between high estrogen levels and increased anterior tibial translation (10, 21, 52). Similarly, a recent study in humans (43) reported that estrogen may inhibit exercise-induced increases in collagen synthesis following strenuous exercise as tendon collagen fractional synthesis rates are lower in women than in men. Hence, a long-term reduction in blood estrogen levels due to MOCP administration in the present study could have contributed to decreased Achilles tendon strain by augmenting exercise-induced collagen fractional synthesis rate. To substantiate this hypothesis, future studies comparing MOCP users to non-MOCP users should include magnetic resonance imaging to determine Achilles tendon cross-sectional area at discrete points along the length of the tendon. While biopsy techniques and scanning confocal microscopy to define the amount of collagen subtypes in the Achilles tendon would also be extremely beneficial from an investigative perspective, this method is not ethically feasible as biopsying healthy tendons may have serious adverse effects (e.g., low-grade tendinopathy) given that the process of acquiring tendon samples from pathological tendons has been found to result in a provocation of symptoms (45). Albeit not in the Achilles tendon, procurement of the small samples of tendon (i.e., patella, semitendinosus or gracilis tendons) that are trimmed from ACL tendon grafts before implantation may, however, be an ethical alternative in assessing the effects of estrogen on tendon collagen subtypes in human females.

Given that there were no significant changes in the strain behavior of the Achilles tendon in non-MOCP users at menstruation and ovulation, it is assumed that short-duration variations in plasma estrogen across the menstrual cycle had a limited effect on Achilles tendon structural metabolism. In contrast, several studies have identified acute estrogen-induced changes in the mechanical behavior of other soft tissues including the ACL (10, 21, 52) and lower limb musculotendinous structures (11). However, in support of the results of the present study and the notion that acute estrogen fluctuations do not alter tendon collagen synthesis, a recent cross-sectional study by Miller et al. (43) on 15 young, healthy non-MOCP users demonstrated that fluctuations in circulating estrogen levels from the follicular to the luteal phase of the menstrual cycle were insufficient to affect synthesis rates of collagen because the small samples of tendon (i.e., patella, semitendinosus or gracilis tendons) that are trimmed from ACL tendon grafts before implantation may, however, be an ethical alternative in assessing the effects of estrogen on tendon collagen subtypes in human females.

In conclusion, the results of this study suggest that in accordance with $H_1$, acute fluctuations in plasma estrogen across the menstrual cycle in non-MOCP users do not alter the strain behavior of the Achilles tendon. Conversely, but in agreement with $H_2$, long-term exposure to attenuated estrogen in MOCP users was associated with a decrease in Achilles tendon compliance, which is thought to be attributed to the effects of estrogen on collagen synthesis. These findings have a myriad of implications pertaining to muscle performance, joint injury, tendon healing, and ACL surgery. Future studies should investigate the cross-sectional area and collagen composition of the Achilles tendon of non-MOCP and MOCP users to clarify some of the assumptions presented in this study.

GRANTS

We gratefully acknowledge Asics Oceania for funding this project.

REFERENCES


