Effect of menstrual cycle phase on the concentration of bioavailable 17-β oestradiol and testosterone and muscle strength

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ABSTRACT

To investigate the effect of changes in sex hormone concentration on muscle strength and the bioavailability of 17-β oestradiol (oestradiol) and testosterone, seven eumenorrheic females were tested during two phases of the menstrual cycle. Maximum voluntary isometric strength of the first dorsal interosseus muscle was measured during the early follicular and mid-luteal phases of the menstrual cycle. These phases were chosen for testing as the concentration of total oestradiol is significantly different in these two phases. Total oestradiol has been repeatedly associated with changes in muscle strength in females, whereas the effects of bioavailable oestradiol are unknown. The concentrations of total and bioavailable oestradiol and testosterone were measured in addition to the concentration of total progesterone. Concentrations of total progesterone and oestradiol were significantly different between the early follicular and mid-luteal phases of the menstrual cycle (P < 0.05 and P < 0.001 respectively). The concentration of total testosterone (0.7 ± 0.2 and 0.8 ± 0.1 nmol·l⁻¹ respectively) and the ratio of total oestradiol to progesterone (153.0 ± 251.2 and 108.5 ± 27.8 respectively) did not change significantly between the early follicular and mid-luteal phases. Bioavailable testosterone (102.2 ± 66.3 and 105.0 ± 90.2 pmol·l⁻¹ respectively) and bioavailable oestradiol (90.5 ± 35.5 and 120.0 ± 60.6 pmol·l⁻¹ respectively) did not differ significantly between phases. There were no significant differences in muscle strength during the menstrual cycle (P = 0.1). Mean maximum voluntary isometric force of the first dorsal interosseus muscle did not correlate significantly with the mean concentration of any reproductive hormone measured. These results indicate that cyclical variation in endogenous reproductive hormones does not affect muscle strength.

INTRODUCTION

Research into muscle periodicity as a result of menstrual cycle phase has been ongoing since 1876 when a Harvard student first noted cyclical changes in muscle strength [1]. Moore and Barker [2] extended this work by making daily observations on muscle efficiency in 19 women across 12 self-reported monthly cycles. Since then, the effects of reproductive hormones on muscle function in females have been widely debated. Various indices of muscle strength have been shown to increase, decrease or remain unchanged at different phases of the menstrual cycle [3–8]. These ambiguous results may be due to conflicting definitions of ‘reproductive status’ [3,4] and ‘strength’ [2,9], inaccurate identification of menstrual cycle phase [5,10], different modalities of strength assessment [8,11],
examination of dissimilar muscle groups [6,7] and recruitment of non-homogenous groups of subjects [12]. In addition, large inter- and intra-individual variability in hormone secretion [8] undermines studies using the menstrual cycle model.

Recently, Janse de Jonge et al. [8] addressed some of these problems by measuring the concentration of total oestrogen, progesterone, luteinizing hormone and follicle stimulating hormone and muscle strength, in conjunction with percutaneous electrical stimulation, throughout the menstrual cycle. They found that maximum isometric strength and fatigability of the quadriceps muscles did not change between the menstrual, late follicular and luteal phases of the menstrual cycle, despite significant changes in hormone concentration.

Vermeulen et al. [13] suggested that (in plasma) the bioavailable fraction, rather than the total concentration of testosterone, is a more accurate representation of the clinical situation. Testosterone is bound specifically to sex hormone binding globulin (SHBG) (66 %) and non-specifically to albumin (30 %), such that only 1–3 % circulates freely [14]. As testosterone has a low affinity with, and is easily dissociated from, albumin, both the albumin-bound and free portion can be considered ‘bioavailable’ [15]. Further evidence to support this hypothesis is provided by Van den Beld et al. [16], who found that albumin-bound testosterone has access to target tissues. As both testosterone and 17-β oestradiol (oestradiol) have a hydroxy group bound to position C-17, the same biochemical principles apply to oestradiol.

The bioavailable part of oestradiol and testosterone is not a set proportion of the total concentration. The amount of steroid carried by a particular binding protein depends not only on its affinity for the protein, but also on its concentration. First, testosterone and oestradiol have higher affinities with SHBG than albumin; therefore the concentration of free hormone in equilibrium with the albumin-bound hormone will always be smaller. Secondly, SHBG becomes saturated with testosterone and oestradiol more easily than albumin, such that large quantities of testosterone and oestradiol should result in an increase in the amount of bioavailable hormone. However, the concentration of SHBG in humans is not constant. Oestrogen administration and the thyroid hormones increase SHBG levels, whereas androgen administration and growth hormone decrease the concentration of SHBG. Gower and Nyman [17] found that oestrogen replacement therapy increased SHBG levels (due to the first-pass effect, the liver is exposed to supraphysiological concentrations of oestradiol) and consequently decreased the amount of bioavailable testosterone in postmenopausal women. Therefore, in theory, the hormonal changes that occur during the menstrual cycle [e.g. the concentration of total oestradiol increases significantly between the early follicular (EF) and mid-luteal (ML) phases of the menstrual cycle] have the potential to change the equilibrium between free, albumin- and SHBG-bound oestradiol and testosterone and, consequently, the concentration of bioavailable oestradiol and testosterone. If the concentration of bioavailable oestradiol and testosterone changes significantly during the menstrual cycle, then this may account for the fluctuations in muscle strength observed previously [4,5]. The mechanisms behind any possible effects are unknown as, to date, no mechanistic work has been performed. Phillips et al. [4] proposed that the effects of oestrogen on muscle strength are mediated by the classical steroid receptor for oestrogen, whereas Sarwar et al. [5] suggested that the membrane receptor model probably controls the action of oestrogen. Sarwar et al. [5] also proposed that changes in the activity of myosin ATPase or the re-uptake of calcium by the sarcoplasmic reticulum might be responsible for these effects.

As previous studies have exclusively reported the concentration of total oestradiol [4,8], the objective of the present study was to examine the effects of menstrual cycle phase on the bioavailability of oestradiol and testosterone. In addition, this study was designed to investigate the possible influence of cyclical changes in bioavailable oestradiol and testosterone on maximum voluntary isometric force (MVIF) of the first dorsal interosseus (FDI) muscle under more rigorous test conditions than have been used previously. We hypothesize that, if after all of the aforementioned methodological flaws are overcome, any changes in maximum force production that are observed will coincide with changes in the concentration of bioavailable oestradiol and testosterone. EF and ML phases of the menstrual cycle were chosen for testing, because the concentration of total oestradiol is low and high respectively, at these times and oestradiol is the hormone most implicated in strength regulation [4,5,11,18]. Testosterone was measured in the present study as (i) few investigators have examined the effects of cyclical changes in testosterone concentration on muscle strength in females, and (ii) testosterone secretion is indirectly influenced by oestrogen concentration [19]. The bioavailability of progesterone was not measured as progesterone is also bound to cortisol-binding hormone and, to date, cannot be separated into its bioavailable and bound portions. Consequently, the concentration of total progesterone was measured and reported.

**METHODS**

**Subjects**

Seven healthy female subjects, with mean (± S.D.) characteristics of age, 25 (± 5) years; height, 1.6 (± 0.1) m; and mass, 62.1 (± 2.7) kg, were recruited from the local University. All subjects reported normal
Table 1  Intra- and inter-assay reproducibility for each hormone

Values are expressed as a percentage coefficient of variation and are from four samples. Coefficient of variation was calculated by dividing the S.D. of the differences between the two tests by the mean of the two tests and multiplying by 100. hCG, human chorionic gonadotropin.

<table>
<thead>
<tr>
<th>Reproducibility</th>
<th>Testosterone</th>
<th>Progesterone</th>
<th>Oestradiol</th>
<th>hCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-assay</td>
<td>5.3, 7.6, 2.7, 3.6</td>
<td>5.7, 3.7, 3.7, 3.9</td>
<td>7.5, 4.6, 5.1</td>
<td>6.8, 4.3, 4.6, 4.8</td>
</tr>
<tr>
<td>Inter-assay</td>
<td>11.5, 11.9, 7.8, 5.8</td>
<td>6.2, 3.7, 3.8, 3.1</td>
<td>9.5, 5.1, 3.2, 4.7</td>
<td>8.7, 5.1, 4.3, 4.8</td>
</tr>
</tbody>
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menstrual cycle function, with mean cycle lengths of 29 ± 1 days. Subjects had not been taking oral contraceptives or any other hormonal treatments for at least 6 months prior to testing. Only non-smokers were included in the study [20]. Subjects with any muscular, neurological or skeletal disorders capable of influencing performance of the hand were excluded. Approval for the experimental protocol was obtained from the institutions Human Ethics Committee and conformed to the Declaration of Helsinki. All subjects provided written consent having read and understood the details of the experiment.

Experimental design

All subjects reported to the laboratory in a ‘normal’ fed state, having abstained from alcohol and caffeine consumption and any strenuous physical exercise for 24 h, due to known effects on muscle strength and reproductive hormone concentration [21–24]. A 10 ml venous blood sample was drawn from each subject prior to any physical testing. All testing was undertaken at the same time of day in order to control for circadian variation in muscle strength [25].

Prior to experimentation, subjects were familiarized, on two occasions, with the experimental environment and procedures. Following familiarization, subjects were tested on two occasions, day 2 and 21 of the cycle. Day 2 (EF phase) was the day after the onset of menses and day 21 (ML phase) was 7 days after ovulation had occurred. Ovulation was determined using 1 month of oral temperature measurement (MC 63B; Omron, Vernon Hills, IL, U.S.A.) and a urinary luteinizing hormone kit (Clearplan, Bedford, U.K.). The muscle was warmed due to known effects of pre-exercise muscle temperature on muscle performance [30]. A reading lamp (60 W bulb) was positioned at a standard distance over the muscle throughout the test session in order to maintain skin temperature.

A custom-built finger dynamometer [31] was used to assess MVIF of the FDI muscle. The test re-test ratio limits of agreement for assessment of MVIF of the FDI muscle using this equipment is 1.2. Based on a 20% error, the nomogram of Atkinson et al. [32] indicates that a sample size of seven is adequate to detect at least a 10% change in MVIF of the adductor pollicis muscle in maximum force as a result of changes in reproductive hormone status.

The dominant arm was placed in a prone position on the finger dynamometer. The forearm was secured to the diagonal slope of the platform, at the wrist, mid-forearm and distal portion of the elbow joint, with Velcro straps. The lateral side of the distal head of the proximal phalanx of the index finger was aligned with the force transducer,
which was attached to a strain gauge (Model UL4000; Maywood Instruments Ltd, Tilehurst, Berks., U.K.). The strain gauge was calibrated with known weights prior to testing. The thumb was secured, with a strap around the shaft of the first phalanx, in a fully abducted position. The remaining fingers were covered in bubble-wrap and restrained using a Velcro strap. An adjustable clamp, tightened to the shaft of the second metacarpal, prevented upward movement of the index finger. Hand position was standardized for each test session. MVIF of the FDI muscle was measured while the index finger was fully abducted. Three sub-maximum isometric contractions were carried out prior to maximum force assessment. Following a rest of 1 min, three maximum voluntary isometric contractions were performed, the best of which was taken as definitive. A rest of 1 min separated each contraction.

Percutaneous electrical stimulation was used to superimpose electrical impulses on to the FDI muscle during each contraction. Two self-adhesive surface electrodes (3S; Healthcare, London, U.K.) delivered 1 Hz twitches, at a tolerable current, throughout the test. Individual tolerable currents were established prior to assessment of maximum isometric force. The anode was placed directly proximal to the head of the second metacarpal and the cathode medially to the head of the first metacarpal. Electrical impulses were applied, using a computer-driven Digitimer Stimulator (Model DS7; Digitimer Ltd, Welwyn Garden City, Herts., U.K.), at 150 V with a pulse width of 100 µs duration. Force output was amplified and displayed visually on an Apple Macintosh computer interfaced with a data acquisition system (MP100WS; Biopac Systems, Goleta, CA, U.S.A.). Maximum activation was confirmed when no extra force could be generated by the superimposed twitches.

### Results

#### Total concentrations

Concentrations of progesterone (3.3 ± 1.5 and 36.2 ± 28.3 nmol·l⁻¹ in EF and ML phases respectively; \( P < 0.05 \)) and oestradiol (110.8 ± 61.6 and 464.7 ± 83.5 pmol·l⁻¹ in EF and ML phases respectively; \( P < 0.001 \)) were significantly different between phases. The concentration of testosterone (0.7 ± 0.2 and 0.8 ± 0.1 nmol·l⁻¹ in EF and ML phases respectively) and the ratio of oestradiol to progesterone (153.0 ± 251.2 and 108.5 ± 27.8 in EF and ML phases respectively) did not change between the phases of the menstrual cycle.

#### Bioavailable concentrations

There were no significant differences in the concentration of bioavailable testosterone (102.2 ± 66.3 and 105.0 ± 90.2 pmol·l⁻¹ in EF and ML phases respectively) or oestradiol (90.5 ± 35.5 and 120.0 ± 60.6 pmol·l⁻¹ in EF and ML phases respectively) between the two phases of the menstrual cycle.

#### Muscle strength

There were no significant differences in MVIF of the FDI muscle between the EF and ML phases of the menstrual cycle (\( P = 0.1 \)). Mean strength was 28.2 ± 4.3 N during the EF phase and 30.8 ± 7.4 N during the ML phase. Mean MVIF did not significantly correlate with the mean concentration of any of the reproductive hormones (either bioavailable or total concentration) measured (Table 2).

### Discussion

Investigators are not in agreement on either the quantitative or directional effects of menstrual cycle phase on strength [3,33,34]. In the present study, muscle strength was measured under strict test conditions and did not differ across the menstrual cycle, despite significant
changes in the concentration of total oestradiol and progesterone. In addition, muscle strength was not significantly correlated with any of the reproductive hormones measured. Janse de Jonge et al. [8] also found no influence of menstrual cycle phase on muscle contractile properties using a similar research design, measuring MVIF of the quadriceps muscles and the concentration of reproductive hormones. These results are consistent with earlier work by Higgs and Robertson [10], who failed to detect any significant differences in handgrip strength throughout the cycle.

The present findings do not agree with previous studies, which have implicated oestrogen and progesterone in strength regulation [4,5]. Phillips et al. [4] demonstrated a 10% increase in MVIF of the adductor pollicis muscle during the follicular phase (days 1–14) of the menstrual cycle. This was followed by a similar drop in strength at the onset of ovulation. Although Phillips et al. [4] did not find a significant relationship between strength and oestrogen status, they suggested that changes in muscle strength across the menstrual cycle were related to, or caused by, fluctuations in oestrogen. However, if oestrogen was the hormone responsible for cyclical changes in muscle strength, then the largest strength differences should occur between the ovulatory and follicular phases in which the highest and lowest concentrations of oestrogen are observed respectively. Indeed, Sarwar et al. [5] found that the greatest differences in strength occurred between the ovulatory (high oestrogen levels) and luteal (high oestrogen and progesterone levels) phases. They suggested that progesterone might inhibit oestrogen's inotropic effect or act directly on the muscle to weaken it. However, this hypothesis is contradicted by the observation that, despite low progesterone levels, post-menopausal skeletal muscle is weak for its size [35]. Furthermore Greeves et al. [31] reported that maximum muscle strength and fatigue did not change when progesterone levels were suppressed and the concentration of oestradiol was raised from hypo- to hyper-oestrogenic levels. Based on this evidence, it is unlikely that either oestrogen or progesterone significantly affects muscle function.

To the best of our knowledge, this is the first investigation of the effects of menstrual cycle phase on the bioavailability of oestradiol and testosterone and their effect on muscle strength in females. As stated previously, the bioavailable fraction of a hormone is not a set proportion of the total concentration. The concentration of bioavailable oestradiol and testosterone largely depends on the amount of circulating SHBG, which is affected by the concentration of thyroid, growth, oestrogenic and androgenic hormones and the concentration of total oestradiol and testosterone. In the present study, the total concentration of oestradiol increased significantly between the EF and ML phases of the menstrual cycle. However, the bioavailability of oestradiol did not change significantly throughout the menstrual cycle. This might suggest that cyclical changes in the total concentration of oestradiol are (i) not sufficient to saturate the concentration of circulating SHBG and hence increase the concentration of free and albumin-bound oestradiol (i.e. bioavailable oestradiol) or (ii) sufficient to cause an increase in the concentration of circulating SHBG, resulting in an increase in the amount of unavailable (or bound) oestradiol. Future research may benefit from measuring the concentration of circulating SHBG. No significant differences in testosterone status (either total or bioavailable) were noted between the EF and ML phases. Given that muscle strength and the bioavailable concentration of oestradiol and testosterone remained unchanged, the possibility that muscle strength could indeed be affected by changes in the bioavailability of oestradiol and testosterone cannot be dismissed. A model that manipulates bioavailability of these hormones might be used to examine this question. In particular, a model that significantly changes the concentration of both total and bioavailable testosterone is warranted, so that the independent effects of testosterone on the maximum force-generating capacity of females can be investigated.

Bioavailable oestradiol and testosterone have been shown to change as a function of age in men. Van den Beld et al. [16] measured free, albumin-bound, SHBG-bound and total oestradiol and testosterone levels in elderly men (>70 years). They found that bioavailable testosterone levels decreased with advancing age, whereas the concentration of SHBG-bound testosterone increased. Conversely, total testosterone did not change as a function of age. Total and bioavailable oestradiol decreased and SHBG-bound oestradiol increased with age. In addition, Van den Beld et al. [16] found that bioavailable testosterone, but not bioavailable oestradiol, was positively related to muscle strength in men. The concentration of total, but not bioavailable, testosterone has been shown to increase with age in women [36], but muscle strength was not assessed in this study.

Previous studies that have measured the concentration of free oestradiol and testosterone during the menstrual cycle have yielded conflicting results. Wu et al. [37] found that free oestradiol remained constant throughout the cycle. Conversely, Stahl et al. [38] reported significant increases in free testosterone (mid-cycle) and oestradiol (two peaks), even though SHBG levels did not change. Mathor et al. [39] found, using equilibrium dialysis of undiluted plasma, that the concentration of free testosterone increased from the follicular to the luteal phase of the menstrual cycle. In contrast, Schijf et al. [40] showed that the free androgen index decreased during the luteal phase, due to a significant increase in the concentration of SHBG during this phase.

In the present study, a large variation (as reflected by the S.D. values) in the concentration of reproductive
hormones was noted. In particular, bioavailable testosterone ranged from 169 to 103 and 195 to 15 pmol·l$^{-1}$ during the EF and ML phases respectively. Although all blood samples were taken at the same time of day, ultradian fluctuations in hormone production may have masked any potential inotropic effects of reproductive hormones on muscle strength.

In conclusion, when measured under strict test conditions, menstrual cycle phase had no effect on the bioavailability of oestradiol, testosterone or MVIF of the FDI muscle. Despite a significant increase in the concentration of total oestradiol and progesterone between the EF and ML phases, no cyclical changes in maximum force-generating capacity were noted. Muscle strength was not significantly correlated with any of the hormones measured, suggesting that cyclical changes in reproductive hormone concentration do not affect muscle function. To reduce the inter-individual variability in hormone concentration caused by the menstrual cycle, future work should utilize more stringent models of reproductive functioning to examine the effects of sex hormones on muscle strength. In addition, the effect of significant changes in the concentration of bioavailable oestradiol and testosterone on muscle strength could be explored using other models besides the menstrual cycle, such as the menopause.

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Muscle strength and menstrual cycle phase

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