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PATELLAR TENDON PROPERTIES WITH FLUCTUATING MENSTRUAL CYCLE HORMONES

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ABSTRACT

Debate continues over whether skeletal muscle performance and injury risk vary over the course of the menstrual cycle. Alterations in tendon properties may play a role in the potential fluctuations of both of these variables. The aim of the current study was to determine any association between menstrual cycle phase and corresponding levels of female sex hormones and tendon properties. Fifteen normally menstruating (28–32-day cycles) healthy females (age 23 ± 1 years, mass 63.1 ± 2.6 kg, height 1.66 ± 0.02 m) not taking any form of hormonal contraceptive took part in this study. In vivo patellar tendon properties and associated circulating hormonal levels were assessed on 3 occasions including days 3 ± 0.4, 13 ± 0.2, and 21 ± 0.3. Dynamometry, ultrasonography, electromyography, and biochemical assessment of circulating levels of estradiol and progesterone were utilized. No significant differences were seen in tendon mechanical properties among the 3 phases of the menstrual cycle ($p > 0.05$). Regressions were carried out and revealed that estrogen and maximal voluntary tendon force explained 17.8% ($p = 0.043$) of the variance in young's modulus. Our findings link estrogen to a chronic, rather than an acute, impact on tendon behavior. These findings are relevant to clinical outcomes, exercise performance, and injury risk. In terms of tendon properties, menstrual cycle phase does not necessarily need to be considered when organizing training and competition schedules.

KEY WORDS: compliance, ELISA, in vivo test, soft tissue biomechanics, tendon

INTRODUCTION

During the female menstrual cycle, not only are there large variations in the levels and ratios of sex hormones such as estrogen and progesterone (16), but also the interplay of these hormones is extremely complex (27). There is a continued debate over whether menstrual cycle phase and corresponding changes in hormonal milieu affect injury risk in women. A recent review suggests that there is an increase in noncontact anterior cruciate ligament (ACL) injuries surrounding the preovulatory phase of the menstrual cycle (25). However, other reports show increased injury risk in the early follicular phase (35,52) or around ovulation (55,56). Owing (a) to the interstudy variations, (b) the intrastudy variability in hormone levels, and (c) the retrospective nature of these studies, it is not possible to make associations between hormone levels and injury risk from the data presented. Equivocal reports also exist concerning menstrual cycle effects on knee joint laxity. However, a recent meta-analysis of 11 studies, which included studies that had found both significant and nonsignificant effects of menstrual cycle phase on knee laxity, reported that overall, there is a significant effect of menstrual cycle phase on knee laxity (57). More specifically, it was concluded that knee laxity measured at days 10 to 14 is greater than laxity at days 15 to 28, with days 1 to 9 exhibiting the highest knee stiffness values (57). In agreement with this observation, changes in knee joint laxity have been correlated with changes in both serum estradiol and progesterone levels (49).

Female sex hormones have been shown to affect the structure and composition of a variety of tissues (55). Connective tissue from women expresses hormone receptor transcripts for both estrogen and progesterone (32,47), with estrogen having measurable effects on collagenous tissue including decreased collagen synthesis and increased degradation (14,18,37); decreased total collagen and protein content, fiber diameter, and density (1,23); increased elastic content (19,48); and decreased tensile strength (6,51). Collectively these findings suggest that the properties of collagenous tissues such as ligament and tendon may be significantly affected when exposed to varying concentrations of sex hormones (49). In general agreement with this, the mechanical properties of tendon have been shown to be different between males and females (29,42). It is possible that the

variance in the circulating levels of hormones between the 2 genders causes this male vs. female difference. Presence rather than absence of relatively high levels of estrogen and/or progesterone has been associated with decreased stiffness of ligamentous tissues (53), although Bryant and colleagues (7) reported lower rates of strain in those females who had been taking the contraception pill for at least a year. In addition, musculotendinous stiffness has been seen to vary considerably over the course of the menstrual cycle (15). However, this latter measure does not distinguish between a predominance of muscle or tendon events.

Changes in tendon properties over the course of the menstrual cycle would have implications for both performance and injury risk. All things being equal, reductions in tendon stiffness would be expected to cause increases in (a) initial muscle-shortening velocity, (b) degree of muscle shortening, and (c) muscle fascicle pennation angle at rest and during contractions (30,33,36). Overall, this would adversely affect force-producing capacity especially in the early stages of muscle contraction. This has been demonstrated practically with tendon stiffness has been shown to be related to the rate of force development (5,8,44). High rates of force development are often crucial in sporting performance because in most dynamic activities there is a limited time available to produce force; for example, contact times in sprinting can be less than 100 m (34).

More compliant tendon structures are also potentially at greater risk of injury because they experience greater strains in response to a set load (40). In addition, Hughes and Watkins (26) have identified the material properties of tendon as an element in the dynamic stability of the knee joint in their risk factor model for ACL injury. In fact, following their observation of increased incidence of ACL injury in females as a result of sex differences in the dynamic stabilizing structures rather than the passive stability structures', these authors proposed that the consistently reported greater incidence in ACL injuries among female athletes in comparison to males may be associated with their differing tendon mechanical properties (26). In light of previous work that reported increased joint laxity in females at phases of the menstrual cycle where estrogen is highest (57) and in light of the suggestion that collagenous tissues may be affected by concentrations of sex hormones (49) the aims of the current study were 2-fold: To investigate whether in vivo tendon mechanical properties in females alter over the course of the menstrual cycle and to determine whether any differences in in vivo tendon properties (both over the course of the menstrual cycle and absolute levels) are associated with circulating serum levels of estrogen and progesterone.

METHODS

Experimental Approach to the Problem

This study used a repeated measures research design, whereby patellar tendon mechanical properties of normally menstruating females were determined at different time points during the menstrual cycle. Participants were tested once during days 1 through 4 (when estrogen and pro-gesterone levels are low), once during days 12 through 14 (when estrogen levels reach their highest and progesterone levels are low), and once during days 20 through 23 (when both estrogen and progesterone are at relatively high levels); day 1 was defined as the first day of menstruation and the starting phase was randomized for all participants. As such, the main dependent variables measured in this study were the mechanical properties of the patellar tendon (i.e., stiffness, young's modulus, stress, and strain). The independent variable was therefore menstrual cycle day and consequently estrogen and progesterone levels.

Subjects

Fifteen healthy recreationally active female university stu- dents (age 23 ± 1 years, mass 63.1 ± 2.6 kg, height 1.66 ± 0.02 m) who (a) had experienced normal menstrual cycles (i.e., 28–32-day cycles for the previous 6 months [17]), (b) reported consistent flow between cycles (27), and (c) had not taken any form of hormonal contraceptive during this time were recruited to participate in the study. The participants were not involved in any kind of formal training program. The investigation was approved by the local Ethics Committee and all participants gave their written informed consent to take part in this study. The study conformed to the principles of the World Medical Association's Declaration of Helsinki.

Participants visited the laboratory prior to the test session to allow familiarization with the protocols. Participants attended 3 testing sessions at distinct time points over the course of a menstrual cycle. Participants conducted their 3 tests at the same time of day, in line with previously reported circadian rhythm in tendon properties (39,44). Furthermore, participants were required to fast overnight for approximately 10 hours in advance of the test session to allow for venous blood sampling and later analyses of serum levels of estradiol and progesterone (see "Assessments of Hormone Circulating Levels" section).

Procedures

Measurement of Tendon Forces

Torque output during isometric knee extensions was determined using a dynamometer (Kin Com, type 125 AP, Chattanooga, Tennessee, USA). Briefly, the knee was fixed at 90 degrees of flexion (full extension = 0 degrees) and the hip was at 85 degrees (supine = 0 degrees). A lever attachment cuff was placed on the lower leg at ~ 3cm above the medial malleolus. The center of rotation of the dynamometer lever arm was aligned with the joint center, and straps were fixed across the chest and the hip and

thigh of the test limb to prevent any extraneous movement. Five submaximal isometric knee extension efforts were carried out to ensure tendon preconditioning prior to recording data. Participants were instructed to perform ramped isometric contractions from rest to maximum over a 3- to 4-second time period. Three trials of the knee extension test were performed with 190 seconds of rest between contractions.

Tendon force was calculated as $F_{tend} = (P + P_{antag})/ T_{arm}$ where F_{tend} = force in the tendon, P = observed torque output, P_{antag} = antagonist (hamstring) co-contraction torque (see later), and T_{arm} = tendon moment arm. The patellar tendon moment arm was determined from previous reports, 44.7 mm (2,28).

Estimation of Co-Contraction Torque Using Electromyographic Activities

During voluntary isometric muscle contractions, in addition to the recorded external torque produced, the agonist muscle group also produces an "additional" torque to overcome the co-contraction action of the antagonistic muscle group. As such, the force experienced by the tendon is greater than the recorded external torque. This "additional" torque therefore also must be determined to calculate the true force in the tendon and consequently tendon properties. Failure to do this leads to underestimations in tendon force and consequently gives artificially lower values of tendon stiffness. This "additional" torque is equal to the co-contraction torque, which is the torque produced by the antagonist muscle group, and this can be determined using electromyography (EMG). In this study the EMG of the long head of the biceps femoris muscle (BF) was measured to ascertain the level of antagonistic muscle co-contraction during the isometric knee extensions (44). Assumptions were that the BF is representative of its constituent muscle group (9) and that the BF EMG relationship with knee flexors torque is generally linear (31). Two self-adhesive Ag-AgCl electrodes (Medicotest UK, type N10A) were placed in a bipolar configuration with a constant interelectrode distance of ~20 mm at a site corresponding to the distal one-third of the length, in the midline of the belly of the BF. The reference electrodes (Medicotest, UK, type Q10A) were placed on the lateral tibial condyle. Prior to electrode attachment the skin was prepared by shaving, abrading, and cleaning with an alcohol-based solution to minimize its resistance below 5KV. The electromyographic signals were high and low pass filtered between 10 and 500 Hz, respectively (Neurolog filters NL 144 and NL 134, Digitimer, UK), preamplified (31000) (Neurolog remote AC preamplifier NL 824, Digitimer, UK), amplified (x2) (Neurolog isolation amplifier, NL 820, Digitimer, UK), and A/D converted at 2000 Hz (KPCI 3101, Keithley Instruments, UK). A series of 3 maximal isometric knee flexions were carried out to obtain the EMG at maximal flexion torque. The root mean square (RMS) EMG activity corresponding to the peak torque period was analyzed over 50-m epochs and averaged for a 1-second period during the plateau of peak torque. This previously has been suggested to be acceptable in terms of signal-to-noise ratio (24). Electro- myographic activity of the BF during knee extension was divided by the maximal flexor EMG. The maximal flexor torque then was multiplied by this value to determine co-contraction torque.

Measurement of Tendon Elongation

Elongation of the patellar tendon was assessed during the graded isometric knee extensions using a 7.5-MHz, 40-mm linear array, B-mode ultrasound probe (AU5, Esaote Biomedica, Italy) with a depth resolution of 49.3 mm. The probe was positioned in the sagittal plane over the patellar tendon at the apex of the patellar (Figure 1). Three efforts to maximum were recorded. An echo-absorptive marker was placed between the probe and the skin to act as a fixed reference from which measures of elongation could be made. Ultrasound images were recorded in real-time onto mini DV via s-video output and captured onto PC at 25 Hz using Quintic Biomechanics (9.03 v11). The ultrasound output was synchronized (using an electronic square wave signal generator) with the force and EMG records to allow temporal alignment among these 3 parameters. Tendon displacements were determined at intervals of 10% of the maximal force (from 10 to 100%) using image J (National Institutes of Health, Bethesda, Maryland, USA).

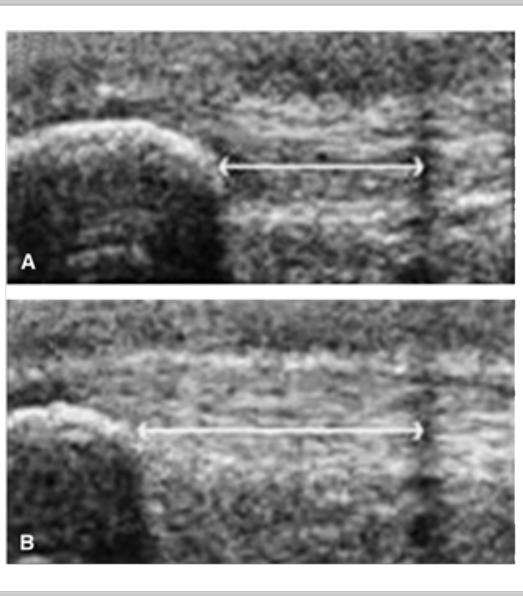

Figure 1. Ultrasound image of the patellar tendon, at rest (A) and 100% MVC (B). White arrows indicate the distance measured between the echo-absorptive marker and the reference point of tendon attachment.

Calculation of Tendon Properties

The tendon force–elongation relationships were fitted with second-order polynomial functions forced through zero. Tendon stiffness measures (K in Nmm^{-1}) were calculated from the slope of the tangents at 10% force intervals.

Patellar tendon cross-sectional area (PT_{CSA}) and resting length (PT_L) also were assessed with the knee joint at 90 degrees. PT_{CSA} was measured as the average from transverse-plane ultrasound images taken at 25, 50, and 75% PT_L. PT_L was determined from sagittal-plane ultrasound images and measured from the inferior pole of the patellar to the superior aspect of the tibial tuberosity.

Young's modulus was calculated as the product of stiffness and the ratio between PT_L to PT_{CSA}. Tendon strain (%) was calculated as the ratio of tendon elongation to the PT_L. Tendon stress was calculated by dividing force in the tendon by PT_{CSA}.

Assessments of Hormone Circulating Levels

Blood samples were taken from the median antecubital vein (5 mL) by a trained phlebotomist at the beginning of each testing session following a 10-hour fasting period. The samples were allowed to clot while refrigerated, centrifuged at 8,000 rpm for 6 minutes, and then the serum was aliquotted and stored at 220°C for later analysis. The samples were coded for each participant and testing session, and the coding system was not revealed to the person performing the biochemical analyses. Hormone (estradiol and progesterone) concentrations were measured using standard enzyme-linked immunosorbent assays (ELISA; Alpha Diagnostic International, San Antonio, Texas, USA), and each sample was assessed 4 times. Assay sensitivities are 10 pg/mL for progesterone and 0.1 ng/mL for estradiol. Ligand values were compared with laboratory reference data to verify correct measurement intervals. Intraassay and interassay variations for progesterone are 7.9% and 9.8%, respectively. For estradiol these values are 9.9 and 10.1%.

Statistical Analyses

Repeated-measures 1-way ANOVA (analyses of variance) were used to determine the presence of significant changes in the variables of interest (muscle/tendon structural/mechanical properties and hormone levels) among the 3 phases of the menstrual cycle. Regression analyses were used to determine the significance of each independent variable (e.g., estradiol level, progesterone level, maximal voluntary force, menstrual cycle day) in predicting the tendon material properties of interest (i.e., stiffness and young's modulus). In all cases, significance was set to $p \# 0.05$. Intraclass correlation coefficients (ICCs) were calculated to determine reliability of the measures. All data are presented as mean ± standard error of the mean (SEM).

RESULTS

Within-session ICCs were 0.911 for patellar tendon elongation, 0.927 for knee extension torque, 0.983 for PT_{CSA}, 0.916 for PTL, 0.986 for estradiol level, and 0.996 for progesterone level. The power of all the tests was ≥0.8.

On average, testing sessions occurred on days 3 ± 0.4, 13 ± 0.2, and 21 ± 0.3 of the menstrual cycle. There were significant differences in the levels of both serum estradiol (p = 0.001) and progesterone (p = 0.030) over the course of the menstrual cycle (Table 1).

TABLE 1. Serum levels of estradiol and progesterone at 3 time points in the menstrual cycle.

	Day of cycle		
	1–4	12–14	20–23
Estradiol (pg/mL)	87.90 6 34.30	159.00 6 67.80*	137.10 6 33.00*†
Progesterone (ng/mL)	0.54 6 0.10	0.89 6 0.20	8.02 6 1.30*†

*Significantly different from days 1 through 4.
†Significantly different from days 12 through 14.

Maximal voluntary tendon force showed no significant differences between testing sessions despite showing a trend to follow the same pattern of changes as estrogen levels (Table 2).

TABLE 2. Patellar tendon structural and mechanical properties during days 1 to 4, 12 to 14, and 20 to 23 of the menstrual cycle. Data are means ± *SEM*.

	Day 1–4	Day 12–14	Day 20–23
Tendon length (cm)	4.7 6 0.1	4.5 6 0.2	4.7 6 0.1
Tendon CSA (mm^2)	69.9 6 3.1	71.9 6 2.6	72.1 6 3.1
Tendon length/CSA ratio (mm^{21})	0.70 6 0.03	0.65 6 0.03	0.66 6 0.03
Maximum voluntary tendon force (N)	7,709.9 6 543.78	8,809.8 6 816.25	8,035.6 6 548.78
Maximum elongation (mm)	4.5 6 0.2	4.5 6 0.3	4.2 6 0.3
Maximum strain (%)	9.6 6 0.6	10.0 6 0.8	9.0 6 0.7
Maximum stress (MPa)	111.4 6 9.6	121.2 6 13.8	109.3 6 8.0
Maximal stiffness (Nmm^{21})	3,777.9 6 321.8	3,871.8 6 345.1	3,879.2 6 316.2
Maximal young's modulus (GPa)	2.6 6 0.3	2.5 6 0.3	2.5 6 0.2

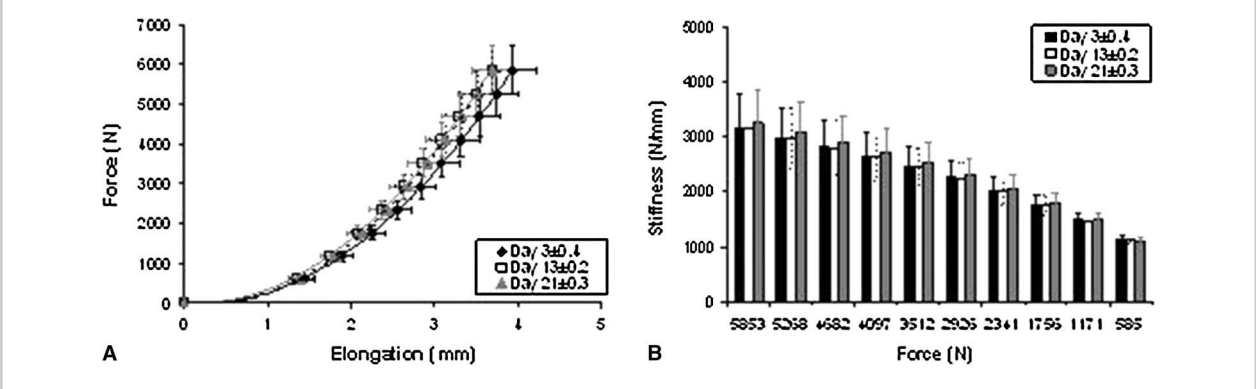

Figure 2. Average patellar tendon properties across the menstrual cycle. A) Mean force-elongation relationships; B) stiffness/force relationships. Data represent mean for all individuals (n = 15) at 3 time points in the menstrual cycle: Day 3 6 0.4, day 13 6 0.2, and day 21 6 0.3.

Similarly, there were no significant differences in the maximal (or indeed submaximal) (Figure 2) tendon mechanical properties among the 3 phases of the menstrual cycle (Table 2). The results from the multiple linear regression, with tendon stiffness as the dependent variable, excluded all but 1 of the entered dependent variables (i.e., menstrual cycle day, estrogen, and progesterone), leaving only maximal voluntary tendon force (MVF_T) as significantly associated with tendon stiffness. Thus 14.4% ($p = 0.024$) of the variance in tendon stiffness can be described by $K = 2298.66 + 0.19\ MVF_T$.

Running a similar multiple linear regression for young's modulus excluded specific dependent variables (i.e., menstrual cycle day and progesterone) from the equation, leaving only estrogen and maximal voluntary tendon force as significantly associated with tendon young's modulus. Thus 17.8% ($p = 0.043$) of the variance in young's modulus can be described by $YM = 1196.82 + 0.69$ Estradiol $+ 0.16\ MVF_T$.

DISCUSSION

The current study aimed to investigate whether females' tendon mechanical properties alter over the course of the menstrual cycle and determine whether any changes in tendon properties would be associated with circulating levels of serum estrogen and/or progesterone. Our findings show that (a) there are no significant changes in the structural and mechanical properties of the patellar tendon in a group of healthy females assessed at days 1 through 4, 12 through 14, and 20 through 23 of their menstrual cycle. However, having taken into account the principle parameters associated with tendon mechanical properties and rejected all collinear parameters, 17.8% ($p = 0.043$) of the variance in young's modulus is explained by a combination of estradiol and maximal voluntary tendon force. Progesterone was not significantly associated with any of the tendon characteristics investigated.

Although no previous study has systematically measured tendon stiffness over the course of the menstrual cycle, Eiling and colleagues (15) examined the changes in lower limb musculotendinous stiffness using a unilateral hopping protocol. Musculotendinous stiffness was measured 4 times over the course of the menstrual cycle: Once on the first day of menses, once on the day of predicted ovulation (14 days prior to estimated menstruation based on the length of the last 3 cycles), once in the midluteal phase (7 days prior to predicted menstruation), and once in the midfollicular phase (halfway between the first day of menses and predicted ovulation). An important methodological difference between that of Eiling and co-workers (15) and the present study is that here hormonal levels were actually measured rather than based on a prediction. The findings from Eiling's study do not necessarily agree with those reported here in that they reported that indices of musculotendinous stiffness were lowest at the time of ovulation and highest during menses (15). In contrast, here we report no changes with menstrual cycle phase. When comparing our data with those of Eiling and colleagues (15), it is also important to consider the differences between the measurements of musculotendinous stiffness and tendon stiffness. In addition to the stiffness of the tendon, musculotendinous stiffness also takes into account the properties of the muscle, muscle activation, fascia, joints, and so on, and these factors may be differentially affected over the course of the menstrual cycle. What is more, results from Eiling and colleagues (15) are somewhat at odds with some previous work in that it has been shown previously that maximal muscle force is increased around the time of ovulation with increased levels of estrogen (45). The finding we present here that menstrual cycle phase has no effect on maximal voluntary tendon force, tendon stiffness, or tendon Young's modulus is in agreement with those previous studies that have found no effect of menstrual cycle phase on various measures of maximal strength (11–13,20,22) and knee laxity (3,4,27,46,54).

The primary function of the tendon is to transmit/dampen forces between muscles and bones. As such, the stiffness of the tendon structure can affect the rate of force development, thereby affecting muscle force output in the early stages of muscle contraction. The direction of any association between tendon stiffness and tissue injuries is, however, a multifaceted issue. Whereas we suggested earlier the case where increased compliance may be linked to increased collagen tissue injuries, primarily as a result of the increased strain experienced for any given load, it also could be argued that the reduced damping ability of a stiffer tendon could result in increased loading (both in terms of magnitude and rate) on muscle and surrounding tissue and thus increase risk of injury to these structures (21).

In addition, it also could be argued that high tendon forces may lead to increased injury risk along the series musculotendinous element owing to potentially greater shear and tensile forces generated. Whatever the case, it should be noted that our analyses revealed that the interaction among hormonal levels, menstrual cycle day, and tendon properties is complex. Our data showed no association between tendon properties and menstrual cycle day, but showed significant correlations between tendon properties normalized for dimension and circulating levels of estrogen. The contrast between the 2 concepts is not obvious, but it is important. Hence, we have shown that whether a female exhibited relatively high or low young's modulus was a factor of her intrinsic levels of estrogen rather than the phase of the menstrual cycle per se. In other words, we observed indications of a chronic, rather than acute, effect of estrogen on tendon structural properties. Estrogen has been linked with deleterious effects in terms of tendon metabolism (14,18,37). Here it may be that there are 2 mechanisms at odds with each other, in that if estrogen has both the ability to increase muscle strength (10,43,50) and to decrease collagen synthesis (14,18,37), the 2 factors may cancel each other out with regard to the effects on the tendon in so far as increased strength may be seen to increase potential loading on the tendon structure and the tendon would adapt by increasing its stiffness. This has been shown previously in resistance training studies (8,41), and such an adaptation is in direct opposition to the changes (1,23) induced in the collagen turnover via the action of estrogen.

The lack of acute changes in tendon stiffness in the presence of increased levels of estrogen here could be for a number of reasons. The first possibility is that there may be a delayed response between hormone fluctuations and tendon property changes. Indeed, previous authors (49) found that the relationship between estradiol and pro-gesterone levels and knee laxity was stronger when the changes in hormone concentrations are compared with changes in knee laxity occurring approximately 3 to 4 days later. However, the study by these authors also highlighted the variability of the time shifts for the knee laxity to be observed and the variability in the degree of response between individuals. With regard to the study by Shultz and colleagues (49), because tendon properties were only measured 3 times during the menstrual cycle in the present study, it is possible that the day(s) when tendon stiffness was truly at its maximum or minimum have been missed. It is also possible that the days in the cycle when these points occur are different between individuals as a result of differing time frames and magnitudes of response to hormone fluctuations and differing hormone fluctuations. It would be a matter for future studies to look into the time course of the changes in tendon properties with the menstrual cycle, with only a day or so gap between assessments. Another possible explanation for the complex relationship between tendon properties and the menstrual cycle phase may be that estrogen interacts with other hormones in vivo (e.g., insulin like growth factors have been associated with tendon properties [38]), thereby making it difficult to predict the effects on tendon properties through measurement of in vivo levels of only 1 hormone/ cytokine. Therefore, it is evident that a detailed study of the time course of the changes in hormones, in relation to tendon mechanical and structural properties, is in order so that these issues may be elucidated.

In conclusion, the findings of the current study align with previous research that demonstrates no significant effect of menstrual cycle phase on maximal strength and injury risk. In addition, our results also demonstrate that in the presence of high circulating levels of estrogen, tendon stiffness increases. The difference between the 2 concepts is crucial in as much as they have potential implications in terms of linking estrogen to a chronic, rather than an acute, impact on tendon behavior. These findings are relevant to clinical outcomes, exercise performance, and injury risk.

PRACTICAL APPLICATIONS

The practical performance applications of the current study are that because tendon mechanical properties do not vary, their effect on muscle output, and hence motor control and injury risk, will remain unchanged over the course of the menstrual cycle. Thus, "time of the month" does not need to be considered when organizing training and competition schedules. Notably, however, our data also suggest that it is only those females who are normally exposed to chronically high levels of estrogen who may be at greater risk of injury.

As such, female athletes may benefit from systematic determination of their estrogen levels so as to allow their coach/team manager to take extra precautions when deciding on a training plan for those "at-risk females." Our current data also may partly explain the gender differences in tendon mechanical properties and injury rates observed in previous studies.

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