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A B S T R A C T

Background: Tendon tissue contains oestrogen receptors and is therefore likely to be responsive to female sex hormones. Here we examine any effect of levels of female sex hormones associated with the menstrual cycle phase on corresponding tendon mechanical properties. *Methods:* Fifteen healthy females aged 23 (SEM 1.0 years) underwent three assessments of medial gastrocnemius tendon mechanical properties. Assessments were carried out once during days 1–4, 12–14 and 20–23 (with day 1 being the first day of menstruation). Venous blood samples were taken on the same days as tendon properties assessments to quantify serum levels of oestradiol and progesterone. *Findings:* There was no significant difference in the stiffness of the medial gastrocnemius tendon over the course of the menstrual cycle (days 1–4, 65.08 (SEM 5.16 Nm m^{-1}), days 12–14, 62.73 (SEM 5.82 Nm m^{-1}), days 20–23, 66.74 (SEM 7.14 Nm m^{-1})). There were also no significant differences in tendon length and cross-sectional area which led to no significant differences in Young's modulus values. No correlations were found between serum levels of oestradiol and/or progesterone and tendon stiffness and/or Young's modulus. *Interpretation:* Acute fluctuations in female sex hormones have no significant effect on medial gastrocnemius tendon mechanical properties. In a context where studies are often limited to selecting only oral contraceptive-users as participants in order to minimise potential noise related to the anticipated effects of menstrual cycle hormones on physical performance, our findings provide the basis for enabling the pooling of female tendon data, regardless of the phase of the menstrual cycle of individual participant.

Keywords: Collagen; Female; Sex hormones; Structural properties

1. Introduction

The structure and composition of a variety of tissues can be influenced by female sex hormones (Wojtys et al., 1998). Skeletal muscle and tendon is known to possess receptors for sex hormones including progesterone and oestrogens (Wiik et al., 2008). Oestrogen has been reported to have a number of measurable effects on collagenous tissue in a variety of animal models. It is associated with reductions in tensile strength (Slauterbeck et al., 1999). Its action has also been shown to bring about a decrement in total collagen content, fibre diameter, and density (Abubaker et al., 1996; Hama et al., 1976). The mechanisms underlying oestrogen presence also cause changes in the production and clearance of collagen, with decreased collagen synthesis and increased degradation being seen (Fischer, 1973; Neugarten et al., 2000). These findings suggest that the properties of collagenous tissues such as ligament and tendon may be affected when exposed to varying concentrations of sex hormones (Shultz et al., 2004).

Naturally occurring variations in sex hormones occur in women during the menstrual cycle (Karageanes et al., 2000). Musculotendinous stiffness has been seen to vary considerable over the course of the menstrual cycle (Eiling et al., 2007), however, this does not distinguish between muscle and tendon changes alone. The effects of the changing hormone levels (over the course of the menstrual cycle) in females on tendon mechanical properties alone have not previously been investigated. In addition, it has been reported that females who have been taking the contraceptive pill for at least a year demonstrate lower levels of tendon strain compared to non-pill taking females, indicating a possible influence of hormonal state on tendon properties (Bryant et al., 2008). In general agreement with this, the mechanical properties of tendon have been shown to be different between males and females (Onambele et al., 2007b) with a suggested explanation being the variance in the levels of circulating sex hormones between genders, as presence rather than absence of oestrogen and progesterone has been associated with decreased stiffness of ligamentous tissues (Uldb-jerg and Ulmsten, 1990).

It has been reported that both strength (Davies et al., 1991; Phillips et al., 1996; Greeves et al., 1999) and injury occurrence (Slauterbeck et al., 2002; Wojtys et al., 1998) can vary over the course of the menstrual cycle. Changes in tendon mechanical properties could have a bearing on both of these factors. Where reductions in tendon stiffness would result in: increases in initial muscle shortening velocity and increases in the amount of muscle shortening with resultant alteration in the degree of fibre pennation angle changes, all of which adversely affect force producing capacity especially in the early stages of muscle contraction. In addition, the ability to maintain balance or stability has previously been associated with lower limb tendon structural and mechanical properties, with stiffer tendon structures associated with increased balance ability (Onambele et al., 2006). A proposed explanation for this finding is related to tendons primary function of force transmission. Stiffer tendon structures enable more rapid force transfers than compliant systems and thus increase the speed at which the muscle–tendon complex corrects the 'catch and throw' actions in-volved in maintaining balance (Loram and Lakie, 2002) and consequently improve balance performance (Onambele et al., 2007a). In fact it has been reported that postural sway is significantly increased in the mid luteal phase in women suffering from premenstrual syndrome (PMS) (Friden et al., 2005; Friden et al., 2003), indicating possible decreased tendon stiffness in this phase. The possible alterations in tendon properties over the menstrual cycle may therefore have important implications due to its capacity to affect muscle function and balance and thus performance and injury risk.

The aims of the current study were therefore twofold: (1) to investigate whether female medial gastrocnemius tendon mechanical properties alter over the course of the menstrual cycle and (2) determine whether any changes in the medial gastrocnemius tendon properties are related to the fluctuating levels of serum oestradiol and progesterone.

2. Methods

2.1. Participants

Fifteen healthy recreationally active females aged 23 (SEM 1.0 years), mass 63.1 (SEM 2.6 kg), height 1.66 (SEM 0.02 m) who were experiencing normal menstrual cycles (reported 28–32 day cycles for the last 6 months) and had not taken any form of hormonal contraceptive during this time participated in the study. The investigation was approved by the local Ethics Committee and all subjects gave their written informed consent to participate. The study conformed to the principles of the World Medical Association's Declaration of Helsinki. Participants visited the laboratory prior to the first test session to allow familiarization with the protocols. Participants attended three testing sessions at set time points over the course of a menstrual cycle, including once during days 1–4, once during days 12–14 and once during days 20–23 (day 1 was defined as the first day of menstruation). The order of testing was randomised and each participant conducted their three tests at the same time of day. During each testing session, data was acquired for later determination of participants' medial gastrocnemius tendon mechanical properties and serum levels of oestradiol and progesterone.

3. Measurement of tendon forces

Torque output during isometric plantar flexion was determined using a dynamometer (Kin Com, type 125 AP, Chattanooga, USA). During the plantar flexion efforts the knee was fully extended and the hip flexed to 90°, the foot was fixed in a neutral anatomical position, where the sole of the foot was at 90° to the tibia. The centre of rotation of the dynamometer lever arm was aligned with the joint centre, and straps were fixed across the chest, hip and thigh of the test limb and around the foot to prevent any extraneous movement. Maximal isometric plantar flexion efforts were carried out to ensure tendon pre-conditioning prior to the test. Participants were instructed to perform ramped isometric contractions from rest to maximum over a 3–4 s time period. Three trials of the plantar flexion were performed with 180 s 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} = antagonistic (tibialis anterior) co-contraction torque, and T_{arm} = tendon moment arm. The moment arm length of the medial gastrocnemius tendon was obtained using the tendon travel method (An et al., 1984). Correction for the relative contribution of the physiological cross-sectional area of the medial gastrocnemius within the plantarflexors (Fukunaga et al., 1992) was applied to the calculation of medial gastrocnemius tendon force.

4. Estimation of co-contraction using electromyographical (EMG) activity)

The EMG of the long head of the tibialis anterior (TA) was measured in order to ascertain the level of antagonistic muscle co-contraction torque during the plantar flexion performances. Assumptions were that TA is representative of its constituent muscle group (i.e. the dorsiflexors) (Carolan and Cafarelli, 1992), and that the TA EMG relationship with dorsiflexion torque is linear (Lippold, 1952). Two self-adhesive Ag–AgCl electrodes (Medicotest UK, type N10A), were placed in a bipolar configuration with a constant inter-electrode distance of ~20 mm, at a site corresponding to the midline on the TA muscle belly, halfway between the centre of the belly and the distal myotendinous junction of the TA. Prior to electrode attachment the skin was prepared by shaving, abrading, and cleaning with an alcohol-based solution in order to minimise the resistance. The reference electrodes (Medicotest, UK, type Q10A) were placed on the lateral malleolus of the ankle. The electromyographic signals were high and low pass filtered between 10 and 500 Hz, respectively (Neurolog filters NL 144 and NL 134, Digitimer, UK), pre-amplified (×1000), (Neurolog remote AC pre-amplifier NL 824, Digitimer, UK), amplified (×2) (Neurolog iso-lation amplifier, NL 820, Digitimer, UK) and A/D converted at 2000 Hz (KPCI 3101, Keithley instruments, UK). A series of three maximal isometric dorsiflexion contractions 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 analysed over 50 ms epochs and averaged for a 1 s period during the plateau of peak torque. This has been previously suggested to be acceptable in terms of signal to noise (Hermens et al., 2000). Electromyographic activity of the TA during plantarflexion was divided by the maximal dorsiflexor EMG, and the maximal dorsiflexflexion torque was then multiplied by this value to determine co-contraction torque.

5. Measurement of tendon elongation

Elongations of the medial gastrocnemius were assessed during the graded isometric plantar flexions 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 placed in the sagittal plane over the myotendinous junction of the medial head of the gastrocnemius muscle (see Fig. 1).

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 v 11). The ultrasound output was synchronized (using an electronic signal generator) with the force and was synchronized (using an electronic signal generator) with the force and EMG records to allow temporal alignment. Tendon excursions were determined at intervals of 10% of the maximal force (from 0% to 100%) using image J (National Institute of Health, Bethesda, MB, USA).

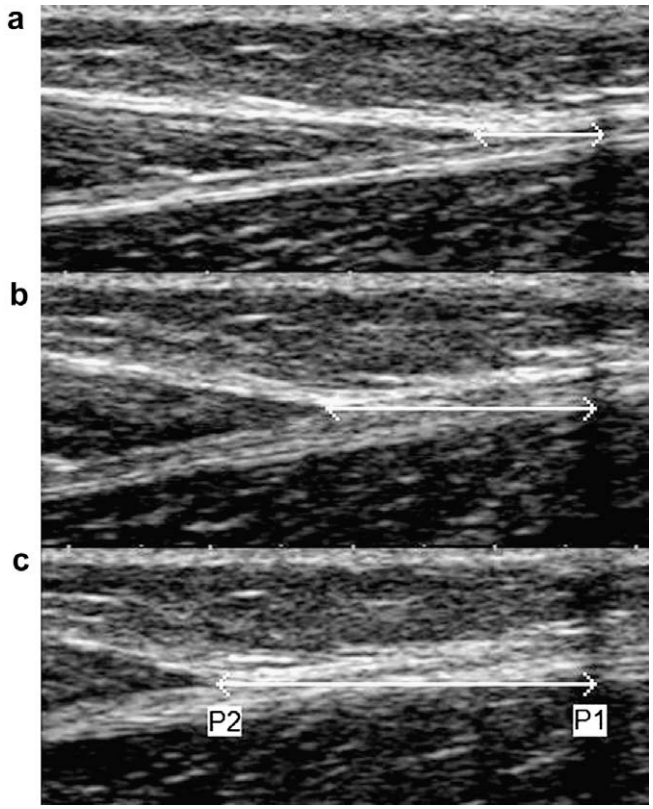

Fig. 1. Typical example of medial gastrocnemius tendon elongation during ramped isometric contraction (a – at rest, b – during contraction and c – maximal contraction). White lines indicating measured distances from echo-absorptive marker (P1) to myotendinous junction (P2).

6. Calculation of tendon properties

The tendon force–elongation relationships were fitted with second order polynomial functions forced through zero. Tendon stiffness measures (K in Nm m^{-1}) were calculated from the slope of a tangent at 100% maximum force (each individual's lowest MVC over the three testing sessions was used as 100% MVC). Medial gastrocnemius cross-sectional area (MGT_{CSA}) and resting length (MGT_L) were assessed with the foot in neutral position (sole of foot at 90° to the tibia) and lower limb straight. MGT_{CSA} was measured as the average from transverse-plane ultrasound images taken at 1,2 and 3 cm above the tendon insertion point in the calcaneus (Burgess et al., 2009) and corrected based on the assumption that the medial gastrocnemius tendon cross-sectional area occupies a fraction of the average achilles tendon cross-sectional area equiva-lent to the relative physiological cross-sectional area of the medial gastrocnemius muscle with respect to the entire triceps surae muscle (Fukunaga et al., 1992). MGT_L was determined from sagittal-plane ultrasound images and measured form the insertion of the Achilles tendon into the calcaneus to the insertion of the myotendinous junction to the medial gastrocnemius.

Young's modulus was calculated as the product of stiffness and the ratio between MGT_L and MGT_{CSA}: Young's modulus thus provides a stiffness value normalised for tendon dimensions. Hysteresis was defined as the area under the ascending force–elongation curve minus the area under the descending curve expressed as a percentage of the area under the ascending curve.

Tendon strain (%) was calculated as the ratio of tendon elonga-tion to the T_L. Tendon stress was calculated by dividing force in the tendon by T_{CSA}.

7. Hormonal measures

Blood samples were taken from the median antecubital vein (5 ml) by a trained phlebotomist at the beginning of each testing session. The blood sample was allowed to clot whilst refrigerated, centrifuged at 8000 rpm for 6 min, and the serum was separated and stored at −20 °C until analysis. The samples were coded for each participant and testing session. Hormone concentrations were measured using Enzyme-Linked Immuno Sorbent Assays (estradiol-1920, progesterone-1860, Alpha Diagnostic International, USA). Oestradiol and progesterone values were compared with laboratory reference values to verify correct measurement intervals.

8. Statistical analyses

A repeated measures ANOVA was performed to determine the effect of menstrual cycle day on tendon properties and serum levels of oestradiol and progesterone. Post-hoc *t*-tests were performed and *P* values Bonferroni corrected to examine any differences highlighted by the ANOVA analysis. Pearson's correlation coefficients were determined to examine relationships between serum hormone levels and tendon mechanical properties. Significance was set to $P \leqslant 0.05$. Intraclass correlation coefficients were calculated to estimate reliability of the measures. All data are presented as mean ± standard error of the mean (SEM).

9. Results

The within-session ICCs were: 0.947 for medial gastrocnemius tendon elongation, 0.917 for plantarflexion torque, 0.980 for MGT_{CSA} and 0.981 for MGT_L.

For three individuals a full data set for all three cycle days was not obtained and thus these individuals have been omitted from the analysis.

There were significant differences in the levels of both serum oestradiol and progesterone over the course of the menstrual cycle ($P < 0.05$) (see Fig. 2). With significant differences in oestradiol occurring between days 1–4 vs. 12–14 ($P < 0.05$) and 1–4 vs. 20–23 ($P < 0.05$), and significant differences in progesterone occurring between days 1–4 vs. 20–23 ($P < 0.05$) and 12–14 vs. 20–23 ($P < 0.05$).

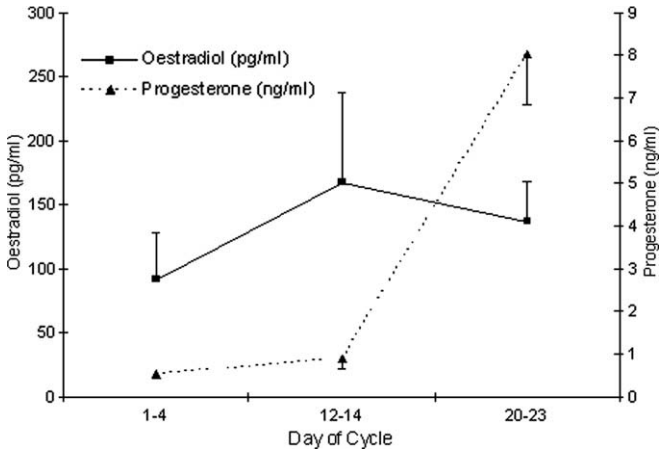

Fig. 2. Changes in serum oestradiol and progesterone over the course of the menstrual cycle.

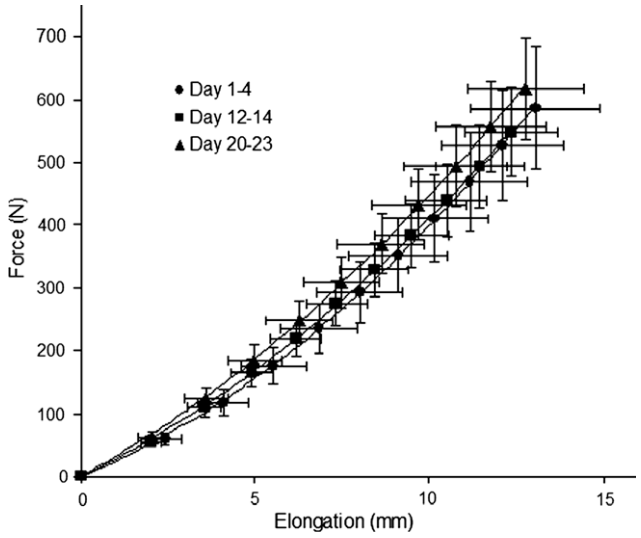

Fig. 3. Mean force–elongation curve for the medial gastrocnemius tendon at three time points in the menstrual cycle, days 1–4 (●), days 12–14 (■) and days 20–23 (▲). Data presented are means ± SEM.

The stiffness values calculated over the three time points are represented graphically in Fig. 3 showing the medial gastrocnemius mean force–elongation curves, the gradients of which denote the tendons stiffness. There were no significant changes in medial gastrocnemius tendon stiffness, Young's modulus, hysteresis, CSA, length, maximum strain or maximum stress ($P > 0.05$) (see Table 1 for values).

As there were no significant differences in both of the tendon's length and CSA this lead to non-significant differences in Young's modulus (see Fig. 4).

Interestingly although non-significant, the medial gastrocnemius showed a trend of lowest stiffness and Young's modulus during days 12–14 and highest during days 20–23 (with Young's modulus being 6.25% lower during days 12–14 than days 20–23). However, neither tendon stiffness nor Young's modulus values and changes were significantly correlated to serum levels and/or changes in oestradiol and progesterone ($P > 0.05$) (see Table 2 for specific details).

Table 1
Medial gastrocnemius tendon structural and mechanical properties for days 1–4, 12–14 and 20–23 of the menstrual cycle. Data are means (SEM).

Variable	Days 1–4	Days 12–14	Days 20–23
Maximum force (N)	677.6 (105.9)	684.9 (87.4)	729.6 (101.4)
Maximum stiffness (Nm m^{-1})	65.08 (5.16)	62.73 (5.82)	66.74 (7.14)
Maximum Young's modulus (GPa)	0.63 (0.06)	0.60 (0.05)	0.64 (0.07)
Hysteresis (%)	19.37 (3.11)	19.95 (3.89)	18.71 (3.78)
Tendon length (cm)	17.73 (0.83)	17.35 (0.53)	17.17 (0.49)
Tendon CSA (mm^2)	17.92 (0.45)	17.92 (0.41)	17.78 (0.38)
Maximum strain (%)	7.6 (1.1)	7.3 (0.8)	7.5 (1.0)
Maximum stress (MPa)	32.51 (5.38)	30.28 (3.73)	34.50 (4.45)

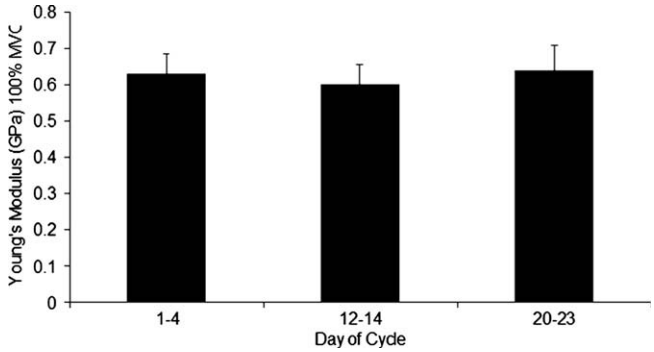

Fig. 4. Young's modulus of the medial gastrocnemius during days 1–4, 12–14 and 20–23 of the menstrual cycle. Data are means ± SEM.

Table 2
Correlation coefficients between oestrogen and tendon properties in absolute levels and in relative terms.

R value for		Serum levels of hormone vs. tendon parameter value	Change in hormone vs. change in tendon parameter
Tendon stiffness	Oestradiol	0.083	0.258
	Progesterone	0.088	0.163
Tendon Young's modulus	Oestradiol	0.258	0.024
	Progesterone	0.093	0.040

10. Discussion

The current study aimed to: (1) investigate whether females' tendon mechanical properties alter over the course of the menstrual cycle and (2) determine whether any changes in tendon properties are related to the fluctuating levels of serum oestradiol and progesterone. Our findings have shown that: (1) there was no significant change in the structural and mechanical properties of the medial gastrocnemius tendon in a group of healthy females between days 1–4, 12–14 and 20–23 of their menstrual cycles ($P > 0.05$) and (2) tendon stiffness and Young's modulus values and changes were not correlated to serum levels and changes in oestradiol or progesterone.

The values for medial gastrocnemius tendon stiffness and Young's modulus reported here are in line with previously reported values, (Kubo et al., 2003; Magnusson et al., 2001; Burgess et al., 2009). The values are similar to those reported by Burgess and co-workers for female gastrocnemius tendon, greater than those of female gastrocnemius tendon/aponeurosis reported by Kubo and colleagues, and lower than those found by Magnusson and colleagues for male gastrocnemius tendon. As aponeurosis has previously been shown to be more compliant than tendon (Arampatzis et al., 2005), and males possess stiffer tendons than their female counterparts (Onambele et al., 2007b), these differences are to be expected.

Although no previous studies have measured tendon stiffness over the course of the normal menstrual cycle, Eiling et al.(2007) 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 three cycles), once in the mid luteal phase (7 days prior to predicted menstruation) and once in the mid follicular phase (half way between first day of menses and predicted ovulation). This study reported similar findings to those reported here in that stiffness was lowest at the time of ovulation; however, unlike our own findings, these authors also reported highest stiffness levels during menses. When comparing the two sets of results it is 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, fascia, joints, etc., these structures may be differentially affected over the course of the menstrual cycle.

It has previously been reported that muscular strength alters over the course of the menstrual cycle, (Davies et al., 1991; Phillips et al., 1996; Greeves et al., 1999; Sarwar et al., 1996) with the majority of studies reporting increases in strength in the late follicular phase or around ovulation (Phillips et al., 1996; Sarwar et al., 1996). These studies suggest that oestrogen has a strengthening action, directly or indirectly, on skeletal muscle and that the rise in serum oestrogen levels during the late follicular phase peaking at ovulation, results in increased skeletal muscle strength performance (Phillips et al., 1996). However, with regards to the effects of oestrogen on tendon tissue, oestrogen has been reported to have negative effects on collagenous tissue (decreased collagen synthe-sis and increased degradation) (Fischer, 1973; Neugarten et al., 2000) so that a trend towards decreased tendon stiffness at the time of ovulation would be expected to impact negatively on muscle force generation. It is important to note that numerous studies have reported no effect of menstrual cycle phase on strength (Dibrezzo et al., 1988; Gur, 1997; de Jonge et al., 2001; Elliott et al., 2003) and that our current results are aligned with these. It may be that several mechanisms which influence muscle strength are affected by hormonal fluctuations. These mechanisms (such as: an increase the force produced by each cross-bridge through a change in the equilibrium between 'low' and 'high' force states (Sarwar et al., 1996), and alterations in tendon stiffness) may counteract one another, perhaps to differing degrees depending on the cycle phase and method used to assess strength, thus contributing to the equivocal nature of previous results.

The trend towards lower levels of tendon stiffness during days 12–14 and highest during days 20–23 reported here concur with the conclusion drawn by (Hewett et al., 2007) in a recent review of injury occurrence over the course of the menstrual cycle that there is a greater risk of injury in the pre-ovulatory phase compared to the post-ovulatory phase. More compliant tendon structures can lead to reduced performance in tasks requiring rapid force generation and thus lead to greater risk of injury especially via the mechanism of falls due to reduced ability to counteract deleterious forces and reduced stability. However, these findings contradict those of Friden and co-workers (Friden et al., 2005; Friden et al., 2003) who showed increased postural sway in the mid luteal phase in females with pre-menstrual syndrome (PMS). The results in the current study indicate that any alteration in balance ability may not necessarily be due to fluctuations in tendon stiffness but may be due to other, as yet unidentified mechanisms.

The non-significant changes in tendon stiffness over the course of the menstrual cycle reported here may be due to a number of factors. Firstly it could be that tendon stiffness does not change over the course of the menstrual cycle and that tendon stiffness is unaffected by oestrogen and progesterone levels, or that the fluctuations in sex hormones experienced during the menstrual cycle are not large enough or long enough to induce a change in in vivo tendon characteristics. Secondly it may be that there is a delayed response between hormone fluctuations and tendon property changes. Indeed (Shultz et al., 2004) found that the relationship

between oestradiol and progesterone levels and knee laxity was stronger when the changes in hormone concentrations are compared with changes in knee laxity occurring approximately 3–4 days later. However, the time shifts were variable between participants and the variance explained by the regression model for each individual participant was substantially greater than that of the group analysis highlighting the large variability in this relationship between subjects. In addition there was also large variability in the degree of response between individuals. As tendon properties were only measured three times during the menstrual cycle in this current study it is possible that the days when tendon stiffness was at its maximum or minimum may have been missed. It is also possible that the days in the cycle when these points occur are different between individuals due to differing time frames and magnitudes of response to hormone fluctuations and differing hormone fluctuations.

11. Conclusion

Menstrual cycle phase and associated variations in female sex hormones, in a muscle–tendon model, have no significant effect on tendon mechanical properties. This has important methodological implications. In a context where studies are often limited to selecting only oral contraceptive-users as participants in order to minimise potential noise related to the anticipated effects of menstrual cycle hormones on physical performance, our findings provide the basis for allowing pooling of female tendon data, regardless of the phase of the menstrual cycle of each individual study participant.

Conflict of interest

The authors are not professionally and/or financially affiliated to any institution that may be perceived as causing a bias in the presentation of their results.

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