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Menstrual cycle variations in oestradiol and progesterone have no impact on in vivo medial gastrocnemius tendon mechanical properties

K.E. Burgess [a,*], S.J. Pearson [a], G.L. Onambélé [b]

[a] Centre for Rehabilitation and Human Performance Research, Directorate of Sport, University of Salford, Salford M66PU, Manchester, UK
[b] Department of Exercise and Sport Science, Manchester Metropolitan University, Alsager, UK

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ABSTRACT

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.

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 (Uldbjerg 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

* Corresponding author.
E-mail address: k.e.burgess@pgr.salford.ac.uk (K.E. Burgess).

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(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 involved 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 pre-menstrual 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 isolation 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