INFLUENCE OF MENSTRUAL CYCLE ON INDICES OF CONTRACTION-INDUCED MUSCLE DAMAGE

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ABSTRACT
Markofski, MM and Braun, WA. Influence of menstrual cycle on indices of contraction-induced muscle damage. J Strength Cond Res 28(9): 2649–2656, 2014—Limited evidence suggests women exhibit a dampened response to contraction-induced muscle damage (CIMD). The purpose of this study was to examine if differences in symptoms of CIMD exist when induced in the menstrual cycle follicular or luteal phase. Sixteen resistance exercise trained women between the ages of 18–37 completed 75 eccentric-biased extension exercises with their nondominant arm. Creatine kinase (CK), elbow joint angles, arm volume, strength, and soreness were measured over 7 days. Estrogen was higher (p < 0.001) in the luteal group. The high estrogen group (luteal) had an overall greater strength decrement and higher CK concentration at 96 hours. Significant time effects were present for CK, elbow extension, elbow flexion, arm volume, and soreness. With the exception of strength and CK, signs and symptoms of CIMD were independent of menstrual cycle phase. Estrogen concentration in women may have limited effects on symptoms associated with muscle damage, but further research in this area is warranted.

KEY WORDS exercise, estrogens, creatine kinase, eccentric, woman

INTRODUCTION
Unaccustomed exercise or exercise involving heavy eccentric bias is associated with contraction-induced muscle damage (CIMD). The soreness and damage associated with CIMD are temporary, but nevertheless unpleasant. Other transient side effects from muscle damage may include soreness, loss of range of motion, and reduction in strength (5). While CIMD can result from a variety of activities, in a laboratory setting eccentric contractions are frequently used to induce CIMD. However, even in a controlled laboratory situation, differences in participants’ response to CIMD can arise for a variety of reasons, including recent prior damage of the same muscle (11,22), activity level (21), type of activity (18), and health status (4). It has also been proposed that estrogen may exert a protective effect on muscle (14,30). Although some researchers have not found a sex-related difference in muscle damage (17,27), a number of recent studies (7,27,29,31,34) suggest a sex difference in response to eccentric loading, such that women may experience less muscle damage than men. If the sex difference is due to women having a higher concentration of circulating estrogen, then differences in response to eccentric muscle loading may exist within the normal hormonal fluctuations of a woman’s menstrual cycle.

Muscle damage has many subjective measures, but an elevation of serum creatine kinase (CK) is often used as an objective indicator of muscle damage. Creatine kinase is present in muscle cells and an elevated presence in the serum indicates muscle damage (3,24). Muscle damage, irrespective of causation, will result in a loss of membrane integrity as reflected by CK release from cells. Creatine kinase concentration cannot quantify muscle damage but indicates its presence (12).

Estrogen may confer protection against CIMD through 2 proposed mechanisms: by supporting integrity of cell membranes and by protecting the cell against free radical assault through antioxidant properties (9,10,19,20,30,35). For example, ovariectomized rats supplemented with estrogen had a lower plasma concentration of CK (30) and less calpain-like activity (34) post-CIMD than nonsupplemented rats. Additionally, an in vitro study of myoblasts found that the presence of estrogen promotes better maintenance of baseline ATP levels (34). This is important because better conservation of ATP homeostasis would offset the damage-induced increase of intracellular calcium ions, which precipitates the events leading to free radical assault on the cell. In addition to the free radical damage, calcium ions will also promote calpain-like activity (2,20,26).

Ayres et al. (2) conducted several in vitro experiments to examine the protective role of estrogen against free radicals and other oxidants. The presence of estrogen significantly inhibited low-density lipoprotein oxidation and protected
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against hydrogen peroxide–induced deoxyribonucleic acid damage. When estrogen was combined with other known free radical scavengers, protection was not magnified. Based on the results of these and other studies (1,6,26), it has been hypothesized that estrogen helps to stabilize cell membranes.

Stupka et al. (31) investigated muscle damage differences between sexes in response to exercise performed at the same relative intensity. Statistical trends existed for sex differences in CK concentrations and leukocyte concentrations, which, when elevated, are indicative of an inflammatory response in damaged tissue. Both CK and leukocyte concentrations trended higher in men. Additionally, only the men had a significant elevation in circulating granulocyte concentrations, another measure of the inflammatory response. Because women have significantly higher circulating estrogen concentrations than men, the results of this study support the hypothesis that estrogen may confer a protective advantage against eccentric muscle work. These sex-related trends are unlikely to be related to differences in total muscle mass, which is often greater in men. Graves et al. (16) reported that although men generally have greater muscle mass than women, the volume of active muscle mass does not seem to influence the magnitude of change in CK in response to muscle damage. In the study of Graves et al. (16), a balanced-design was used to administer damage to 1 arm, both arms, or 1 leg. No significant differences in circulating CK were observed—thus concluding that the rise in CK is independent of the involved muscle area.

Although studies using animals and men are important, they may not be representative of the response in human women. There is evidence to suggest that estrogen influences markers of CIMD; however, it is unknown if eccentric muscle actions, when performed during periods of high estrogen vs. low estrogen, will result in detectable differences in indices of muscle damage. Therefore, the purpose of this study was to examine if symptoms of muscle damage differ when eccentric loading is administered during the follicular vs. the luteal phase of the menstrual cycle. The luteal phase is associated with higher amounts of estrogen than the follicular phase. If estrogen confers a protective role, then muscle damage symptoms may differ between the 2 phases, with the luteal phase group experiencing less severe signs and symptoms of muscle damage.

**METHODS**

**Experimental Approach to the Problem**

Regularly menstruating young women participated in an eccentric exercise bout on their nondominant arm. A repeated measure (phase × time) design was used to determine if there were menstrual cycle phase-dependent differences in response and recovery to CIMD. Soreness, arm volume, elbow extension, elbow flexion, and relaxed elbow joint angle were measured before the eccentric exercise and immediately, 24, 48, 72, 96, and 162 hours after exercise. Creatine kinase was measured before the eccentric exercise and 48, 72, 96, and 162 hours after exercise. Estradiol was measured to confirm menstrual cycle phase (Table 1).

**Subjects**

Eighteen regularly menstruating resistance trained women between the ages of 18–38 years were recruited for this study. All subjects reported a regular menstrual cycle of between 21 and 35 days for at least the preceding 12 months. Although the cycle length was variable across subjects, within each subject the participant reported consistent cycle length, and this was used as a criterion for inclusion. Eight participants were hormonal birth control (HBC) pill users and the remaining 10 were not (non-HBC). Resistance exercise trained participants were employed in this study because they would have previous experience with muscle soreness and discomfort and would therefore better tolerate the extent of muscle damage. Each participant provided written informed consent, and all testing procedures and the informed consent were approved by the Institutional Review Board at California State Polytechnic University, Pomona.

To be eligible to participate, volunteers had to engage in a minimum of 2 resistance training sessions (which included upper-body exercises) per week for a minimum of 3 continuous months preceding the study and were asked to refrain from resistance exercise while undergoing testing. Participants were not using nonsteroidal anti-inflammatory drugs (NSAIDs) and were free of upper-body injury in the past 6 months. All participants were tested within a 2-month window. To minimize variability, each participant reported to the laboratory at the same time of the day. The start dates of recent menstrual cycles and HBC use were self-reported. Phases of the menstrual cycle were confirmed by a verbal discussion with the participant. Participants randomized to the follicular group were tested on day 2 or 3 of the follicular phase; participants in the luteal phase were tested on day 1 or 2 of the luteal phase. Randomization into luteal or follicular phase groups was performed in a way that helped ensure equal distribution of HBC and non-HBC users. Participant characteristics are described in Table 1.

**Procedures**

At least 4 days before the eccentric exercise bout, the participant reported to the laboratory for a 1 repetition maximum (1RM) biceps curl test on the nondominant arm. To help keep the joint angles the same for all trials, a standard preacher curl bench (Life Fitness, Inc., Schiller Park, IL, USA) was used for the 1RM testing, eccentric exercise bout, active soreness testing, and follow-up strength assessments. After a warm-up consisting of 8 repetitions using a light load, the maximum weight the participant could lift was determined. Weight was added to a dumbbell, in 2.5 lb (~1.14 kg) increments, until the participant could no longer lift the weight. Between each attempt, the participant was allowed 2 minutes of rest. The 1RM was used to determine the loads for eccentric exercise and for setting a test load to be employed during active soreness testing.

On the day of the eccentric exercise session, baseline measurements were collected immediately before the
exercise. These included determination of baseline soreness, arm volume, a strength assessment (described below), and relaxed elbow joint angle and passive flexion. In addition, a 5 ml venous blood sample was collected from an antecubital vein for later determination of CK concentration and estrogen concentration (described below). The participants were instructed to refrain from taking NSAIDs; performing any upper-body or high-intensity lower-body weightlifting or downhill running; and icing, heating, stretching, or massaging the arm for the duration of the study.

The active soreness rating was performed with a weight corresponding to approximately 40% of the participant’s 1RM. After the participant raised and lowered the weight, they indicated on a 10-cm visual analog scale how painful the eccentric and concentric phases of the lift were for them to perform. Arm volume was measured by water volumetry, which is an established method for arm volume and swelling (32,36). Briefly, the participant would submerge her arm into a long water-filled freestanding plastic tube and displaced water volume was measured. The measurement was repeated twice, with a third trial if the measurements varied by more than 20 ml. A waterproof marker was used to make a mark approximately over the inferior end of the deltoid muscle belly. The mark was reapplied daily to ensure that the participant was submerging her arm to the same depth every day. Relaxed elbow joint angle and passive flexion were measured with a goniometer. To measure relaxed elbow joint angle, the participant was instructed to relax her arm at her side; for elbow flexion, the participant held her upper arm parallel to the ground and let her forearm relax against her upper arm.

The eccentric exercise bout consisted of 5 sets of 15 repetitions each, with a 2-minute rest between each set. Because the eccentric exercise bout, the participant performed a light 2-set warm-up. The first set was 6–8 repetitions at ~30% of 1RM, followed by a second heavier warm-up (4–6 repetitions at ~60% 1RM). The first set of the eccentric muscle action corresponded to 140% of the participant’s 1RM. The participant was seated at the preacher curl bench to perform the exercise. An investigator assisted the participant in lifting the weight but did not aid in the eccentric work. A metronome was used to set the repetition rate of a 4-second eccentric muscle action and 2-second aided concentric contraction. Once the participant was no longer able to sustain a controlled 4-second eccentric muscle action, the weight was reduced to 130% of 1RM. Once this elicited failure, the load was further reduced to 120% of 1RM. All participants were able to complete all sets with a weight corresponding to at least 120% of their 1RM. The reduction of weight ensured that all participants were able to complete 75 contractions.

All concentric strength measurements conducted after eccentric exercise were assessed as the percentage of the initial 1RM. The first attempt used a mass that was approximately 40% of the participant’s 1RM. After a 2-minute rest period, the next attempt was at 50% of 1RM and continued to rise

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<th>TABLE 1. Subject descriptives (mean ± SE).</th>
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<tr>
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<td>Age, y</td>
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<tr>
<td>Estrogen, pg·ml⁻¹</td>
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*The luteal group had significantly (p = 0.0008) higher estrogen than the follicular group.
RM, repetition maximum.

Figure 1. A moderate negative correlation was present between baseline estrogen concentration and strength recovery on day 7. Data points for 2 sets of subjects overlap closely, so they cannot be distinguished in this figure.
in increments of 10% until the participant was no longer able to lift the weight.

Blood samples were collected from an antecubital vein from the nondamaged arm using sterile procedures. The samples were allowed to clot and subsequently centrifuged at 3,000 rpm for 10 minutes. Serum was collected and stored at $-70\degree$ C for later analysis.

Baseline estradiol was measured using an enzyme immunoassay kit (Diagnostic Systems Laboratories, Webster, TX, USA). Estradiol was measured to not only quantify estradiol,
A moderate negative correlation was observed in Table 1. Ratios involving more than 7% of the participants were followed by post hoc multiple pairwise comparisons using the Tukey method to locate the inequities. There were no statistically significant differences between the follicular and luteal groups for any variable except estrogen.

Statistical Analyses
All statistics were calculated with SAS v.9.1 (SAS Institute, Cary, North Carolina, USA). PROC GLM was used to determine possible baseline differences between groups. Repeated-measures analysis of variance (ANOVA), PROC MIXED was used. The data were evaluated for assumptions of normality (Shapiro-Wilk), homogeneity of variance (residual plots), and independence of observations before evaluation of main effects and group interactions. As needed, a Box-Cox transformation was used to reduce skewness and stabilize variance. Hormonal birth control use was treated as a covariate.

Creatine kinase measures were subjected to a $2 \times 5$ (phase $\times$ time) factor ANOVA. Arm volume, range of motion, relaxed elbow flexion, relaxed elbow joint angle, muscle soreness, and strength recovery were subjected to separate $2 \times 7$ (phase $\times$ time) factor ANOVA. Significant $F$ ratios involving more than 2 means were followed by post hoc multiple pairwise comparisons using the Tukey method to locate the inequities. A criterion alpha level of 0.05 was adopted for all tests. Correlations were measured with Pearson’s product-moment correlation coefficient. All results are reported as mean and $SEM (s_x)$.

Results
Participants
During statistical analysis, 2 participants were removed from the data set due to incomplete data (1 noncompliance; 1 unrelated illness), the final sample size was 16 participants (follicular $n = 9$; luteal $n = 7$) and 6 of these participants were HBC users (follicular $= 4$; luteal $= 2$). Hormonal birth control use was determined to be a covariate and thus was treated as one in the statistical models. There were no statistically significant differences between follicular and luteal groups for any variable except estrogen.

Estrogen Concentrations
The luteal group had significantly ($p = 0.0008$) higher estrogen concentration than the follicular group ($187.8 \pm 27.8$ vs. $77.3 \pm 10.7$) (Table 1). A moderate negative correlation was present between baseline estrogen concentration and strength recovery, as calculated by percent of 1RM the participant was able to lift (Figure 1).
Strength Assessment
Strength was evaluated as a percent change from the initial 1RM. This allows for baseline strength to be taken into account without having to use baseline as a covariate. A significant \((p < 0.0001)\) main effect was present for time. Strength recovered over time, but full strength recovery did not occur during the 1-week course of the study (Figure 2). A significant interaction \((p = 0.04)\) was present, in that the follicular group had greater strength recovery at 96 hours after \((p = 0.009)\) and at 168 hours after \((p = 0.0013)\) than the luteal group.

Contraction-Induced Muscle Damage Exercise Bout
The eccentric load and the number of eccentric repetitions performed at each percentage of 1RM (140%, 130%, 120%) did not statistically differ between participant groups.

Elbow Flexion and Extension
A significant time effect was present for relaxed elbow joint angle (Figure 3). A main effect for time \((p = 0.04)\) was present for elbow flexion (Table 2). No other effects or interactions were observed. A main effect for time \((p = 0.0002)\) was present for elbow extension (Table 2). No other effects or interactions were observed.

Arm Volume and Soreness
Because of a large range of individual arm sizes, arm volume has been expressed as a percent change from baseline. A main effect for time \((p < 0.0001)\) was present. No other effects or interactions were observed for arm volume. A main effect for time \((p < 0.0001)\) was present for soreness. No other effects or interactions were observed.

Creatine Kinase
A main effect for time \((p = 0.0014)\) was present. There was also a significant interaction, with the luteal group showing a significantly higher \((p = 0.016)\) CK than the follicular group at 96 hours after (Figure 4). Creatine kinase remained elevated 168 hours post. No other effects or interactions were observed.

DISCUSSION
Significant time effects for elbow flexion and extension, arm volume, soreness, and CK were present, indicating that the protocol elicited changes in common markers of muscle damage. However, a higher concentration of estrogen was not associated with a significant improvement in signs and symptoms of CIMD. Contrary to the hypothesis, the participants’ physiological concentrations of estrogen were not related to a protective effect for the variables measured in this study. Based on the results of the study, it seems likely that menstrual cycle phase has little impact on CIMD in resistance training women. This unexpected result may be partially explained by some recent studies and some variations of studies in the present body of literature.

Despite estrogen previously being shown to have a protective effect on cells (2,25,33,34), strength recovery was found to be better in the follicular (low estrogen) group. It was hypothesized that because estrogen appears to protect cells, then participants with higher estrogen concentrations would exhibit a less severe strength decrement; however, the opposite was observed in this study. A higher concentration of circulating estrogen was associated with poorer strength recovery. The estrogen concentration at the time of damage may not have an effect on strength; instead, the estrogen concentrations during recovery may play a more crucial role. The days that the participants were tested were selected because of the anticipated difference of estrogen concentrations at those points in the menstrual cycle. During the recovery week, the estrogen concentrations of the follicular group would be naturally increasing, whereas the luteal group would have revealed declining estrogen levels. Further research of this question would be needed before a conclusion could be drawn.

Postexercise elbow flexion and extension each showed a significant time effect. There was also a significant time effect for arm volume. These are common signs of muscle damage (15) and were not unexpected.

Creatine kinase concentrations significantly increased across time, suggesting that the exercise caused enough CIMD to disrupt membrane integrity. It was surprising that the only interaction was the luteal group’s large CK increase.

Figure 4. Creatine kinase concentration over time. A significant \((p = 0.0014)\) time effect was present. *Luteal was significantly \((p = 0.016)\) higher than follicular phase at 96 hours.
concentration at 96 hours later. We had hypothesized that the luteal group would have fewer signs of muscle damage, but found the opposite. The magnitude of difference at this time was also unexpected.

One study that served as the driving force for the present study was conducted by Stupka et al. (31). Women in the follicular phase, who were taking HBC, were compared with men for signs and symptoms of CIMD. Following damage induction, the men had significantly higher circulating granulocyte concentrations than the women. Other trends were present that indicated that women may be less susceptible to muscle damage, but low power from a small sample size was identified as being a possible study limitation. A possible explanation for the lack of group differences in our study is that women, regardless of phase, may have sufficient estrogen present to offer some level of protection against muscle damage. Although this study does not directly provide evidence for this, a threshold for estrogen’s protective effect could exist. Ayres et al. (2) observed a potent antioxidant effect of estrogen, but they did not see any additive benefits when other known antioxidants were used in conjunction with estrogen. Along with the literature supporting a sex difference, the results of this study and the study of Ayres et al. indicate the effect of estrogen on signs and symptoms of muscle damage is a complex puzzle. It is possible that it is not just the amount of estrogen that has an effect on muscle damage, but also the interaction of estrogen with estrogen receptors. Other researchers (8,13,23) have reported sex differences in estrogen receptor activity, and the interaction of estrogen with estrogen receptors may be responsible for the sex difference response to CIMD that others have reported.

Savage and Clarkson (28) compared muscle damage in women who did or did not use HBC. The only difference was that the birth control group had a delayed strength recovery. We treated birth control as a covariate and blocked for its use. However, 2 participants had to be excluded from the data set, and both of these participants were birth control users in the luteal group. This resulted in the follicular group having more HBC users. Despite this, the follicular group had greater strength recovery than the luteal group.

Differences in response to CIMD vary between men and women, and the few published studies directly addressing this topic are in conflict. Participants in this study had a poorer strength recovery if they had higher estrogen at the time of the CIMD bout. This was in conflict with the hypothesis; yet, it is still possible that estrogen concentration influences CIMD outcomes. This area would benefit from additional research studies that further explore the complex role of estrogen in protection against CIMD, given the lengthy time course of events that are characteristic of muscle damage models.

**Practical Applications**

Based on the findings of this research, there is some indication that the response of women to a bout of CIMD is dependent on their menstrual cycle phase. This may have implications for women who do exhaustive or maximal weightlifting as part of their exercise training, in that their exercise program could be timed in accordance to their menstrual cycle. Specifically, that there may be some benefit to exhaustive or maximal weightlifting during the follicular, as opposed to the luteal, phase of the menstrual cycle. However, given the lack of strong evidence in support of a protective role of estrogen against muscle damage in the present study, it seems likely that menstrual phase will have small impact on symptoms of CIMD.

**Acknowledgments**

This work was conducted in the Department of Kinesiology and Health Promotion at California State Polytechnic University, Pomona, California No outside funding source was used to support this research. The results of the present study do not constitute endorsement of the product by the authors or the National Strength And Conditioning Association.

**References**


