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Original Article

The effect of the oral contraceptive pill on the passive stiffness of the human gastrocnemius muscle *in vivo*

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Abstract

The aim of this investigation was to determine the effect of sustained monophasic oral contraceptive pill (MOCP) use on the *in vivo* passive stiffness of the gastrocnemius medialis (GM) muscle-tendon unit. Twenty four females volunteered for this study (age range 20-25 yrs); twelve participants had been taking the combined MOCP for a minimum of 12 months, and twelve participants, who had never taken the MOCP, formed a control group. Distal displacement of the GM myotendinous junction (MTJ) was measured during passive dorsiflexion at 2 Nm increments to 20 Nm, and at end range of motion using ultrasonography. In addition, GM MTJ displacement was measured at passive torques equivalent to 5, 10 and 15% of plantarflexion maximal voluntary contraction (MVC) torque, and relative to GM length. MOCP users had significantly greater GM MTJ displacement at all passive torques ($P<0.01$), reaching 40% more at 20 Nm; these displacements remained significantly different when MVC and GM length were accounted for ($P<0.01$). Passive muscle stiffness from 0-20 Nm was 31% less in MOCP users compared to non-users ($P<0.01$). In conclusion, based on the *in vivo* assessment of GM MTJ displacement, passive muscle stiffness is less in MOCP using females, compared to non-pill users.

Keywords: Gastrocnemius Medialis, Oral Contraceptive Pill, Passive Stiffness

Introduction

Passive stiffness of the muscle-tendon unit (MTU), often defined by the passive torque-angle relation, provides a gross estimate of the viscoelastic properties of the MTU as a whole, and is often used as a surrogate for the assessment of flexibility[1]. Recently, triceps surae stiffness, assessed using frequency oscillations of the MTU, has been shown to be less in females than males[2]. More specifically, muscle stiffness (assessed through passive stretch) and tendon stiffness (assessed through graded isometric contractions) have both been shown to be lower in females than males[3,4]. These gender differences in stiffness of the muscle and tendon, could be attributed to the reduction in tendon collagen fractional synthesis rates[5], and decreased tendon collagen density[6] associated with elevated oestrogen levels in females compared to males.

Although gender differences in tendon stiffness have been attributed to circulating oestrogen[7] and an associated suppression of tendon collagen synthesis rates[5]; menstrual fluctuations in circulating oestrogen have shown no impact on *in vivo* stiffness of the tendon[8,9]. In contrast passive knee joint laxity, a contributing factor to anterior cruciate ligament injury, demonstrates a menstrual variation in accordance with the elevation in oestrogen during the luteal phase[10,11]. However this does not rule out an accumulating effect of oestrogen fluctuation in normally menstruating woman, when contrast to females who experience sustained suppression of oestrogen through monophasic oral contraceptive pill (MOCP) use.

In women aged 16-49 years, the use of low-dose oral contraceptives (<50 μg ethinyl estradiol) is widespread, approximately 40% of 26 yr old females use some form of hormonal contraceptive[12]. MOCP results in a significant down regulation of endogenous oestrogen, with attenuated plasma oestrogen levels throughout the menstrual cycle, in contrast to this, endogenous oestrogen levels in non-MOCP users are considerably higher[13]. Indeed it is the suppression of endogenous oestrogen that has been attributed to stiffer tendons in pill users compared to non-MOCP users when assessed through graded isometric contrac-

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tions[13]. Yet it remains unclear whether exogenous administration of female sex steroids impacts on the passive stiffness of the muscle and the associated functional characteristics, such as flexibility and passive torque. It is these functional characteristics which have previously been associated with a lower incidence of muscle damage[14] and may reflect the overall predisposition to ligament injury in non-MOCP using females compared to males[15]. Indeed the suppression of oestrogen through MOCP confers some protection from the markers of muscle damage following eccentric contraction[16]. Yet the *in vivo* passive properties of the MTU remain to be elucidated in MOCP users.

During passive dorsiflexion to end range of motion (endROM) the increase in gastrocnemius MTU length is distributed evenly between the muscle and tendon[17]. Tendon compliance is a predominant factor in the capacity of the musculotendinous unit to harness elastic strain energy during stretch-induced work[18], but the measurement of tendon stiffness under active loading may be of little significance to the passive properties of the muscle[19]. In terms of passive muscle lengthening, it has been proposed that an acute increase in passive tendon stiffness could contribute to an apparent decrease in passive muscle stiffness following static stretching[17]. However, at present it is not known to what extent MOCP use may alter passive muscle stiffness directly or whether the previously observed increase in active tendon stiffness from MOCP use[13] may influence the muscle under stretch indirectly. Therefore the aim of the present study is to determine the effect of sustained MOCP use on the *in vivo* passive stiffness of the human gastrocnemius muscle-tendon unit.

Materials and Methods

Ethical approval

Twenty four healthy females volunteered for this study. Twelve participants had been taking the combined MOCP containing synthetic oestrogen (either 20 or 30 μg ethinyl oestradiol) and progesterone (3 mg drospirenone), for a minimum of 12 months (age 20.3±0.8 years, stature 1.65±0.05 m, mass 65.7±1.0 kg). Twelve participants who had never taken the MOCP, and all reported normal menstrual patterns (here defined as a cycle length of 24-35 days, with no incidence of amenorrhea in the preceding 2 years), were allocated to a control group (CTRL, age 19.8±2.1 years, stature 1.63±0.05 m, mass 64.7±8.4 kg). All participants self-reported as "recreationally active" (undertaking less than 1 hour of moderate intensity physical activity per week) and were free from any lower extremity injury. Written informed consent was obtained from all participants. All procedures conformed to the standards set by the latest revision of the Declaration of Helsinki, and were approved by the local Ethics Committee of Manchester Metropolitan University, Cheshire.

Experimental setup

Participants attended the laboratory on two occasions, on the first for maximum isometric voluntary contraction (MVC) familiarisation and anthropometric testing and on the second for assessment of passive stiffness and MVC.

All tests were performed with the participants seated on an isokinetic dynamometer (Cybex Norm, Cybex International. NY, USA) with hip extension set at 65º and the knee secured at 180º for all participants; which has been shown to minimise any influence of the hamstrings group[20]. The left foot was securely fastened to the foot plate of the dynamometer with the lateral mallioli of the ankle joint aligned with the rotational axis of the dynamometer. As with previous passive stiffness measurements limb dominance was ignored. The rationale for ignoring limb dominance was due to the fact that no group difference in limb dominance was observed (1 Left foot dominant participant in the CTRL group and 2 in the MOCP group); furthermore unless undertaking frequent, strenuous physical activity there is no observed difference in tendon morphology between dominant and non-dominant limbs[21]. An electrogoniometer (K100, Biometrics Ltd, UK) was attached at the ankle, and the foot was strapped to minimize heel displacement during dorsiflexion. All reported angle measurements refer to the ankle joint assessed with the goniometer, not the angle of the dynamometer foot plate, where 0° refers to the foot at a right angle to the tibia. All participants completed testing in the same order (range of motion, passive stiffness and then MVC).

The passive range of motion was determined using an approach similar to that adopted by others[17,22,23]. This involved passive isokinetic dorsiflexion at $1°.s^{-1}$ from 30º plantarflexion for all participants to volitional end range of motion (endROM). Volitional endROM was defined as the point when the participant felt mild discomfort (such as during a static stretch) and caused the participant to stop the dynamometer using a safety trigger. Participants were instructed to stay relaxed throughout, upper body strapping was utilized to minimise displacement. Following determination of endROM, electromyography (EMG), passive torque, and measures of myotendinous junction (MTJ) displacement were recorded during passive dorsiflexion at $1°.sec^{-1}$ to end ROM.

Passive torque

Torque was displayed on a computer screen, interfaced with an acquisition system (Acknowledge, Biopac Systems, Santa Barbara, CA, USA) used for analogue-to-digital conversion. The sampling frequency was 2000 Hz. Each torque signal was filtered with a low-pass fourth order Butterworth filter with a 30 Hz cut-off frequency. The validity of the measurement techniques has been described previously[3,17]; in brief, torque was calibrated as linear up to 180 Nm. As the torque experienced with passive loading is much lower than MVC, smaller 7 calibration increments of 0.9 N masses were added to the cybex, providing calibration torques up to 3 Nm. Over this range typical error of estimate was 0.03 Nm with 90% confidence limits of ±0.04 Nm, R^2 was 0.999. Signal to noise ratios were confirmed as 0.15 Nm at a calibration load of 10.8 Nm.

In vivo measurements of muscle and tendon properties

B-Mode ultrasonography (AU5, Esaote Biomedica, Genoa, Italy) was used to determine the displacement of the MTJ of the gastrocnemius medialis (GM) during passive dorsiflexion. The MTJ was identified as described by Maganaris and Paul[24], as the distal join between the deep and superficial aponeuroses of the GM muscle. For clarity (and consistency with others e.g. Magnusson et al[25]) we refer to the tendon as the in series elastic components distal to the GM MTJ, and the elastic components proximal to the GM MTJ as the muscle. Distal displacement of the GM MTJ was visualized and recorded as a continuous sagittal plane, ultrasound video, using a 10 cm, 7.5 MHz linear-array probe. The ultrasound image was time locked with the torque and goniometer outputs. Displacement was measured relative to an acoustically reflective marker (a thin strip of micro-pore tape) secured to the skin proximal to the GM myotendinous junction (the validity of which has been discussed previously[17]). Images were recorded directly to a PC at 30-Hz, and analyzed offline every 2 Nm from 0-20 Nm (20 Nm was the highest passive torque achieved by all participants at endROM). Digital tracking software was used to measure the distal displacement of the MTJ under passive stretching (Dartfish, Friburg, Switzerland). In addition to absolute torques at 2 Nm intervals and to account for any possible difference in PF strength between the participants, distal displacement of the GM MTJ was also measured at passive torques equivalent to 5, 10 and 15% of MVC torque.

As tendon compliance measured under concentric conditions has been shown to be unrelated to passive stiffness[19], the contribution of the tendon to the movement of the MTU under passive loading was estimated. This was achieved using the difference between the measured GM MTJ displacement and the estimated increase in the MTU from the measured change in joint angle throughout the passive stretch, as described previously[17,26]. The total MTU length was determined at the start of the experiment with an inextensible tape laid over the surface of the muscle and tendon and using ultrasound to identify the anatomical landmarks of the GM origin, the insertion at the MTJ and the insertion of the Achilles tendon. The change in MTU length at each joint angle was estimated using a cadaveric regression model[27,28]. The change in MTU length (ΔL) was calculated as follows:

$$\Delta L = -22.185 + 0.30141(90+qA) + 0.00061(90+qA)^2$$

where qA is the ankle angle (°), measured from the neutral position with the foot at a right angle to the tibia, and estimating the change in length based on the change in joint angle from the angle at which 0 torque was measured to endROM. Using this approach the change in MTU length was found to be 0.80 mm.$^{\circ -1}$, over the 20 Nm torque range in the present study, which is similar to the value of 0.83 mm.$^{\circ -1}$ quoted by Herbert et al[26]. The estimated tendon elongation was calculated by subtracting the measured GM MTJ displacement from the estimated MTU elongation. As tendon elongation is essentially the inverse of the GM muscle elongation it is only reported at a passive torque of 20 Nm.

Validity of the static and dynamic ultrasound measurements has been discussed previously[3,17]. Based on the visual tracking technique adopted in the present study, Mahieu et al[29] reported that during gastrocnemius contraction there was a 9% Standard Error of Measurement, with a 6.2 mm proximal displacement of the MTJ, and ICC of 0.96. However, in order to ensure validity of MTJ displacement under smaller passive torques, we have previously assessed the accuracy of the US by recording 1 mm increments[3]. It was reported that over 1 mm increments between 1-10 mm the mean error using the visual tracking US technique was 0.06 mm, with 90% confidence limits (CL) ±0.02 mm, and R^2 of 0.999. Therefore, as with previously adopted visual tracking techniques, the US technique in the present study should be considered sensitive enough to identify the visual tracking of the MTJ during distal displacement under stretch, previously observed at 9-12 mm in the GM[3,17].

It should be noted that although it is possible to allocate contractile forces based on relative physiological cross sectional area under loading[30], it is as yet not possible to determine accurately the total contribution of the GM to the measured passive plantar flexion (PF) torque; therefore with this caveat in mind nominal muscle stiffness is presented as the ratio of passive PF torque (Nm)/distal displacement of the GM MTJ (cm). To account for any difference in GM muscle length and MVC, muscle stiffness is also presented as distal displacement relative to GM muscle length at 0°, and at passive torque values equivalent to 5, 10, and 15% of MVC.

Isometric maximal voluntary contractions (MVC) torque.

Maximal isometric voluntary plantar flexion contractions (MVC) were performed 30 mins after the passive stretch took place. Participants performed a series of three submaximal isokinetic contractions (90°.s^{-1}) and two submaximal isometric contractions at an ankle angle of 0° as a warm up. MVC's were performed at 0° and maintained for 4 seconds (sufficient to reach a plateau) with 2 min of rest in between each contraction to prevent development of fatigue. To maximize performance, visual feedback of the torque signal and verbal encouragement were given to all participants. The highest torque reached during a contraction was recorded, and the highest value of the two contractions was given as the MVC.

Electromyography

In order to quantify the degree of muscle activity during the passive stretch, EMG activity was recorded from the GM throughout passive DF and MVC. Two pre-gelled, unipolar, 10 mm, Ag-AgCl percutaneous electrodes (Medicotest, Denmark) were placed along the mid-line of the muscle having defined the GM muscle boundaries using ultrasonography consistent with Morse et al.[17] Electrodes were placed at two thirds muscle length with 25 mm between the centres, and a reference electrode placed over the lateral epicondyle of the femur. Prior to placement of the electrodes, the skin was shaved to remove hair and the recording sites were rubbed lightly using abrasive gel and cleaned using alcohol swabs to reduce inter-electrode impedance below 5 KΩ. The raw EMG activity was acquired with a sampling frequency of 2000 Hz and processed with a multi-channel

analogue-digital converter (Biopac Systems Inc., USA). The raw EMG signal was filtered with low and high-band pass filters set at 500 Hz and 10 Hz respectively, and amplified with a gain of 2000. EMG activity was calculated as the integral of the root mean square of the raw signal over 0.5 s either side of each torque value. From this value baseline EMG, recorded under static conditions, was subtracted. EMG activity is reported as a percentage of the EMG activity recorded during MVC.

Time-of-day

In order to account for circadian fluctuations in endogenous oestrogen concentration[31], all testing sessions were performed between 12 and 4.00 pm. This ensured some homogeneity for endogenous oestrogen fluctuations, and avoided the considerable fluctuations in serum concentration of ethinyl oestrodial following morning MOCP consumption[32]. However, it should be noted that participants were not tested on a specific day such as the rationale utilised by Bryant et al[13], as evidence produced by Burgess et al[8,9] and Bryant et al[13], showed that fluctuating female hormone levels do not influence tendon strain patterns across the menstrual cycle. This would appear to be in conflict with data showing menstrual fluctuations in knee joint laxity, however although ligament laxity has been observed to show menstrual variations, within those same participants there was observed to be no menstrual variation in the mechanical properties of the ligamentous structures[11,33].

Statistics

Descriptive data (means±SD) for subject characteristics together with passive stiffness and elongation data were calculated for both groups (MOCP and non MOCP). After confirming normality (Shapiro-Wilk test) and equal variance (Levene test), the following statistical analysis were implemented.

For variables with between and within measures (distal displacement, relative displacement, and EMG) significant difference was determined using a mixed measures ANOVA; between participant: MOCP vs CTRL; within participant variables: passive torque (10 levels), or relative torque (3 levels). If a significant interaction was found, the location of group differences was determined with independent t-tests. For variables with only a between group main effect, with no within group comparison, impendent t-tests were performed (e.g. ROM, MVC, static muscle length, stiffness). Differences were considered significant at an alpha level of $P \leq 0.05$. Values are presented in the results and figures as means±SD, with the exception of EMG data which is presented as means±SEM for clarity. All analyses were conducted using Statistical Package for Social Sciences (SPSS, version 19.0.1; Chicago, IL) for Windows.

Results

No significant difference was observed in ankle joint angle at endROM between MOCP and CTRL participants (Table 1). Similarly, PF torque at endROM and during PF MVC was not significantly different between the two groups (Table 1).

At 2 Nm intervals throughout passive DF, distal displacement of the GM MTJ was significantly greater in MOCP than in CTRL at all torque levels from 2 Nm (Main effect was observed for group (MOCP & CTRL), within group (2 Nm passive torque increments); and a significant interaction effect was observed (group x passive torque), $P<0.01$, Figure 1). At 20 Nm of passive DF torque, GM MTJ displacement was 45% greater in MOCP than in CTRL ($P<0.05$). When expressed relative to resting GM length (relative strain), distal MTJ displacement was significantly higher in MOCP users than CTRL (main effect was observed for group, passive torque and group x passive torque interaction, $P<0.01$, Figure 2). At 20 Nm or passive dorsiflexion torque, the relative GM muscle strain was 6.6±1.2 % in MOCP and 4.7±0.8 % in CTRL ($P<0.01$).

	MOCP	CTRL
endROM (°)	12.8±9.9	11.8±7.9
endROM torque (Nm)	38.8±11.8	36.3±10.5
PF MVC (Nm)	144±28	133±17
Resting GM length (cm)	26.4±1.0	25.6±1.6
Distal displacement of GM MTJ at 20 Nm (cm)	1.72±0.31*	1.19±0.22
Muscle stiffness ($Nm.cm^{-1}$)	11.9±2.1*	17.3±3.2
Estimated MTU elongation at 20 Nm (cm)	2.13±0.42	2.14±0.36
Estimated tendon elongation (cm)	0.41±0.28*	0.95±0.35

*Volitional end range of motion (end ROM) during passive dorsiflexion, Plantarflexion maximal voluntary contraction torque (PF MVC), Myotendinous junction (MTJ). Values are presented as means±SD *denotes significant difference from CTRL, P<0.05.*

Table 1. Between group comparisons of the gastrocnemius medialis (GM) muscle-tendon unit (MTU) in monophasic oral contraceptive pill users (MOCP) and non-users (CTRL).

Distal displacement of the GM MTJ, measured at passive torque values equivalent to 5, 10 and 15% of PF MVC, was significantly greater in MOCP than in CTRL group (Main effect was observed for group, passive torque and group x passive torque interaction, $P<0.01$, Figure 3). Nominal GM muscle stiffness from 0-20 Nm was 31 % lower in MOCP than CTRL (Table 1, $P<0.01$). Based on the fact that no difference was observed in passive ROM from 0-20 Nm between MOCP and CTRL, estimated MTU elongation over 20 Nm between MOCP and CTRL was not different (Table 1). Estimated tendon elongation at a passive torque of 20 Nm was significantly less in MOCP than in CTRL ($P<0.01$, Table 1).

EMG activity within the GM remained below 2% of that recorded during MVC throughout passive stretch in both groups. There was no difference in EMG activity relative to that recorded during MVC between MOCP and CTRL (Figure 4).

Discussion

The main finding from the present investigation was that passive muscle stiffness was significantly lower in MOCP users

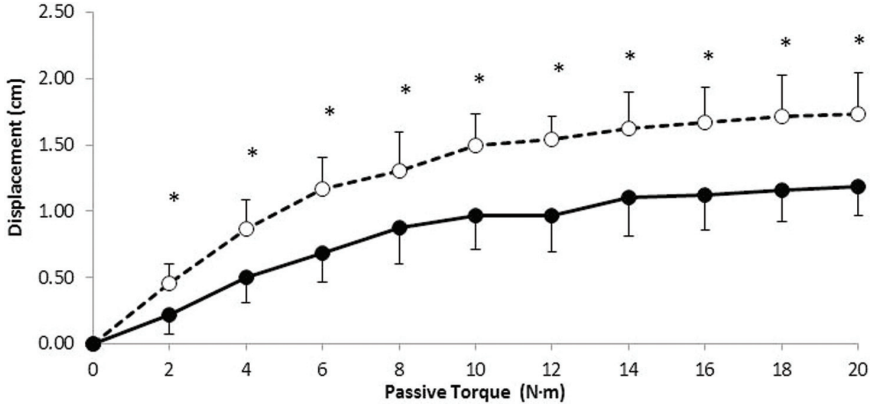

Figure 1. Distal displacement of the Gastrocnemius medialis myotendinous junction (GM MTJ) at 2 Nm intervals during continuous passive stretch. CTRL (filled circles) and MOCP users (open circles). All points were significantly different between groups from 2 Nm ($P<0.05$).

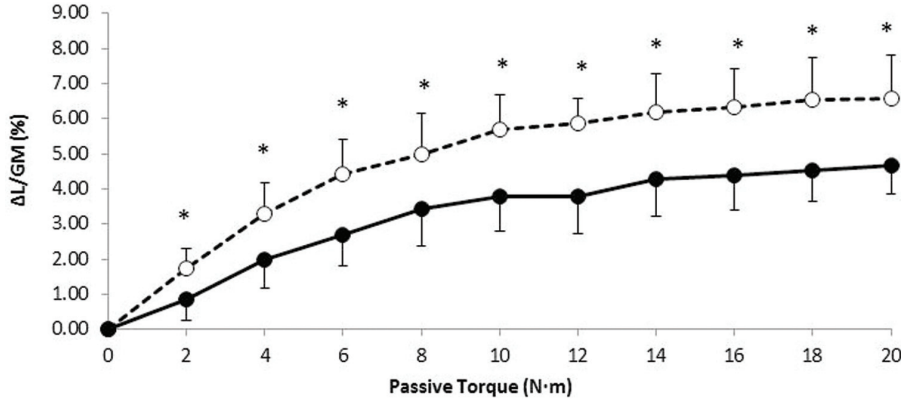

Figure 2. Distal displacement of the Gastrocnemius medialis myotendinous junction (GM MTJ), relative to resting GM length, at 2 Nm intervals during continuous passive stretch. CTRL (filled circles) and MOCP users (open circles). All points were significantly different between groups from 2 Nm ($P<0.05$)

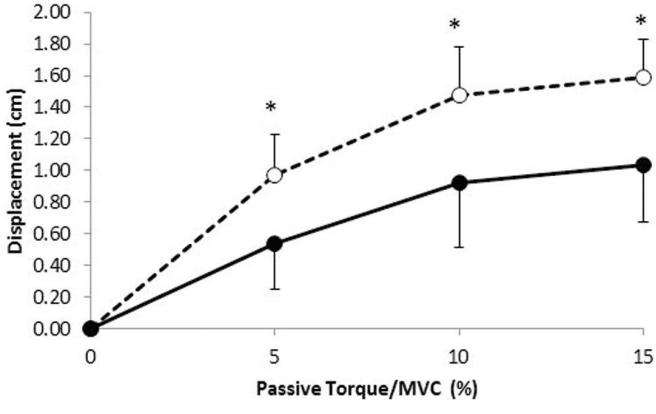

Figure 3. Distal displacement of the Gastrocnemius medialis myotendinous junction (GM MTJ) during passive dorsiflexion, assessed at passive torque relative to PF MVC torque. CTRL (filled circles) and MOCP users (open circles). * denotes significant difference from CTRL group ($P<0.05$).

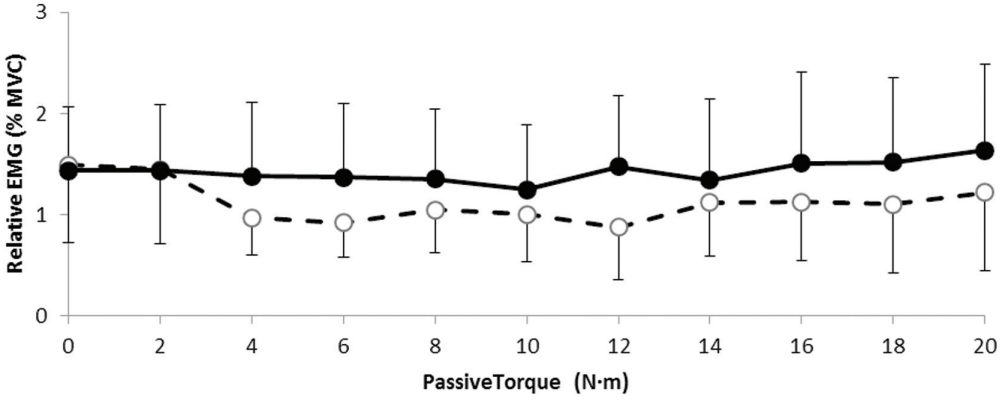

Figure 4. EMG activity of the Gastrocnemius medialis at 2 Nm intervals continuous passive stretch. Values are presented relative to EMG during PF MVC. Error bars denote SEM.

than CTRL participants. However, there was no difference in ankle joint angle or passive torque recorded at endROM.

Based on the *in vivo* determinants of passive stiffness and previous observations on the distal displacement of the GM MTJ[17,23], we propose that there may be four possible mechanisms by which MOCP use could contribute to the finding of lower muscle stiffness observed in the present study; these are: 1) a direct alteration in viscoelastic properties of the muscle, 2) an increase in tendon stiffness contributing to greater displacement of the GM MTJ, 3) a reduction in EMG activity in MOCP and 4) a smaller GM muscle in MOCP.

The suppression of oestrogen through oral contraceptives has been shown to decrease myofibrillar protein fractional synthesis rates (FSR) in humans[34]. It is possible that any structural modification of the muscle through oestrogen suppression could contribute to the observed decrease in passive stiffness in MOCP in the present study. It should be noted however that the impact of MOCP on the muscle is not consistent, with no effect of MOCP on myofibrillar FSR reported by Hansen et al[35]. This discrepancy is due to the fact that the suppression of myofibrillar FSR is only present in females taking the third generation pill as opposed to the second generation pill used by the females in the present study[34]. It is likely therefore, that changes in myofibrillar FSR observed by Hansen et al. (2011), may not contribute to the differences in passive stiffness observed between the MOCP users and non-users in the present study. A possible contributory factor to the lower passive stiffness in the MOCP users could be through suppression of muscle collagen synthesis rates in response to loading[35].

The tendon also demonstrates a change in structure and function when circulating oestrogen levels are altered. In regards to gender differences in tendon properties, oestrogen is known to reduce tendon collagen synthesis rates[36], increase tendon collagen degradation[37], reduce tendon collagen synthesis[5], and ultimately contribute to the greater tendon compliance observed in the Achilles tendon of females compared to males under loading[4]. In contrast, suppression of oestrogen through MOCP use is thought to contribute to an increase in tendon stiffness in MOCP users[13]. It is possible that these reported changes in tendon properties from MOCP use may play a role in the observation of reduced muscle stiffness in the present study.

In accordance with the series elastic model of the MTU (e.g. Hill[38]), and observations on the consequences of increasing tendon stiffness on the displacement of the GM MTJ[17]; any increase in tendon stiffness as a result of MOCP use, is likely to contribute to a reciprocal reduction in muscle stiffness as observed in the present study. Indeed it has previously been reported that MOCP users have a greater Achilles tendon stiffness than non-users, when assessed during PF MVC[13]. Initially this would appear to be in contrast to the present investigation, where we have observed lower passive muscle stiffness in this group. However, as has been demonstrated previously, when the MTU is passively stretched, the increase in length is evenly distributed between the muscle and tendon, furthermore, an acute increase in tendon stiffness was shown to result in greater distal displacement of the GM MTJ[17]. Hence in the present study, it is possible that a stiffer GM tendon from MOCP use[13], could result in a greater displacement of the GM MTJ, and contribute to our finding of reduced muscle stiffness in MOCP.

Based on the estimated contribution of the tendon to length changes in the MTU at 20 Nm of passive torque, it can be proposed that MOCP users in the present investigation have stiffer tendons than the CTRL group. However it should be noted that the present technique of measuring the displacement of the GM MTJ, does not allow us to state the extent to which MOCP use has either a direct influence on the GM muscle, or whether there is greater GM MTJ displacement as a result of the aforementioned increase in tendon stiffness. It can be stated therefore that the passive viscoelastic properties of the GM MTU are altered through MOCP use in females; however, the relative contribution of decreased muscle stiffness or increased tendon stiffness remains to be elucidated.

In addition to the viscoelastic properties of the MTU, pas-

sive muscle stiffness is determined by the degree of muscle activity during stretch, and muscle CSA[23]. Similar to previous data from passive stretch[17], the EMG activity of the GM remained below 2% of the value recorded during MVC, and was not significantly different between groups. Therefore, GM muscle activity during stretch is unlikely to contribute to the observed differences in MOCP and CTRL in the present study. Muscle CSA was not measured in the present investigation; however the contribution of CSA to the observed differences between MOCP and CTRL groups can be approximated based on a surrogate measure of muscle mass such as MVC. Indeed, it has previously been demonstrated that PF MVC is significantly correlated with passive torque during stretch[19]. In the present investigation there was no significant difference in MVC between the groups, and no difference in passive torque at endROM. However, to account for any possible contribution that variability in strength could make to the results, distal displacement was also expressed relative to MVC (as is often reported in measures of tendon compliance[4]). When expressed relative to MVC the distal displacement of the MTJ was ~50% higher in MOCP users compared to CTRL. It is likely therefore that the observed difference in distal displacement between MOCP and CTRL is attributable to difference in viscoelastic properties of the muscle or tendon, with no observed difference in muscle stiffness relative to MVC, GM muscle length, and no difference in EMG between the groups.

An assumption of the present study is that endogenous oestrogen levels of the MOCP participants is suppressed. Although oestrogen levels were not reported within the present study, Bryant et al[13], showed that oestrogen levels in MOCP users compared to non-MOCP users was significantly lower, and remained lower throughout the entire cycle. In contrast, endogenous oestrogen levels in non-MOCP users were considerably higher with average serum levels of 433 pg/mL in non-users compared to 117 pg/mL in MOCP users. Similarly, average serum ethinyl oestradiol concentrations of ~130 pg/mL are reported across a 25 day cycle in MOCP users who consumed an oral contraceptive pill containing 30 μg of ethinyl oestradiol[32]. Therefore, we would argue that it is acceptable to assume that the MOCP participants in the present investigation were in a state of suppressed Oestrogen levels compared to the CTRL participants.

Although suppression of Oestrogen is likely to be the main candidate for changes to the passive properties of the MTU as a result of MOCP use, the role that progesterone plays remains unclear. As discussed by others[35], the potential collagen stimulating effect of progesterone suppression in MOCP users is offset by a direct inhibition of collagen FSR from suppression of oestrogen. With this in mind it should also be considered that circulating levels of progesterone in MOCP users and CTRL participants is reported as being physiologically negligible[35,39]. Whereas oestrogen may play a greater role, due to the fact that oestrogen receptors are known to be more numerous (at least in the ligament), and through modulation, are known to influence ligament laxity to a greater extent than progesterone receptors[40]. However, this remains to be determined from measurements in the muscle or tendon.

In conclusion, the main findings from the present investigation support previous work that has demonstrated a role of oestrogen in altering MTU stiffness, specifically we have demonstrated that use of the MOCP can result in a decrease in passive muscle stiffness, and/or impact on muscle properties as a result of an increase in tendon stiffness. Although currently contentious, any change in MTU stiffness may have an impact on the muscle's response to eccentric contractions, and consequently, the degree of damage in individuals with lower passive joint torque[14]. It remains to be seen whether any previous findings of a prophylactic role of MOCP in reducing symptoms of muscle damage[16], can be attributed to the changes in muscle stiffness observed in the present investigation.

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