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Endogenous and Exogenous Estrogens on Biochemical and Performance Indicators of Exercise Induced Muscle Damage in Users and non-Users of Oral Contraceptives

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By

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Endogenous and Exogenous Estrogens on Biochemical and Performance Indicators of Exercise Induced Muscle Damage in Users and Non-Users of Oral Contraceptives

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It has been proposed that endogenous estrogens may protect skeletal muscle integrity and promote the repair and recovery process after acute muscle damage. The purpose of this study was to examine both biochemical and performance indices of exercise-induced muscle damage (EiMD) across menstrual cycle phases and compare oral contraceptive pill users (OCP) and eumenorrheic non-users (EUC). A total of 12 women (5 EUC; 7 OCP) underwent a prolonged eccentric running EiMD protocol and were evaluated for biochemical markers of skeletal muscle damage including creatine kinase (CK), C-reactive protein (hsCRP), and myoglobin (MgB) as well as performance markers including strength, range of motion (ROM), soreness perception, and swelling during early follicular (EF), late follicular (LF), and luteal (LU) phases. Measurements were taken pre- and post-exercise and at 24, 48, and 72 h post exercise. No differences were observed in ROM, swelling, or pain perception in response to EiMD across menstrual cycle phases. The EUC and OCP groups showed similar increases in CK, MgB, and soreness perception in response to EiMD across menstrual cycle phases. Strength recovery differed across menstrual cycle phases and between the EUC and OCP groups (p=0.011), being optimal in phases with higher endogenous estrogen levels (LF and LU). We conclude that endogenous estrogens produced during the natural menstrual cycle may support improved strength recovery, despite immediate increases in muscle cell damage, regardless of oral contraceptive pill use.

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CHAPTER 1: Introduction

It is generally understood that males and females respond differently to exercise. Females are more susceptible to lower limb injuries (Renstrom, 2013; Zazulak, Paterno, Myer, Romani, & Hewett, 2006) particularly ankles, knee, and hips (Engstrom, Johansson, & Tornkvist, 1991; Wong & Hong, 2005). This may be due to hormone interactions with connective tissues and more drastic hip angles (Zazulak et al., 2006), which place greater mechanical stress across the knee joint. However, research regarding muscle recovery and injury prevention in females, remains limited, particularly with regard to how cyclic changes in the hormonal milieu influence muscle recovery in the exercising female. It is well established that estrogen plays an anti-inflammatory role within the body, yet studies have failed to provide clear evidence of estrogens' impact on the muscle recovery and repair process in the active female (St Pierre Schneider, Correia, & Cannon, 1999; Stupka & Tiidus, 2001). Many studies examining the muscle recovery process across different phases of the menstrual cycle have displayed procedural flaws, methodological issues, and inappropriate study designs. Furthermore, oral contraceptive pill use confounds the relationship between estrogen and skeletal muscle recovery and repair (Lee, Petrofsky, & Yim, 2015) and few studies have compared oral contraceptive pill users and non-users in one study.

The menstrual cycle encompasses a fluctuation of endogenous female hormones that occur throughout a timeline of approximately 28 d. It is comprised of three components: early follicular phase (menses), late follicular phase (periovulatory), and luteal phase (post ovulatory). The menses occurs due to a shedding of the uterine lining and is marked by a fall in concentrations of both estrogen and progesterone. The follicular

phase is characterized by low levels of progesterone and a rising concentration of estrogen while the luteal phase is characterized by a high level of progesterone and a moderate concentration of estrogen (Shangold & Mirkin, 1998). Due to constant fluctuations in hormonal concentrations, the exercising female has vastly different responses to exercise compared to her male counterpart (Kerksick, Taylor, Harvey, & Willoughby, 2008). To date, very few studies have investigated muscle damage unique to the menses since it is often considered a part of the follicular phase (Hayashida, Shimura, Sugama, Kanda, & Suzuki, 2015). Yet, this phase is critical to our understanding of the muscle damage and repair process since the menses reflects a highly inflammatory phase involving feedback loops that enhance proinflammatory pathways (Evans & Salamonsen, 2012).

Delayed onset muscle soreness (DOMS) is a product of exercise induced muscle damage (EiMD) and is marked by pain, swelling, loss of function, heat, redness, accumulation of intramuscular proteins, and decrements in physical performance. Biochemical markers of EiMD include creatine kinase (CK) and myoglobin (MgB) leakage into plasma circulation as well as C-reactive protein (CRP) (Franklin et al., 1992; Peake et al., 2005). DOMS begins to present symptoms 24 h post-exercise (Keane, Salicki, Goodall, Thomas, & Howatson, 2015) and is considered the most common sports injury (Cheung, Hume, & Maxwell, 2003), being classified as a type I muscle strain (Gulick, Kimura, Sitler, Paolone, & Kelly, 1996). In addition to causing joint and musculoskeletal pain, DOMS may contribute to decreased muscle function and altered joint mechanics (Rowlands, Eston, & Tilzey, 2001). An increase in EiMD following exercise increases one's risk for musculoskeletal injury due to structural physical disruption and a heightened inflammatory state (Cheung et al., 2003).

There is compelling evidence to support the fact that females fare better against EiMD than their male counterparts (Minahan, Joyce, Bulmer, Cronin, & Sabapathy, 2015; Oosthuyse & Bosch, 2017; Shumate, Brooke, Carroll, & Davis, 1979; Stupka et al., 2000) putting them at lower risk for DOMS. Data suggest that estrogen preserves the integrity of muscle cell membranes (Schwartz et al., 1996) conferring greater protection against EiMD. Yet, it is not known which phase of the menstrual cycle may confer optimal protection from unaccustomed exercise (Dannecker et al., 2012). Oosthuyse and Bosch (2017) found that after exercise there were no differences in CK, a marker of muscle damage, across the menstrual cycle. Yet women reported faster pain recovery in their luteal phase than in their early and late follicular phases. Unfortunately, with a small sample size and limited power using a cross sectional design, results could only be considered preliminary in nature. These findings however, merit special attention since women have lower rates of exercise participation than men and EiMD results in an inflammatory response that may negatively impact a woman's adherence to regular physical exercise (Howatson & van Someren, 2008).

Approximately 37.2% of women under the age of 44 are currently taking oral contraceptive pills (Cooper & Adigun, 2018) and it is unclear whether the exogenous estrogens contained in those pills provide the same anti-inflammatory protection against muscle damage as endogenous estrogens. The beneficial effects of oral contraceptive pill use on EiMD are supported by Carter, Dobridge, and Hackney (2001) who demonstrated attenuated CK levels after exercise and by Rao, Ranganekar, and Saifi (1987), and Thompson, Hyatt, De Souza, and Clarkson (1997) who found lower levels of reported muscle soreness after exercise. In the absence of exercise, these pills have been found to

suppress the beneficial effects of endogenous estrogens contributing to chronic low-grade inflammation thereby predisposing women to greater physical and neuromuscular stress (Cauci, Buligan, Marangone, & Francescato, 2016; Roth, Gajdosik, & Ruby, 2001). Thus, it is not surprising that other investigators have shown oral contraceptive pills to have a deleterious effect on DOMS in exercising women (Roth et al., 2001; Savage & Clarkson, 2002). Presently, it is unclear as to whether or not oral contraceptive pills confer greater protective properties against the muscle damage and repair process.

The purpose of this study was to examine both biochemical and performance indices of exercise-induced muscle damage (EiMD) across menstrual cycle phases and compare oral contraceptive pill users (OCP) and eumenorrheic non-users (EUC).

CHAPTER 2: Methods

Subjects

A total of 18 women between the ages of 18 and 28 volunteered to participate in this study. All subjects were screened with a health history, physical activity, and a menstrual cycle history questionnaire. During screening subjects completed the pre-activity readiness questionnaire (PAR-Q). Exclusion criteria included a) previous lower body musculoskeletal injuries b) irregular menstrual cycles c) long-term contraceptive use other than monophasic oral contraceptive pills d) reliance upon anti-inflamamtory medicines e) body fat percentage over 34% or f) participated in less than three sessions of structured physical activity per week.

A total of 12 women (5 OCP users; 7 EUC) completed baseline testing and were deemed eligible for participation in the study. There were six subjects excluded from the study due to failure to meet baseline criteria. The University of Miami Institutional Review Board approved all testing procedures.

Experimental Design

The study was based on a repeated measures of outcome variables at four timepoints across three phases for two independent groups. Following completion of baseline testing, subjects were placed in the OCP or EUC group according to their current birth control methods. Subjects must have kept their current birth control method stable for at least 6 mo prior to enrolling in the study (Casey et al., 2016; Savage & Clarkson, 2002;

Sim et al., 2015). Subjects completed an EiMD protocol and a three-day follow up period during each of their three menstrual cycle phases. Menstrual cycle phase order was randomly assigned using a computer-generated program.

Determination of Menstrual Cycle Phase

Participants completed a health history and menstrual cycle history questionnaire to determine average length of menstrual cycle, verify that subjects were either eumenorrheic or taking a monophasic third or fourth generation oral contraceptive pill with a 28-d cycle. EUC subjects were asked to track their menstrual cycle for two months, reporting dates of menstruation and ovulation using over-the-counter commercially available ovulation kits detecting changes in luteinizing hormone in the urine (Pregmate, Florida, USA). The event of ovulation divided the menstrual cycle into follicular and luteal phases (Casey et al., 2016). In both EUC and OCP subjects, early follicular phase (EF) testing began 1-2 d after the first day menstrual bleeding and late follicular phase (LF) testing began 2-3 d after cessation of menstrual bleeding. In EUC subjects, luteal phase (LU) testing began 2-4 d after the presence of a positive ovulation test. Since OCP subjects were on constant 21-d hormones, results of LF were duplicated to reflect LU in OCP subjects. The three phases of the menstrual cycle were confirmed with serum concentrations of 17-beta estradiol (E2) and progesterone.

Physical Characteristics

Height was assessed using a wall-mounted stadiometer (Seca, Hamburg, Germany) and body mass index was calculated as $BMI = kg/m^2$. A non-invasive bioelectrical

impedance body composition analyzer, Seca mBCA 115 (Seca, Hamburg, Germany) was used to assess body fat percentage.

Determination of Cardiovascular Fitness

A treadmill running test was performed on a motorized treadmill (Lode, Valiant Sport, Groningen, The Netherlands) during LF. This test was initiated with a running speed between 6.44-8.85 kph/h at a 2% gradient and was increased 2% every 2 minutes until volitional exhaustion. Prior to the treadmill test, each subject was fitted with a sealed oro-nasal mask (Hans Rudolf, 7450 series V2, Kansas City, MO) connected to a two-way nonrebreathing valve (Hans Rudolf 2700 series, Kansas City, MO). The breathing valve was connected to the metabolic systems on the expired sides with a corrugated flexible plastic hose with a 3.2 cm-diameter. Continuous gas exchange measurements were made by using a computerized metabolic system (TrueMax 2400, ParvoMedics, Salt Lake City, UT) and were used to determine maximal oxygen consumption (VO_2 max) (Bassett et al., 2001).

Downhill Treadmill Running

All downhill running sessions and follow-up evaluations were conducted after an 8-h overnight fast with the exception of a standardized meal (Clif Bar and Company, Emeryville, CA) two hours prior to evaluations. All testing and evaluations were performed during the same time of day. Subjects were again fitted with a sealed mask and two-way nonrebreathing valve connected to the metabolic systems. After a 5-min warm up, subjects ran continuously for 30 min at a speed corresponding to 60% of their VO_2 max at a -10%

gradation (Carter et al., 2001). Prior to phase testing, subjects performed a familiarization session to ensure they could adequately handle the exercise load and tolerate the muscle damage. All downhill treadmill sessions were separated by a minimum of 10 d to allow for full recovery.

Determination of EiMD

All EiMD evaluations occurred immediately before and after downhill running exercise, and 24, 48, and 72 h following downhill running exercise.

Measurement of Biochemical Indices

Approximately 10 mL of blood was taken from the antecubital vein pre-exercise and 5 min, 24 h, 48 h, and 72, post exercise to determine plasma levels of CRP, CK, and MgB.

Measurement of Performance Indices

Performance indices were obtained pre-exercise, and 5 min, 24 h, 48 h, and 72, post exercise. Subjects rated the intensity of their overall muscle soreness using a 10 cm visual analog scale (VAS) (Chaffin et al., 2011; Nieman et al., 2005). Swelling was determined by taking leg circumference with a Gulick spring-loaded measuring tape (Country Technology Inc., Gays Mills, WI, Model 67019). Upper leg circumference was taken at the mid-point between the greater trochenterion and the lateral epicondyle (Eston & Reilly, 2009). ROM was assessed about the hip joint using a joint goniometer. Maximal isometric strength was measured using a manual muscle test dynamometer (Lafayette, Model 01165, Lafayette, IN) of the leg extensors (Mentiplay et al., 2015).

Plasma Analysis

Participants abstained from exercise for 24 h and caffeine for 12 h before testing. After an overnight fast, with the exception of a standardized pre-exercise meal, 10 mL of antecubital venous blood was drawn into vacutainer tubes containing silica clot activator, polymer gel, and a silicone-coated interior. Samples were given 20 min to clot before being centrifuged for 15 min at 2400 rpm. Plasma was aliquoted and stored at -80° C until analysis. Biomarker quantification followed guidelines established for quality control and proficiency testing; all analyses conformed to the multirule criterion for quality control (Warren, Lowe, & Armstrong, 1999). Plasma levels of hsCRP (using the high-sensitivity assay), CK, MgB, E2, and progesterone were measured on an automated analyzer using immunoelectrochemiluminescence.

Dietary Recall

During the pre-exercise and the 24-h follow-up testing, subjects completed a 24-h dietary recall to ensure consistent eating behaviors throughout the study. Subjects refrained from food and beverages other than water for two hours following the downhill treadmill run and were given a list of foods to avoid during the first 24-h following the downhill run. These foods included plant-based products known to contain high levels of anti-inflammatory or soy properties. Subjects also avoided taking anti-inflammatory drugs throughout testing days.

Statistical Analysis

Results were analyzed using SPSS 24.0 software (IBM, Armonk, NY), and are presented as means and standard error of the mean (SEM), unless otherwise indicated. A two (group: EUC vs OCP) by three (phase: EF, LF, LU) by four (timepoint) mixed-design

ANOVA were conducted to test the significance of the within-subjects factor and the between subject's factor on dependent variables including plasma contents, swelling, pain, flexibility, and strength. Due to the exploratory nature of this study, LSD post hoc analyses were used to determine the source where any significant interactions were detected. Significance for all analyses were set a priori at alpha <0.05.

Chapter 3: Results

Subject Characteristics

There were no significant differences between EUC and OCP groups for any physical variables at baseline (Table 1).

In EUC, E2 concentrations were significantly higher during LU compared to EF (141.38, SE=46.46, t=3.04 p=0.038) with no significant changes in progesterone found across any menstrual cycle phase (Table 2). In EUC participants, E2 was numerically but not significantly higher during LF (110.3 ± 47.9) compared to EF (25.9 ± 47.9). In this group, progesterone levels were low during both follicular phases (EF=0.3 ± 0.1, LF=0.3 ± 0.0) and increased during the luteal phase (4.8 ± 2.9). In the OCP group, there were no significant differences across menstrual cycle phase in E2 or progesterone. Numerically, the OCP group had higher E2 levels during EF (12.0 ± 2.5) compared to LF/LU (7.6 ± 2.5) with E2 being lower than the EUC group across menstrual cycle phase. In the OCP group, progesterone levels remained low during all phases and were not significantly different (EF= 0.4 ± 0.4, LF/LU=0.3 ± 0.0). During LU, the E2 levels were significantly higher in EUC compared to the OCP group (MD= 159.72, SE=41.74 t=3.83, p=0.003).

Plasma Inflammation and Muscle Protein

In the EUC group there were no significant differences in hsCRP during any of the three phases compared to baseline (Table 3). In the OCP group, CRP levels were elevated immediately post-exercise compared to baseline during LF/LU (MD=0.22, SE=0.08, p=0.035). An overall analysis of variance for repeated measures showed no significant differences between hsCRP concentrations at each recovery timepoint across phases

between the OCP and EUC group. Highlighted in Figure 1 are the differences in baseline hsCRP levels among the different phases in EUC and OCP groups. In the EUC group, hsCRP levels were significantly higher during EF compared to LF (MD=2.46, SE=1.12, p=0.05) with a trend toward higher levels during EF compared to LU (MD=2.40, SE=1.12, p=0.057). In OCP users, hsCRP levels were numerically lower during EF compared to LF/LU, however, this did not reach the level of statistical significance.

In the EUC group, CK concentrations increased immediately after exercise in all phases (EF: MD=25.67, SE=4.52, p=0.001, LF: MD=21.33, SE=7.19, p=0.025, LU: MD=33.0, SE=3.50, p=0.004). The CK levels remained elevated during LF at 24 hours (MD=134.67, SE=39.48, p=0.014). In EUC participants, the CK levels did not remain elevated at any additional time points during LU. In OCP users, CK concentrations increased immediately after exercise during both EF (MD=24.40, SE=3.50, p<0.001) and LF/LU (MD=26.08, SE=5.57, p=0.003). An overall analysis of variance for repeated measures showed no significant differences in CK concentrations at each recovery timepoint across phase between EUC and OCP users.

In the EUC group, MgB increased significantly immediately after exercise during LF (MD=20.03, SE=3.50, , p=0.001) and LU (MD=29.40, SE=2.81, p<0.001). In OCP users, MgB increased immediately after exercise in both EF (MD=22.48, SE=8.74, p=0.042) and LF/LU (MD=20.81, SE=2.71, p<0.001). An overall analysis of variance for repeated measures showed no significant differences in MgB concentrations at each recovery timepoint across phase between EUC and OCP users.

ROM, Soreness Perception, Strength, and Edema

There were no significant changes in ROM across menstrual cycle phase in either EUC or OCP groups (Table 4). An overall analysis of variance for repeated measures showed no significant differences in ROM at each recovery timepoint across phase between EUC and OCP users.

For both EUC and OCP groups, soreness perception increased similarly across menstrual cycle phase. In all phases and in both EUC and OCP groups, soreness ratings were elevated during the 24- and 48-h evaluation EUC: (EF 24: MD=4.68, SE=0.96, p=0.001; EF 48: MD=4.06, SE=0.96, p=0.001; LF 24 MD=3.67, SE=0.89, p=0.002; LF 48 MD=3.77, SE=0.79, p=0.001; LU 24 MD=4.22, SE=0.87, p=0.001; LU 48 MD=4.00, SE=0.91, p=0.004). In this group, soreness perception was also significantly elevated at 72 hours in EF (MD=2.16, SE=0.73, p=0.039) and immediately post-exercise during LF (MD=2.02, SE=0.70, p=0.016). In the OCP group, soreness ratings were similarly elevated: (EF 24: MD=3.87, SE=0.81, p=0.001; EF 48: MD=3.76, SE=0.72, p<0.001; LF/LU 24 MD=3.22, SE=0.75, p=0.002; LF/LU 48 MD=3.09, SE=0.60, p=0.001). An overall analysis of variance for repeated measures showed no significant differences in soreness perception at each recovery timepoint across phase between EUC and OCP users.

In the EUC group strength significantly declined immediately post exercise during EF (MD=-9.04, SE= 3.72, p=0.035). In this group, strength was significantly higher than baseline during LU at 72 h (MD=16.02, SE= 7.17, p=0.05). In OCP users, strength significantly declined immediately post-exercise during EF (MD=-9.83, SE=3.14, p=0.011). An overall analysis of variance for repeated measures showed a significant

difference in recovery of strength between EUC and OCP groups ($F(6,60)=3.05$, $p=0.011$, $n_p^2=0.234$). The follow-up post hoc comparison yielded significant differences between EUC and OCP groups at 72 hours during LU, in which the EUC group experienced greater strength recovery than the OCP group (MD=22.69, SE=9.39, $p=0.036$). Furthermore, Cohen's effect size value ($d=1.55$) suggested high practical significance supporting the aforementioned post-hoc comparison. At 48 h, the EUC group showed a trend toward greater strength recovery than the OCP group during LF (MD=23.317, SE=11.36, p=0.067). Again, Cohen's effect size value ($d=1.12$) suggested high practical significance supporting this trend. In contrast, a trend was shown in which the OCP group experienced greater strength recovery than the EUC group at 48 hours during EF (MD=-12.89, SE=6.07 p=0.060) with Cohen's effect size value ($d=1.36$) suggesting high practical significance. Figure 2 illustrates changes in strength recovery between EUC and OCP groups across menstrual cycle phase.

In both EUC and OCP groups, there were no significant increases in swelling of the upper leg. An overall analysis of variance for repeated measures showed no significant differences in swelling at each recovery timepoint across phase between EUC and OCP users.

CHAPTER 4. Discussion

The current study found that inflammatory responses and muscle protein leakage were not different between EUC and OCP users during any phase of the menstrual cycle after EiMD. Furthermore, muscle soreness, ROM, and muscle swelling in response to EiMD were not different across menstrual cycles nor different between EUC and OCP users. However, there was a significant difference between EUC and OCP users in strength recovery after EiMD, favoring menstrual cycle phases with higher estrogen levels (LF and LU).

The hormone concentrations of E2 and progesterone confirmed variations in the hormonal milieu typically observed during the menstrual cycle (Carr & Wilson, 1987). In EUC participants, E2 was lowest during EF followed by an approximate 4-fold increase during LF, and a 6-fold increase during LU. As expected, E2 levels were consistently lower in the OCP group, demonstrating a 2-fold reduction during EF, a 14-fold reduction during LF, and a 22-fold reduction during LU. However E2 was 58% higher during the pill withdrawal phase (EF) versus the hormone supplementation phase (LF/LU). During the withdrawal phase, a woman's endogenous production of estrogen rises since exogenous estrogen supplementation has been withdrawn. This study supports the fact that consumption of exogenous estrogens using oral contraceptive pills suppresses endogenous production of estrogen (Roth et al., 2001; Thompson et al., 1997). In the EUC group, progesterone levels were low during the follicular phases and, as expected, increased after ovulation by 18-fold during LU. In OCP users, progesterone levels remained low and unchanged across menstrual cycle phase, which can be attributed to the pharmacological influence of monophasic oral contraceptive pills.

In the present study, prolonged eccentric running at 60% of aerobic capacity effectively induced EiMD, as evidenced by increases in CK and MgB during all phases, independent of OCP use. This was reinforced by the significant increase in soreness perception across menstrual cycle phase, supporting the occurrence of DOMS.

While strength recovery across the menstrual cycle and with oral contraceptive pill use remain highly controversial, the present study showed that strength recovery was greater during menstrual cycle phases with higher E2. This was evidenced by a rapid return of strength during LU in the EUC group while OCP strength values declined steadily. These are novel findings that support the positive relationship between strength recovery and E2, since E2 is significantly greater during LU in EUC compared to the OCP group. This relationship was further supported with a large effect size (d=1.12) at 48 h during LF. The EUC group recovered 10% beyond baseline strength while the OCP group only recovered 94% of baseline strength at this time. This indicates improved strength recovery during LF in the EUC compared to the OCP group, which coincides with the higher endogenous estrogens produced during this phase as well. Declines in strength are a hallmark feature of muscle damage. Previous research suggests that estrogen serves to stabilize muscle cell membranes and prevent muscle damage (Enns & Tiidus, 2010). In the present study, the preservation of cell membrane integrity may present itself through improved strength recovery in response to EiMD.

It should be noted that during EF in OCP users, E2 levels are highest, at 58% above LF/LU while E2 is lowest during this same phase in EUC users. These dynamics supported greater strength recovery in the OCP group during EF (d=1.36). While both the EUC and

OCP groups evidenced significant strength losses immediately post-exercise during EF, strength recovered 4% beyond baseline strength at 48 h in OCP, while EUC strength remained 10% below baseline at this time. This signifies that higher E2 levels may be associated with greater strength recovery, independent of oral contraceptive pill use. Our findings are in contrast to previous research, demonstrating that return of strength is impaired in OCP users compared to EUC during EF (days 2-6) (Minahan et al., 2015; Savage & Clarkson, 2002). However, these studies did not include prolonged eccentric aerobic exercise, which is considered the gold standard for inducing EiMD. Although this relationship failed to reach statistical significance (p=0.06), the effect size is large, suggesting important practical implications for the contribution of endogenous estrogens to recovery of strength post exercise. Our results suggest that estrogen may not necessarily serve to protect against immediate EiMD, but may play a role in promoting muscle recovery. We know of no other studies examining this relationship outside of the EF phase.

It is important to look at performance measures, such as strength, in concert with biochemical markers of muscle damage and inflammation. CRP is a sensitive, nonspecific marker of systemic inflammation (Pepys & Baltz, 1983) and has been implicated in the etiology of coronary artery disease (CAD) (Haidari, Javadi, Sadeghi, Hajilooi, & Ghanbili, 2001; Ridker, 2003), viral respiratory illness (Melbye, Hvidsten, Holm, Nordbo, & Brox, 2004) and acute musculoskeletal injury including EiMD (Taylor et al., 1987). It has also been proposed that women taking oral contraceptive pills have higher resting levels of hsCRP (Doring, Frohlich, Lowel, & Koenig, 2004). Since increases in CRP are associated with decreased endogenous estrogens (Wander, Brindle, & O'Connor, 2008), this may explain why the OCP group evidenced higher hsCRP levels during the hormone

supplementation phase (higher exogenous E2; lower endogenous E2) but not during the withdrawal phase (higher endogenous E2; no exogenous E2). In the OCP group, there was a 58% rise in E2 during the withdrawal phase concomitant with a 74% reduction in hsCRP levels during this phase. In the present study, the EUC group possessed temporarily elevated CRP levels during EF consistent with increased CAD risk, supporting claims that menses is an inherently inflammatory process. Once again, this supports the inverse relationship between hsCRP levels and endogenous estrogen levels in both eumenorrheic and oral contraceptive pills users. Since findings were not significant, further investigation using a larger sample size should be conducted to further examine this relationship.

Myoglobin, a muscle cell protein that appears in the blood in response to muscle damage (Brancaccio, Lippi, & Maffulli, 2010), increased similarly across all phases of the menstrual cycle regardless of OCP use. As supported by Peake et al. (2005), MgB is largely cleared from the blood within 24 h of EiMD. Creatine kinase is not cleared as quickly as myoglobin. It leaks into the plasma when muscle cells are damaged or disturbed, leading to elevated levels of CK in circulation. Both MgB and CK increased similarly across menstrual cycle phase in both EUC and OCP groups. Our data suggest that the immediate increase of inflammatory markers following an EiMD protocol occurs similarly in both EUC and OCP users across menstrual cycle phases.

Other performance measures that affect return to training were examined along with functional measures of strength and plasma indices of skeletal muscle damage. Decreased ROM is another adverse side effect of DOMS that may delay return to exercise. Increased swelling reflects an acute inflammatory response noted by increases in calcium

infiltration into the damaged muscle causing small, involuntary contractions that limit ROM (Kano, Sato, Kobayashi, & Ishiguro, 2012; McBride, Kraemer, Triplett-McBride, & Sebastianelli, 1998). Despite increases in indices of muscle damage (MgB and CK) in both groups and differences in functional strength between groups, there were no changes in ROM or swelling across menstrual cycle phases and between EUC and OCP groups.

Pain perception across the menstrual cycle is a highly debated topic. Riley, Robinson, Wise, and Price (1999) performed a meta-analysis and found that muscle pain tolerance was highest in the follicular phase, while others have found decreased pain tolerance during this time (de Tommaso, 2011). Oosthuyse and Bosch (2017) reported prolonged VAS scores in women in both the EF and LF but not in LU after EiMD, while Thompson et al. (1997) found that OCP users have lower soreness compared to non-users. In the present study, there were no changes in soreness perception after EiMD across the menstrual cycle or with OCP use. Furthermore, a correlation matrix performed on our data revealed no significant correlations between peak VAS scores and either baseline E2 or progesterone levels.

Limitations

Our sample size was powered to perform within-between interactions, not with-in group changes across the menstrual cycle. Furthermore, E2, which is a key player in the skeletal muscle damage process, was only measured at baseline and not during all recovery timepoints. In the future, it would be beneficial to measure E2 during all timepoints, in order to detect subtle changes within phase and further elucidate E2's role in the muscle damage and recovery process. The measurement of gonadotropic hormones (luteinizing and follicle stimulating hormones) would also add to our knowledge of the muscle damage and recovery process in women. The sensitivity of the MgB assays were limited only detecting this protein at a minimum of 21 ng/mL or greater. Therefore concentrations below this threshold would have been missed and may have influenced our findings of EiMD. Since there were eight timepoints where blood could not be withdrawn from subjects, multiple imputation was performed to account for missing values. However, these missing timepoints did not include any baseline measures.

Conclusion

To our knowledge, this was the first study to investigate biochemical and performance indices of EiMD across three phases of the menstrual cycle and two phases of oral contraceptives in one study. Furthermore, we used a graded exercise test to maximum and gold-standard procedures known to elicit maximum EiMD (prolonged eccentric aerobic exercise) at individualized intensities of 60% VO_2max. Our findings showed that endogenous estrogens produced during the natural menstrual cycle may support improved strength recovery following EiMD, despite immediate increases in muscle cell damage, and that this occurs independent of oral contraceptive pill use.

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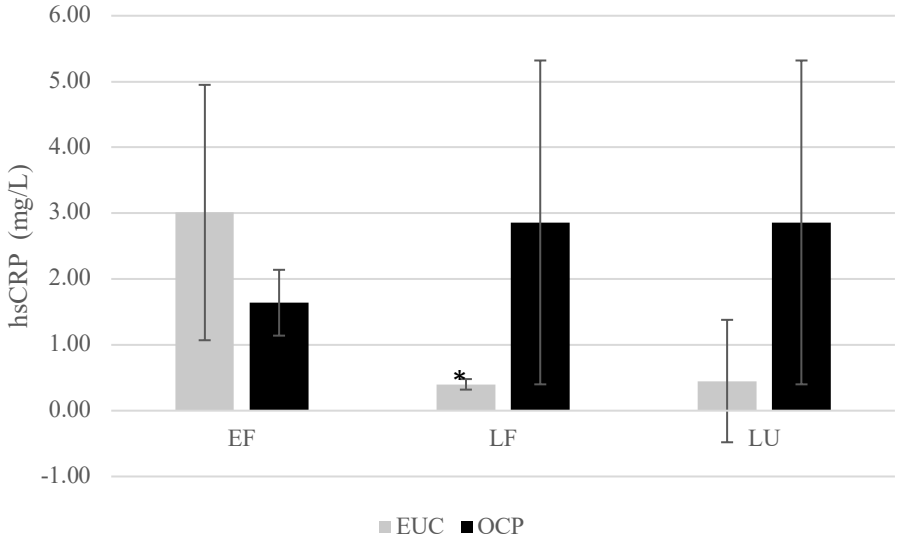
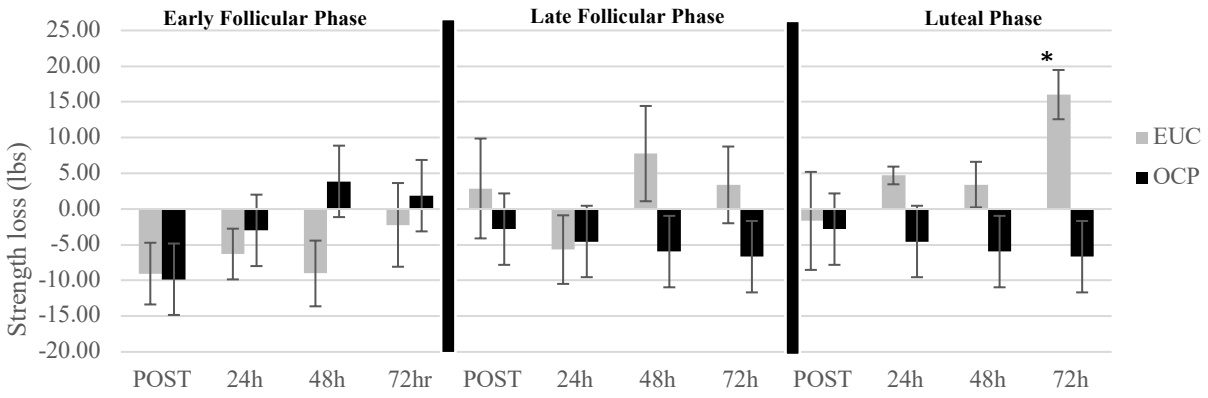
FIGURES

Figure 1. Baseline hsCRP levels between EUC and OCP groups across menstrual cycle phase



Baseline high-sensitivity c-reactive protein (hsCRP) levels across menstrual cycle in eumenorrheic (EUC) and oral contraceptive users (OCP).
EF=early follicular; LF=late follicular; LU=luteal phase.
*denotes significantly different than EF at $p \leq 0.05$

Figure 2. Changes in strength recovery from baseline between EUC and OCP across menstrual cycle phase



Strength recovery changes from baseline across menstrual cycle in eumenorrheic (EUC) and oral contraceptive users (OCP).
EF=early follicular; LF=late follicular; LU=luteal phase.
*denotes significantly different than the OCP group

TABLES

Table 1. Physical characteristics in EUC and OCP groups at baseline

	EUC (n=5)	OCP (n=7)	*p*
Age (y)	23.4 ± 1.5	21.7 ± 1.4	0.26
Height (cm)	166.4 ± 4.1	163.3 ± 1.3	0.43
Weight (kg)	58.1 ± 2.7	61.2 ± 2.9	0.47
BMI	23.0 ± 0.7	22.9 ± 0.8	0.13
Percent Body Fat (%)	25.2 ± 1.5	25.7 ± 0.5	0.84
Maximal oxygen uptake (mL/kg/min)	44.6 ± 1.2	40.4 ± 2.1	0.15

Subject physical characteristics in eumenorrheic (EUC) and oral contraceptive users (OCP). Data presented as Means ± SE.

Table 2. Hormone concentrations in EUC and OCP groups across menstrual cycle phase

	17-β estradiol		Progesterone	
	EUC	OCP	EUC	OCP
EF	25.9 ± 9.8	12.0 ± 2.5	0.3 ± 0.1	0.4 ± 0.4
LF	110.3 ± 65.2	7.6 ± 2.5	0.3 ± 0.1	0.3 ± 0.1
LU	167.3 ± 50.4*	7.6 ± 2.5†	4.8 ± 2.3	0.3 ± 0.1

17-β estradiol and progesterone concentrations in the early follicular (EF), late follicular (LF), and luteal (LU) in eumenorrheic (EUC) and oral contraceptive users (OCP). Data presented as Means ± SE. *denotes significantly different than EF within group, $p \leq 0.05$. †denotes significantly different than EUC, $p \leq 0.05$.

Table 3. Plasma Concentrations in EUC and OCP groups before and after EiMD across menstrual cycle phase

	EUC	OCP
High-Sensitivity C-Reactive Protein (mg/L)		
EF		
Pre	3.0 ± 4.3	1.6 ± 0.2
Post	2.9 ± 1.9	1.8 ± 0.2
24hrs	2.5 ± 1.5	1.9 ± 0.3
48hrs	2.2 ± 1.4	1.4 ± 0.1
72hrs	1.9 ± 1.0	1.3 ± 0.1
LF		
Pre	0.4 ± 0.1	2.9 ± 0.9
Post	0.4 ± 0.1	3.1 ± 1.0*
24hrs	0.5 ± 0.1	3.1 ± 0.9
48hrs	0.4 ±0.1	3.5 ± 1.3
72hrs	0.4 ±0.1	5.6 ± 2.6
LU		
Pre	0.5 ± 0.1	2.9 ± 0.9
Post	0.5 ± 0.2	3.1 ± 1.0*
24hrs	0.6 ± 0.1	3.1 ± 0.9
48hrs	0.5 ± 0.1	3.5 ± 1.3
72hrs	0.5 ± 0.1	5.6 ± 2.6
Creatine Kinase (U/L)		
EF		
Pre	121.4 ± 22.3	102.9 ± 20.6
Post	144.2 ± 24.5**	135.8 ± 25.2**
24hrs	146.1 ± 18.4	139.2 ± 21.3
48hrs	131.4 ± 15.1	143.7 ± 48.6

72hrs	120.5 ± 11.6	139.4 ± 41.3
LF		
Pre	119.0 ± 20.5	151.1 ± 38.3
Post	150.0 ± 20.47*	185.0 ± 45.2**
24hrs	236.0 ± 50.1*	191.7 ± 28.0
48hrs	183.0 ± 19.3	97.5 ± 7.8
72hrs	130.6 ± 14.6	108.6 ± 26.2
LU		
Pre	120.8 ± 20.1	151.1 ± 38.3
Post	146.6 ± 21.9**	185.0 ± 45.2**
24hrs	223.5 ±47.3	191.7 ± 28.0
48hrs	179.3 ± 46.4	97.5 ± 7.8
72hrs	188.0 ± 51.2	108.6 ± 26.8
Myoglobin (ng/mL)		
EF		
Pre	21.1 ± 0.1	23.8 ± 1.8
Post	40.1 ± 5.3	46.1 ± 9.4*
24hrs	23.4 ± 1.6	21.1 ± 0.1
48hrs	21.4 ± 0.8	21.9 ±0.9
72hrs	21.3 ± 0.3	27.2 ± 5.0
LF		
Pre	23.8 ± 2.0	22.3 ± 1.3
Post	54.4 ±10.8**	43.7 ± 2.2**
24hrs	31.9 ± 4.0	21.6 ± 0.4
48hrs	28.3 ± 5.9	21.8 ± 0.8
72hrs	22.8 ± 1.4	36.6 ± 11.9
LU		
Pre	21.5 ± 0.5	22.3 ± 1.3
Post	45.5 ± 5.5**	43.7 ± 2.2**

24hrs	21.9 ± 0.9	21.6 ± 0.4
48hrs	25.1 ± 4.1	21.8 ± 0.8
72hrs	37.8 ± 9.9	36.6 ± 11.9

Raw plasma data across menstrual cycle at baseline and recovery time points in eumenorrheic (EUC) and oral contraceptive users (OCP).
EF=early follicular; LF=late follicular; LU=luteal phase.
*denotes value significantly different than baseline at $p \leq 0.05$
** denotes value significantly different than baseline at $p \leq 0.01$
†denotes significantly different than EUC.
Data are presented at Means ± SEM

Table 4. Performance measures before and after EiMD across the menstrual cycle

	EUC	OCP
ROM Hip Joint (deg)		
EF		
Pre	105.0 ± 6.1	114.0 ± 5.1
Post	110.0 ± 4.5	116.0 ± 6.2
24hrs	104.4 ± 5.4	111.3 ± 6.3
48hrs	105.9 ± 5.4	116.1 ± 4.3
72hrs	110.8 ± 6.7	117.4 ± 6.1
LF		
Pre	104.6 ± 2.8	116.2 ±6.0
Post	109.8 ± 4.8	114.0 ±7.1
24hrs	110.6 ± 7.4	115.6 ±6.0
48hrs	109.6 ± 8.5	111.7 ±6.3
72hrs	112.8 ± 7.1	113.7 ±6.3
LU		
Pre	117.4 ± 4.7	116.2 ± 6.0
Post	108.4 ± 5.5	114.0 ± 7.1
24hrs	109.2 ± 5.7	115.6 ± 6.0
48hrs	112.8 ± 4.4	111.7 ± 6.3
72hrs	115.6 ± 7.2	113.7 ± 6.3
Soreness Perception (mm)		
EF		
Pre	0.4 ± 0.2	0.5 ± 0.3
Post	2.5 ± 1.2	2.0 ± 0.8
24hrs	5.1 ± 1.2**	4.4 ± 0.7**
48hrs	4.5 ± 1.2**	4.3 ± 0.5**
72hrs	2.2 ± 1.0*	1.5 ± 0.4
LF		

Pre	0.7 ±0.2	0.7 ± 0.4
Post	2.7 ± 0.9*	1.7 ±0.5
24hrs	4.3 ± 0.6**	3.9 ± 1.0**
48hrs	4.4 ± 0.8*	3.8 ±0.6**
72hrs	1.2 ± 0.3	1.2 ± 0.3
LU		
Pre	0.6 ± 0.2	0.7 ± 0.4
Post	1.6 ± 0.3	1.7 ± 0.5
24hrs	4.8 ± 0.7**	3.9 ± 1.0*
48hrs	4.0 ± 1.1**	3.8 ± 0.6*
72hrs	2.0 ± 1.1	1.2 ± 0.3
Strength Knee Extensors (lbs)		
EF		
Pre	92.5 ± 7.0	93.2 ± 5.3
Post	83.5 ± 5.6*	83.4 ± 4.1**
24hrs	86.2 ± 8.9	90.2 ± 4.6
48hrs	83.5 ± 6.4	97.1 ± 5.5
72hrs	90.3 ± 6.7	95.1 ± 6.0
LF		
Pre	77.3 ± 5.5	99.6 ± 6.8
Post	80.4 ± 5.3	96.8 ± 7.7
24hrs	71.6 ± 4.4	95.1 ± 9.7
48hrs	85.0 ± 3.2	93.6 ± 5.8
72hrs	80.6 ± 1.7	92.9 ± 7.5
LU		
Pre	74.3 ±4.2	99.6 ± 6.8
Post	72.7 ± 6.9	96.8 ± 7.7
24hrs	79.0 ± 3.4	95.1 ± 9.7
48hrs	77.7 ± 5.1	93.6 ± 5.8

72hrs	90.3 ± 3.4*	92.9 ± 7.5†
Swelling of the Upper Leg (cm)		
EF		
Pre	49.0 ± 1.8	50.8 ± 1.2
Post	49.0 ± 1.9	50.8 ± 1.2
24hrs	49.5 ± 1.9	51.1± 1.3
48hrs	49.6 ± 1.8	51.0 ± 1.3
72hrs	49.4 ± 1.7	50.7 ± 1.2
LF		
Pre	49.2 ± 1.9	51.0 ± 1.1
Post	49.2 ± 1.9	51.4 ± 1.2
24hrs	48.9 ± 1.7	51.4 ± 1.1
48hrs	49.2 ± 1.8	51.0 ± 1.3
72hrs	49.0 ± 2.0	50.7 ± 1.2
LU		
Pre	48.7 ± 1.4	51.0 ± 1.1
Post	48.7 ± 1.5	51.4 ± 1.2
24hrs	49.2 ± 1.5	51.4 ± 1.1
48hrs	49.5 ± 1.4	51.0 ± 1.3
72hrs	48.9 ± 1.6	50.7 ± 1.2

Raw performance data across menstrual cycle at baseline and recovery time points in eumenorrheic (EUC) and oral contraceptive users (OCP).
EF=early follicular; LF=late follicular; LU=luteal phase. ROM measured using joint goniometer, soreness perception using a 10 mm visual analog scale, strength measured using a muscle dynamometer, swelling measuring using a Gulick spring loaded tape measure.
*denotes value significantly different than baseline at $p \leq 0.05$
** denotes value significantly difference than baseline at $p \leq 0.01$
†denotes significantly different than EUC.
Data are presented at Means ± SEM