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Interleukin-6 and Delayed Onset Muscle Soreness Do Not Vary During the Menstrual Cycle

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The purpose of this study was to determine if a difference in interleukin-6 (IL-6) and delayed onset muscles soreness (DOMS) exists in two different phases of the menstrual cycle. Nine runners performed one 75-min high-intensity interval running session during the early follicular (EF) phase and once during the midluteal (ML) phase of the menstrual cycle. Estrogen and progesterone levels were significantly reduced in the EF phase when compared to the ML phase. IL-6 levels increased from pre- to postexercise in the EF and ML phases ($p < .001$). There was no relationship between the IL-6 level and DOMS. The results suggest that menstruating female runners need not vary training throughout the month to reduce DOMS.

Key words: estrogen, high-intensity exercise, progesterone

Cytokines are low-molecular weight proteins involved in the interactions between immune and nonimmune cells (Gomez-Merino et al., 2006; Nieman et al., 2001). Cytokines are responsible for the influx of lymphocytes, neutrophils, monocytes, and other inflammatory cells into injured tissue at the site of inflammation (Pedersen et al., 2001; Petersen & Pedersen, 2005). It has been proposed that the inflammatory marker interleukin-6 (IL-6) is a product of the muscle cell myoblasts and satellite cells in response to muscle injury. Plasma IL-6 increases at a constant rate during exercise and is correlated to exercise intensity, duration, the muscle mass recruited, and the athlete's endurance capacity (Petersen & Pedersen, 2005). Furthermore, endurance exercise is known to induce pro-inflammatory cytokines that have negative effects on performance (Bruunsgaard et al., 1997; Nieman et al., 2005).

The large number of women participating in endurance events warrants study of the impact of female physiology on these inflammatory responses. Much research on female endurance athletes has focused on menstrual disturbances associated with bone mineral density, body weight, body temperature, and menstrual abnormalities (Bonen et al., 1983; Burrows & Bird, 2000; Garcia et al., 2006; Jurkowski, 1982; Lebrun, McKenzie, Prior, & Taunton, 1995). However, limited research has focused on how the menstrual cycle affects muscle damage, exercise-induced inflammation, and delayed onset muscle soreness (DOMS). Hormonal fluctuations during different phases of the menstrual cycle may influence cytokine production during exercise (Timmons, Hamadeh, Devries, & Tarnopolsky, 2005), but research is needed to determine if this hormonal variation is associated with inflammation and DOMS.

The fluctuation of sex hormones across the menstrual cycle may affect production of these inflammatory markers. The menstrual cycle has two different phases: follicu-

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lar and luteal. In the early follicular (EF) phase, estrogen and progesterone levels are low, and in the midluteal (ML) phase both are elevated. Angstwurm, Gartner, and Ziegler-Heitbrock (1997) reported that resting IL-6 levels are lowest in the luteal phase, when progesterone levels are elevated, and highest in the follicular phase during normal menstruation, when estrogen and progesterone are low. In contrast, the luteal phase has been associated with an increase in the immune cells leukocytes and lymphocytes, which are associated with cytokine production (Bouman, Moes, Heineman, de Leij, & Faas, 2001; Faas et al., 2000).

Several studies suggested that increased 17β-estradiol levels have a protective effect against inflammation and muscle damage. E2 may provide protection through its membrane stabilizing capabilities and antioxidant properties (B. Kendall & Eston, 2002). Previous research also found that estrogen may significantly impair the inflammatory cascade through gene regulation by reducing the molecule adhesion expression and suppressing neutrophil and macrophage production (B. Kendall & Eston, 2002). As a membrane stabilizer, estrogen may decrease neutrophil free radical production by limiting the fluctuations of intracellular calcium homeostasis (Tiidus, 2000).

Several animal studies have focused on estrogen's protective role and the occurrence of muscle damage after exercising at intensities found to induce oxidative stress. Rodents were used to investigate the effects of estrogen supplementation on exercise-induced muscle damage using the biomarker creatine kinase (CK; Bär, Amelink, Oldenburg, & Blankenstein, 1988; Tiidus et al., 2001). Both studies observed that oral E2 supplementation significantly suppressed the CK-efflux. Recent work found that muscle damage in the quadriceps femoris muscle was less evident in female rats after eccentrically based downhill running, in comparison to male rats (Komulainen, Koskinen, Kalliokoski, Takala, & Vihko, 1999). Current animal research has measured oxidative stress and neutrophil level but not plasma cytokine concentrations (Komulainen et al., 1999; Tiidus, et al., 2001), and it is possible a significant difference in cytokine levels could have occurred in these studies. Protection was found in animals that had elevated estrogen levels; therefore, it is possible the estrogen level may affect the production and release of IL-6.

Studies on the influence of estrogen and progesterone levels and the occurrence of muscle damage in women who performed exhaustive eccentric exercise during different menstrual cycle phases had mixed results. Studies examining the influence of gender and hormonal concentrations after exercise found that women have decreased levels of muscle damage when compared to men (Apple et al., 1987; Janssen et al., 1989; Shumate, Brooke, Carroll, & Davis, 1979; Stupka et al., 2000; Tiidus, 1995). Resting CK levels were lower in women than men, which may relate to increased estrogen levels in women (Hortobagyi & Denahan, 1989; Meltzer, 1971; Norton, Clarkson, Graves, Litchfield, & Kirwan, 1985; Shumate et al., 1979). Studies that found no significant differences in the IL-6 concentration after E2 supplementation or between sexes after exercise may have used exercise protocols insufficient to induce a significant amount of damage (Edwards, Burns, Ring, & Carroll, 2006; Timmons, Hamadeh, & Tarnopolsky, 2006). It appears that prolonged high-intensity eccentric endurance exercise may be needed to detect a difference in muscle damage during different menstrual cycle phases.

Estrogen variation across the menstrual cycle and its potential to attenuate muscle damage has received limited attention. Increased cytokine level during a specific menstrual cycle phase could limit physical performance by increasing musculoskeletal damage and alternating the perception of pain associated with DOMS (Carter, Dobridge, & Hackney, 2001). If the menstrual cycle influences cytokine production, it may be beneficial for healthy, physically active women to adjust their competitive and training schedules to optimize training and performance. Therefore, the purpose of this study was to determine if there is a difference in IL-6 and DOMS in the EF and ML phases of the menstrual cycle in a group of competitive female distance runners following an exhaustive run. We hypothesized that a healthy woman in the EF phase would show a greater increase in IL-6 and report a higher DOMS rating than in the ML phase.

Method

Participants

Fourteen women completed a portion of the test sessions but were unable to complete all sessions due to musculoskeletal injury or menstrual cycle abnormality. Nine competitive, physically active women completed all three exercise sessions, and their data were used for this study. Participant characteristics were: *M* age = 26.8 years, *SD* = 4.3; *M* body mass = 57.2 kg, *SD* = 4.2; *M* height = 166.1 cm, *SD* = 6.1; *M* maximum oxygen uptake = 49.7 ml/kg/min, *SD* = 6.0; *M* % body fat = 19.1%, *SD* = 4.4; *M* menstrual cycle length = 30.6 days; *SD* = 4.6. Participants were not currently taking any oral contraceptives or medications that would affect their menstrual cycle. All women were competitive runners and had run a minimum of 25 miles (40.2 km)/week for the previous 3 months leading up to their first testing session. We used these inclusion criteria to increase the probability of completing the two intense training sessions. Participants were informed of the experimental protocol and possible risks, and all provided informed consent in accordance. The university's institutional review board approved this study.

Menstrual Cycle Phase Determination and Basal Body Temperature

The participants were asked to record their menstrual history and monitor their basal body temperature for 3 months prior to their first testing session. They recorded the start and end of menses to document the average length of their menstrual cycle. Using a digital thermometer (Mabis Healthcare, Inc., Model # 15-691-000), they assessed basal body temperature every morning on waking to verify ovulation and, therefore, menstrual cycle phase.

In an average 28-day cycle, Day 1 represents the first day of menses, when estrogen and progesterone are at their lowest (EF phase). Days 9 through 13 correspond to peak estrogen surge (late follicular phase), and Days 18 through 24 correspond to peak progesterone surge (ML phase; Boron & Boulpaep, 2005). We considered the last day of the menstrual cycle as the day before the next menses. Exercise sessions occurred at approximately Days 1–3 and Days 20–22 of the cycle. Because menstrual cycles may be shorter or longer than 28 days, we used documentation of previous cycles and temperature recordings to determine their cycle length so they could perform the exercise during the EF and ML phases, when estriadol (E2) and progesterone (P4) reach their lowest and highest levels, respectively.

Design

This study was a repeated measures design in which participants performed two strenuous exercise sessions, one during the EF phase and the second during the ML phase. To counterbalance the order in which the participants performed the sessions, 5 participants started during the EF phase and 4 began during the ML phase. Participants reported to the exercise physiology laboratory 2–4 weeks before their first test to become familiar with the experimental protocol. Following this, their body composition was measured and they performed a maximum oxygen update (VO_2peak) test to determine their aerobic fitness. At each visit, participants' body mass was measured, and blood samples were collected after 5 min of sitting. The strenuous exercise sessions consisted of interval training at specified percentages of VO_2peak. Following the exercise protocol, blood samples were again collected within 5 min postexercise.

Body Composition and VO_2peak

Harpenden skinfold calipers (Model 68875; Baty International West Sussex, England) were used to assess body composition. The Jackson, Pollock, and Ward (1980) equation was used to calculate body density. Skinfold sites included the triceps, thigh, and suprailium. Percent body fat was calculated using the formula established by Siri (1956).

Each participant performed a continuous graded exercise test to assess VO_2peak. A TrueOne 2400 Metabolic Measurement System (ParvoMedics, Inc., Sandy, UT) was used to measure oxygen uptake. The test began with a 3-min walking stage at 80.6 m/min and progressed to 160.9 m/min, with speed increased 26.8 m/min every 3 min until 241.4 m/min. Then speed was increased 13.4 m/min every 3 min until participants reached volitional exhaustion. Participants were verbally encouraged in the later stages of the test. Criteria for reaching VO_2 peak were three of the following: (a) $VO_2 \leq 2$ ml/kg/min increase in the last two consecutive 30-s time periods, (b) a respiratory exchange ratio exceeding 1.10, (c) a heart rate within 10 bpm of age-predicted maximum, or (d) a rating of perceived exertion (RPE) ≥ 19 (Armstrong et al., 2006; Howley, Bassett, & Welch, 1995). Six women achieved all the criterion and 3 achieved three of the four.

Exercise Protocol

Participants performed two 75-min high-intensity, flat running sessions on a Quinton MedTrack CR60 motorized treadmill (Quinton Cardiology, Inc., Bothell, WA). The protocol was designed to increase the likelihood of producing muscular stress. A 10-min warm-up run occurred at 60% of the velocity at VO_2peak (vVO_2peak), followed immediately by 5 min at 70% of vVO_2peak and then 5 min at 75% vVO_2peak. Following a 3-min walk recovery, the interval training portion of the session began. It consisted of three 2-min runs at 100% vVO_2peak, each alternated with a 2-min jog or walk at 50% vVO_2peak, for a total of 6 min hard running and 6 min jogging or walking. Following a 3-min walk recovery, participants repeated the same protocol. Following a 5-min recovery at a self-selected pace, they performed a series of five 1-min bouts at 110% vVO_2peak, with a 3-min walk recovery after each. During each exercise session, heart rate was measured and recorded every 3 min using a Polar Vantage heart rate monitor (Polar Electro Inc., Kempele, Finland). Participants also provided their RPE every 3 min using the Borg 6-20 scale (Borg, 1982).

DOMS Assessment

The women rated their intensity of muscle soreness using a 10-point Likert-type scale (Nieman et al., 2005; Smith et al., 1993) immediately postexercise, and at 24–48 hr following each testing session. The scale used in this study consists of seven adjectives describing pain sensation (Nieman et al., 2005). The runners based their responses on three different lower extremity muscle groups using the following scale: 1 = no soreness, 2.5 = dull, vague ache, 4 = slight soreness, 5.5 = more than slight soreness, 7 = sore, and 10 = unbearably sore. The muscle groups were the anterior thigh, posterior thigh, and posterior leg. Nie-

man et al. (2005) used this scale to measure DOMS during the week following a 160-km running race and found that athletes with the greatest muscle damage had higher post-race plasma levels of IL-6. Because of its greater sensitivity (Vickers, 1999), this type of scale is recommended for assessing muscle soreness rather than a visual analog scale.

IL-6 and Hormone Concentrations

A trained phlebotomist used sterile techniques to take blood samples from an antecubital vein. They collected plasma samples prior to and 5 min following exercise completion to verify an increase in IL-6. Total plasma concentrations of IL-6 were determined using a human IL-6 immunoassay kit (R & D Systems, Inc., Minneapolis, MN). The assay sensitivity was 0.7 pg/ml, and the intra- and interassay coefficients of variance were 4.2% and 6.4%, respectively. E2 and P4 concentrations were determined using ELISA kits (BioQuant, San Diego, CA). The E2 assay sensitivity was 10 pg/ml, and the P4 assay sensitivity was 40 pg/ml; the intra- and interassay coefficients of variance were 10.9%, 12.5%, and 5.4%, 9.7%, respectively. The samples were run in duplicate and calculated from their respective standard curve. The correlation of determination (r^2) for E2 = .98, P4 = .95, and IL-6 = .99. A microplate reader (BioTek instruments, Inc., Winooski, VT) was set at a wavelength of 450 nm for all readings.

Statistical Analysis

Means, standard deviations, and range were calculated for each measure. A 2-way (2 x 2) analysis of variance was used to investigate the influence of menstrual cycle phase on IL-6 pre- and posttest. Dependent *t* tests were used compare E2 and P4 levels in both phases of the menstrual cycle and DOMS. If analyses revealed significant differences, effect size was calculated to assess the magnitude of difference. Pearson *r* correlations were used to compare the relationship between DOMS with the change of IL-6 pre- to postexercise. Statistical significance was set at $p \leq .05$ for all analyses. Data were analyzed using Statistical Package for Social Sciences (Version 17.0, SPSS, Inc., Chicago, IL).

Statistical power analyzing IL-6 was estimated at .70 (Thomas, Nelson, & Silverman, 2005) assuming effect size was moderate to large, as reported in other related studies. Previous studies on this topic detected statistically significant findings with sample sizes similar to $N = 9$ in our study. For example, Thompson, Hyatt, De Souza, and Clarkson (1997) compared the effect of oral contraceptives versus no contraceptives on CK levels and reported an ES of .5 with 6 and 7 participants per group, respectively. Roth, Gajdosik, and Ruby (2001) obtained an ES of .8 in comparing CK levels in women using or not using oral contraceptives with $n = 5$ per group; Shumate et al. (1979) obtained an ES of .8 using a sample size of 9 and 11 in comparing CK levels in women and men, respectively. We also reduced the likelihood of the study being underpowered by using a design in which we analyzed only two effects (IL-6 concentration and DOMS; Hopkins, Marshall, Batterham, & Hanin, 2009). In addition, our participants were relatively homogeneous in regard to age, training status, and VO_2peak, thus, reducing error variance and increasing statistical power. Last, the fact that we found significant elevations in E2, P4, and IL-6 in the pre- versus postexercise condition indicates adequate statistical power for this comparison. However, it is certainly possible that the study was underpowered for comparing IL-6 and DOMS in the two phases. This limitation in identified in the Discussion section.

Results

Hormone Concentrations

E2 and P4 data are presented in Table 1. E2 levels were significantly lower in the EF phase when compared to the ML phase ($p = .032$). The effect size of 1.2 indicates a large effect. P4 levels were also significantly lower in the EF phase when compared to the ML phase ($p = .010$). Likewise, the effect size of 1.5 represented a large difference. All participants demonstrated lower E2 and P4 levels in the EF phase and elevated E2 and P4 levels in the ML phase. These data verified that participants were in the EF (Days 1–3) or ML (Days 20–22) phase during each exercise session.

Interleukin-6

IL-6 concentration data are presented in Figure 1. IL-6 was significantly increased from pre- to postexercise in both phases, $F(32, 3) = 33.68$, $p < .001$ (see Figure 1). No significant difference occurred between phases, $F(32,$

Table 1. Summary of plasma hormone levels during the early follicular and midluteal phases of the menstrual cycle ($N = 9$)

Variable	*M*	*SD*	Range
E2 (pg/ml)			
EF	68.44	28.73	18.98–98.28
ML	85.78*	14.75	73.95–106.68
P4 (ng/ml)			
EF	8.70	3.42	2.04–13.03
ML	13.73*	2.54	9.00–16.63

Note. *M* = mean; *SD* = standard deviation; E2 = estrogen; EF = early follicular; ML = midluteal; P4 = progesterone.
*Significantly greater than the EF phase, $p < .05$.

3) = 1.00, p =.32, and the interaction between pre- and postexercise measures and the menstrual cycle phase was also not significant, $F(32, 3) = 1.07$, $p = .31$.

DOMS Ratings

The higher ratings immediately postexercise in both menstrual cycle phases approached significance ($p = .081$). In the EF phase, the mean rating = 4.67 ($SD = 2.14$); in the ML phase, the mean rating = 3.67 ($SD = 1.64$). The DOMS ratings at 24 and 48 hr did not differ significantly between the two phases. In addition, muscle soreness ratings immediately, at 24 hr, and at 48 hr postexercise did not correlate ($r = .032$, .097, and .136, respectively) with the change of IL-6 from pre- to postexercise ($p > .05$).

Discussion

As anticipated, E2 and P4 levels were reduced in the EF phase when compared to the ML phases. Although IL-6 levels increased following strenuous exercise bouts in both phases, IL-6 levels from pre- to postexercise did not differ between the phases as hypothesized. Likewise, the present study did not demonstrate significant differences in DOMS in the two menstrual cycle phases.

A potential explanation for these findings is that the elevation of E2 in the ML phases did not differ enough from the EF phase in our sample to provide a protective musculoskeletal benefit during and after exercise. Research suggests that estrogen may be protective because it may work as an antioxidant and membrane stabilizer (Bär & Amelink, 1997; B. Kendall & Eston, 2002; Tiidus, 1995; Wiseman & Quinn, 1994; Wiseman & O'Reilly, 1997). A study on the pattern of circulating steroids in the normal menstrual cycle explains that E2 exhibits great individual variation (Guerrero et al., 1976). Values for the E2concentration in healthy young women were $M = 31.5$ pg/ml ($SD = 10.0$) and $M = 118.0$ pg/ml ($SD = 54.7$); $M = 54.4$ pg/ml ($SD = 12.0$) and $M = 147.2$ pg/ml ($SD = 25.5$); $M = 67$ pg/ml ($SD = 10.4$) and $M = 113.3$ pg/ml ($SD = 27.1$); and $M = 98.6$ pg/ml ($SD = 22.2$) and $M = 112.9$ pg/ml ($SD = 46.3$) in the EF and ML phases of the menstrual cycle during the control period and during intervention with soy protein (Cassidy, Bingham, & Setchell, 1994; Chung, Goldfarb, Jamurtas, Hegde, & Lee, 1999; Meendering, Torgrimson, Houghton, Halliwill, & Minson, 2005). Thong, McLean, and Graham (2000) examined E2 in recreationally active and elite female athletes. The recreationally active women had concentrations of $M = 44.5$ pg/ml ($SD = 8.5$) and $M = 105.8$ pg/ml ($SD = 10.2$) EF and ML phases, respectively, and the elite women had $M = 38.6$ pg/ml ($SD = 4.9$) and $M = 97.8$ pg/ml ($SD = 11.7$) in the EF and ML phases, respectively. Although the relative fluctuations in E2 and P4 are similar between women, the absolute values have a high degree of variability. The female runners in the current study followed the relative fluctuation trend, with lowest E2 and P4 in the EF phase and higher E2 and P4 in the ML phase.

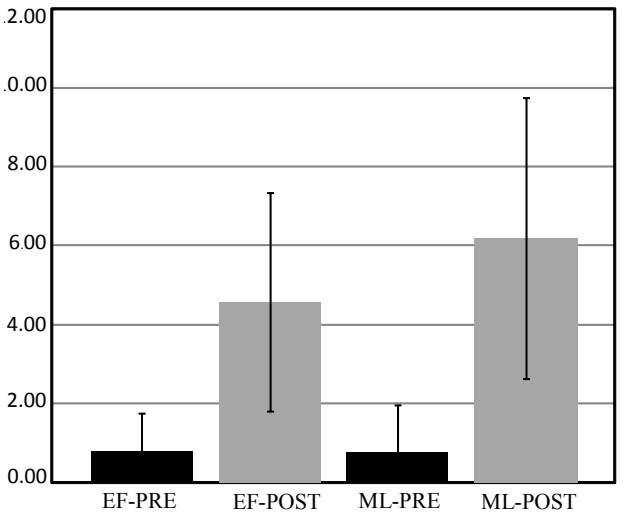

Figure 1. The plasma concentration of interleukin-6 (IL-6) before (pre) and immediately (post) after exercise in the early follicular and midluteal phases of the menstrual cycle; IL-6 was significantly greater post than pre in both phases of the cycle; $p < .001$; bars represent standard deviation.

Some female athletes may become hypoestrogenic with increased levels of physical activity (Warren & Perlroth, 2001). Also, E2 levels have been reported to be lower in physically active women compared to less active women (De Souza et al., 1998; Mitsuzono & Ube, 2006). For example, female distance runners had significantly lower E2 values than nonathletes $M = 112.9$ pg/ml ($SD = 66.2$) versus $M = 30.5$ pg/ml ($SD = 11.3$), respectively ($p < .01$) in the ML phase (Mitsuzono & Ube, 2006). Another study reported a high frequency of luteal phase deficiency and anovulation in recreational female runners (De Souza et al., 1998). The researchers examined three consecutive menstrual cycle phases in a group of sedentary and recreationally active women who ran at least 2 hr per week during the previous year. Estrone conjugates excretion during Days 2–5 was lower in the recreationally active women when compared to the sedentary women and remained lower in the active women during the luteal-follicular transition. Although the women in our study were regularly menstruating, their estrogen levels may not have varied significantly enough between the menstrual cycle phases to provide a difference in musculoskeletal damage protection. The lower E2 concentration in the EF phase may be sufficient to offer protection, and, therefore, the elevated concentration observed in the ML phase may not add further protection.

Others have noted that estrogen's protective role may be different between human and animal models. Bär et

al. (1988) and Tiidus et al. (2001) examined CK in rats that performed endurance running. Following 2 hr of running, the ovariectomized female rats had significantly elevated CK when compared to the ovariectomized female rats that had estrogen supplementation (Bär et al., 1988). After 60 min of running, serum CK was lower in female rats with estrogen supplementation than female rats that received the placebo (Tiidus et al., 2001). Therefore, estrogen supplementation may provide significant protection in the animal model when the estrogen source has been completely removed.

Recent studies on humans demonstrated that hormone replacement therapy (HRT) and estrogen supplementation have positive effects on skeletal muscle in postmenopausal women (Dieli-Conwright, Spektor, Rice, Sattler, & Schroeder, 2009, 2010; Ronkainen et al., 2009; Tiidus, 2009). Ronkainen and colleagues used monozygotic twins to study HRT effects. The HRT users had five times the estrogen levels of the nonusers. The HRT users walked 7% faster, jumped 16% higher, and exhibited 8% greater relative lean tissue content, and had 5% less relative fatty tissue content compared with their nonusing counterparts (Ronkainen et al., 2009). Postmenopausal women using HRT may also experience a greater myogenic response to maximal eccentric exercise (Dieli-Conwright et al., 2009) and develop greater skeletal muscle strength (Greising, Baltgalvis, Lowe, & Warren, 2009).

Limited research has examined the interaction of estrogen and progesterone and their ability to work synergistically to influence muscle damage. One study examined the time-to-fatigue of eccentrically contracted plantarflexor muscles in mice implanted with .05 mg-17β E2 pellets (OE), 15 mg P4 pellets (OP), or E2 and P4 pellets (OEP). Plantarflexor muscle fatigue took 30% and 41% longer to occur in the OP group than it did in the OE and OEP groups, respectively. The authors suggested that P4 reduces muscle fatigue in response to eccentric exercise and that this effect is blunted when estradiol is present concurrently (Schneider, Fine, Nadolski, & Tiidus, 2004). Therefore, the interaction of E2 and P4 could influence an individual's ability to perform strenuous physical activity.

In our study, we found that IL-6 significantly increased from pre- to postexercise in both menstrual phases. This finding was clearly demonstrated in previous literature examining endurance exercise (Bruunsgaard et al., 1997; Gomez-Merino et al., 2006; Nieman et al., 2005; Nieman et al., 2001; Pedersen et al., 1998). Epidemiological studies found a negative correlation between physical activity level and basal plasma IL-6 (Pedersen & Febbraio, 2008). Athletes subsequently may have a lower postexercise IL-6 level than nonathletes. The physically active women in the current study all ran at least 25 miles per week, which may have limited the amount of muscle damage and in turn dampened a rise in IL-6. Currently, it is unresolved whether training influences exercise-induced IL-6 increase. Research has shown that elite competitive skiers have lower plasma IL-6 levels during the training season than off-season (Ronsen et al., 2001). Ten male international Nordic skiers, ages 20–29 years performed an incremental treadmill test to exhaustion at the same time of day (±1 hr), during the competitive season and off-season. IL-6 concentrations were elevated postexercise, 15, 30, and 60 min after the off-season exercise test when compared to levels during the competitive season. It has also found that IL-6 deficient mice have reduced endurance and decreased VO_2 during exercise, compared to control mice (Faldt et al., 2004). The authors speculated that IL-6 may be necessary for normal exercise capacity and that endogenous IL-6 is required to maintain high oxygen consumption levels, thereby permitting the ability to maintain skeletal muscle work during strenuous exercise.

Increased IL-6 production during exercise may be an acute physiological response to increase metabolism rather than a biomarker of muscle cell damage or inflammation (Pedersen et al., 2004). For instance, low muscle glycogen is associated with an increase in IL-6 gene expression resulting in greater IL-6 production. Because the IL-6 gene is rapidly activated in the exercising muscle, it has been suggested to act as an energy sensor by receiving signals based on the muscle's glycogen content (Pedersen et al., 2004). When muscle glycogen stores reach critically low levels, IL-6 may signal the exercising muscle to increase glucose uptake for Glut-4 translocation. It appears IL-6 production may be principally associated with contracting muscle and may not have a large systemic effect. Therefore, it may be informative to measure IL-6 levels in muscle microcirculation. These findings relating to IL-6 are paradoxical, as it is also associated with inflammation and reduced insulin action in obese individuals. This suggests that IL-6 levels during and postexercise may have multiple effects, some of which are related to acute metabolic responses.

We found that menstrual cycle phase had no statistical effect on the plasma IL-6 level. Few studies have investigated the effect of gender on IL-6 production during and after exercise. Edwards et al. (2006) observed that women and men showed similar IL-6 concentrations after a maximal cycling bout, but the women's concentrations were significantly elevated when compared to the men. The authors speculated that a high E2 concentration could contribute to an increased production of IL-6 in women exposed to strenuous exercise. Because IL-6 is a biomarker of muscle damage and inflammation as well as muscle metabolism, interpreting the IL-6 level with exercise is difficult. Furthermore, women's estrogen concentrations may also affect their exercise metabolism (Hackney, 1999). During the luteal phase, there was reduced use of glyco-

gen as compared to the follicular phase (decrease: M = 46.4%, SD = 8.4, and M = 21.2%, SD = 16.3, respectively; $p < .05$), suggesting a glycogen-sparing effect and enhanced lipid metabolism with higher circulating estrogen.

In the present study, we found no significant differences between DOMS and the menstrual cycle phase. In addition, muscle soreness ratings immediately postexercise and at 24 and 48 hr postexercise did not correlate with the change of IL-6 from pre- to postexercise. The results suggest that change in IL-6 level is not related to muscle soreness. However, it is possible there was no correlation because the study was underpowered. Additionally, other inflammatory markers may better represent muscle damage and related muscle soreness. For example, in a study of 30 min downhill running at 60% VO_2 peak there was a significant increase in perceived DOMS. CK was significantly less in women with high estrogen levels than those with low estrogen, but there were no significant difference in DOMS ratings between the groups (Carter et al., 2001). The authors suggested that estrogen protected against muscle tissue damage during exercise. Hence, CK may be a better measure of muscle damage than plasma IL-6 levels, which may explain the discrepancy in the current study. Limited differences in DOMS have been observed between genders (Dannecker, Koltyn, Riley, & Robinson, 2003). Studies have shown that women using oral contraceptives have lower DOMS than nonusers (B. K. Kendall & Eston, 2002; Roth, Gajdosik, & Ruby, 2001; Thompson et al., 1997); however, others found no significant differences (Savage & Clarkson, 2002). Rao et al. (1987) investigated pain perception across a broad range of participant groups. They showed the pain threshold was low in women not on HRT, low in boys and girls, intermediate in men, and high in oral contraceptive users and normally menstruating women. Fluctuations in pain thresholds occurred in menstruating women, with higher thresholds midcycle, when estrogen concentrations are highest (Rao, Ranganekar, & Saifi, 1987).

It has been suggested that E2 and P4 work synergistically to modulate pain perception (Thompson et al., 1997). In the present study, the higher DOMS ratings immediately postexercise in both menstrual cycle phases indicated a nearly significant trend (p = .081). Immediately postexercise, ratings in the EF and ML phases were M = 4.67, SD = 2.14, and M = 3.67, SD = 1.64, respectively.

The sample size in this study may have prevented statistical significance in DOMS between the ML and EF phases. A trend with greater DOMS in the EF phase (p =.081) suggests the possibility the study was underpowered. Additionally, a major limitation to this study is that we did not measure CK. Future studies researching this topic should analyze CK in conjunction with other markers of muscle damage.

From the study results, we concluded that plasma IL-6 levels increase following strenuous running in healthy women in both the EF and ML phases of the menstrual cycle. However, IL-6 and DOMS are not higher in the EF phase than in the ML phase. Our results do not warrant that physically active, eumenorrheic healthy women need to adjust their training to reduce DOMS because of fluctuation in E2 and P4.

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