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Exercise-Induced Inflammation during Different Phases of the Menstrual Cycle

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Abstract

Objective: To examine the effects of menstrual cycle on exercise-induced inflammation, we investigated exercise-induced changes in circulating cytokines and leukocyte responses during different phases of the menstrual cycle.

Methods: Ten healthy sedentary females (20.5 ± 0.7 years) performed 60 min of cycling at 75% of their individual anaerobic threshold (AT) during the three different phases of the menstrual cycle (menstrual, follicular and luteal phases). Blood and saliva were sampled baseline, post- and 30 min post-exercise. The salivary concentrations of female sex hormones, progesterone and 17β-estradiol, and plasma concentrations of pro-inflammatory cytokines, interleukin (IL)-6 and IL-8, and markers of leukocyte activation, calprotectin and myeloperoxidase, were measured in all the blood and saliva sampling intervals.

Results: The plasma concentration of IL-6 increased significantly post-exercise (p<0.001) and the plasma concentration of calprotectin increased significantly 30 min post-exercise (p<0.05) in all the three menstrual cycle phases. A positive correlation was found between exercise-induced changes in plasma IL-6 and calprotectin concentrations in the menstrual phase, suggesting the possibility of enhanced leukocyte reactivity to IL-6 during this phase.

Conclusion: These findings suggest that 60 min of strenuous exercise at an intensity of higher than 75% AT may induce inflammation in sedentary females, especially during the menstrual phase of the menstrual cycle.

Introduction

Moderate aerobic exercise is important for maintaining and improving health. However, strenuous exercise can cause muscle damage and induce leukocytosis and the pro-inflammatory response [1]. Estrogen can play a protective role against inflammation [2], suggesting that changes in the secretion of estrogen during the menstrual cycle can influence inflammatory reactions following exercise. Considering the increasing number of women participating in exercise, it is important to investigate the dynamics of the interaction between the physiological changes occurring over the menstrual cycle and the inflammatory response during exercise. The information generated through such investigations is potentially useful for prescribing safe and effective exercise guidelines to minimize the risk of negative health outcomes during different phases of the menstrual cycle.

Differences in immune functions have been reported between genders and between the follicular and luteal phases of the menstrual cycle. For example, exercise-induced changes in lymphocytes were greater during luteal phase than in the follicular phase [3]. In the luteal phase, women showed a distinctly different pattern of gene regulation during exercise compared with during the follicular phase, and with men [4]. Despite the evidence suggesting differences in immune and inflammatory response during exercise over the course of the menstrual cycle, in-depth investigation into this domain of study has been scarce.

There is some evidence suggesting an increase in leukocytes and the related macrophage activities during the menstrual phase of the cycle. Both neutrophil infiltration [5] and increasing number of macrophages arising from the differentiation of circulating monocytes [6] have been detected in the endometrium in the menstrual phase. These findings suggest that the reactivity of leukocytes within tissues may be increased during menstruation, which can predispose female athletes to greater risk of muscular injury and inflammation.

Moreover, although both moderate and intense exercises can induce neutrophil activation, moderate intensity exercise was shown to induce a larger cytokine response than intense exercise [7]. The evidence presented suggests the possibility that moderate intensity exercise may cause different immune responses across the different phases of the menstrual cycle.

Previous studies in male subjects reported that prolonged exercise increases plasma concentrations of calprotectin [8-10] and myeloperoxidase [1,10], which imply neutrophil activation. Calprotectin is released upon neutrophil activation or endothelial adhesion of monocytes and is detected in plasma as an inflammatory marker [11,12]. These leukocyte responses could be mediated by the systemic release of cytokines such as interleukin (IL)-6 and IL-8 [1,13].

Whereas exercise-induced changes in IL-6 and IL-8 are well documented, there is limited data on the influence of the menstrual cycle on pro-inflammatory cytokine responses during exercise. Therefore, the aim of the present study was to examine the influence of the phases in the menstrual cycle on the exercise-induced inflammatory response. We investigated exercise-induced changes in pro-inflammatory IL-6, IL-8 responses and in leukocyte degranulation responses in terms of calprotectin and myeloperoxidase during different phases of the menstrual cycle.

Methods

Subjects

Ten healthy sedentary females, mean age 20.5 ± 0.7 years, participated in this study. None of the participants were using oral contraceptives or were pregnant during the study. All participants were considered eumenorrheic based on basal body temperature (BBT), which was monitored every morning for two months prior to the study. BBT increased by approximately 0.4°C in the luteal phase, which was maintained for at least twelve days. The menstrual cycle was broken into three phases. The first 4 days of the menstrual flow was considered the "menstrual phase," followed by the "follicular phase," the 7th-11th day after the start of the menstrual flow, and the "luteal phase," which is 5-9 days before the next menstrual flow.

The participants performed an exercise at 75% of anaerobic threshold (AT) i.e., cycling for 60 min, in each phase of the menstrual cycle. The menstrual cycle phases were validated with salivary 17β-estradiol and progesterone concentrations. Written informed consent was obtained from each participant and they were free to withdraw from the study at any time. The experimental procedure was approved by the Research Ethics Committee at Waseda University.

Exercise protocols

Anaerobic Threshold (AT) for each participant was determined using an incremental cycling ergometer test one month before the start of the experiments for the menstrual cycle. The participant was familiarised with the exercise protocol and equipment used during the test before attempting the AT test. The AT test was conducted using a cycle ergometer (Aerobike 75 XL, Combi, Shinagawa, Japan). After a 3 min warm-up period, participants began cycling at a rate of 60 revolutions per minute (rpm) and a work rate of 20 watts, with increments of 2 watts every 10 second, continuing until volitional fatigue.

Expired air volume and content were analysed on a breath-by-breath basis using indirect calorimetry (Aeromonitor AE-300S, Minato, Yodogawa, Japan) and AT was determined using the modified V-slope method, plotted from volume of expired carbon dioxide ($\dot{V}CO_2$) as a function of corresponding oxygen consumption ($\dot{V}O_2$) [14].

Following the measurement of AT, the participant performed 60 min of exercise test on a cycle ergometer at 75% AT in each phase of the menstrual cycle i.e., total of 3 exercise tests. In each of the exercise test, the participant refrained from strenuous physical activity and consumption of alcohol for >24 hours before each exercise test. On the day of test, the participant rinsed her mouth with water before collection of baseline saliva (1.5 mL) and blood samples (400 μl). Body weight and percent of body fat were measured using bioimpedance method (Omron HBF-903, Omron Healthcare, Japan).

During the exercise test, the participant pedaled for 60 min at a rate of 60 rpm against a resistance corresponding to 75% AT. Fluid was consumed *ad libitum* to maintain to ensure that the participant was well-hydrated. Heart rate and blood pressure were monitored using an automated monitor (Bedside monitor BSM-2401, Nihon Kohden, Japan).

The subjective fatigue was measured using a visual analogue scale [15]. To control for diurnal variations, all the testing sessions in the respective menstrual phases were conducted between 1400 h to 1700 h.

Blood and saliva sampling

For each sampling interval, the participant rinsed her mouth with water before 1.5 mL of unstimulated saliva sample was collected by passive drool into pre-weighted sterile plastic containers for measurement of 17β-estradiol and progesterone. Saliva samples were centrifuged at 3,000 rpm for 15 min at room temperature and were stored at -20°C until the day of analysis.

All saliva samples were centrifuged again on the day of the assay to remove particulate matter. Salivary 17β-estradiol and progesterone concentrations were measured using commercial enzyme-linked immunosorbent assays (ELISA) kits (Salimetrics, PA, USA) according to the instructions of the manufacturer and the optical density was read with a microplate reader (VersaMax, Molecular Devices Inc, CA, USA).

Capillary blood samples were collected at three time points: before (baseline), immediately after (post-EX) and 30 min after exercise (30 min post-EX). Capillary blood samples were collected from the fingertip into at least five heparinized capillary tubes for hematocrit measurement. The capillary tubes were immediately centrifuged at 12,000 rpm for 5 min at room temperature. After the hematocrit measurement, the plasma was separated into microtubes and stored at -20°C until the day of assay to determine plasma parameters concentration.

The plasma concentration of IL-6 was measured using a commercial ELISA kit (Quantikine High Sensitivity, R&D Systems, MN, USA). Plasma IL-8 concentration was measured using a commercial immunoassay kit (OptEIA, Becton Dickinson, CA, USA). Plasma myeloperoxidase and calprotectin concentrations were measured using ELISA kits (Hycult Biotechnology, The Netherlands). To account for fluid shifts known to occur with exercise and dehydration, all values were corrected for changes in plasma volume. Percent change in plasma volume was calculated using hematocrit measurements, according to the formula of van Beaumont [16].

Statistical Analysis

All the data are presented as means ± standard errors (SE). Data were first tested for normality with the Shapiro-Wilk tests. Non-normally distributed data were log-transformed to natural log before

the data were analysed. To examine the effect of menstrual cycle, a two-way repeated-measures analysis of variance (ANOVA) was applied to assess the main effects and interactions between time and the 3 menstrual phases. When a significant difference was established in the ANOVA, post-hoc pairwise comparisons were analysed using the Bonferroni test. Pearson/Spearman correlation coefficients were computed between the changes in plasma IL-6 and calprotectin concentrations. The threshold for statistical significance was set at $p < 0.05$. Statistical analysis was carried out using PASW Statistics 18 software (SPSS, Chicago, IL, USA).
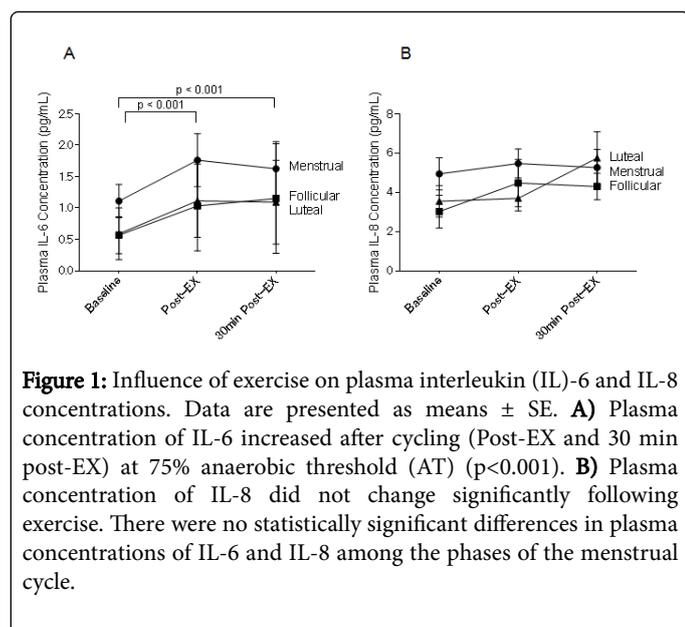
Results

Basal body temperature was higher in the luteal phase (36.55 ± 0.24°C) compared with the menstrual (36.30 ± 0.19°C) and follicular (36.21 ± 0.12°C) phases ($p < 0.05$). Resting salivary 17β-estradiol concentration was significantly higher in the luteal phase (3.26 ± 1.22 pg/mL) compared with the menstrual (2.25 ± 0.73 pg/mL) and follicular (2.37 ± 0.61 pg/mL) phases ($p < 0.05$).

Resting salivary progesterone concentration was significantly higher in the luteal phase (253.27 ± 135.56 pg/mL) compared with the menstrual (97.07 ± 47.75 pg/mL) and follicular (89.16 ± 37.12 pg/mL) phases ($p < 0.001$). These results indicate that the participants had normal menstrual cycles.

Body mass (48.91 ± 5.41 kg) and percent body fat (23.90 ± 3.43%) were similar between the conditions. $\dot{V}O_2$ at the individual AT was 20.47 ± 3.95 mL/kg/min. The moderate aerobic exercise was performed for 60 min at 75% of AT (work rate 45.60 ± 5.74 watts).

The participants remained under AT during steady-state exercise in all three trials. The heart rate was significantly elevated from baseline (70 ± 8 bpm) to post-exercise (127 ± 20 bpm) ($p < 0.001$). Changes in heart rates and subjective fatigue determined by visual analogue scale were similar during all three phases.

Figure 1: Influence of exercise on plasma interleukin (IL)-6 and IL-8 concentrations. Data are presented as means ± SE. **A)** Plasma concentration of IL-6 increased after cycling (Post-EX and 30 min post-EX) at 75% anaerobic threshold (AT) ($p < 0.001$). **B)** Plasma concentration of IL-8 did not change significantly following exercise. There were no statistically significant differences in plasma concentrations of IL-6 and IL-8 among the phases of the menstrual cycle.
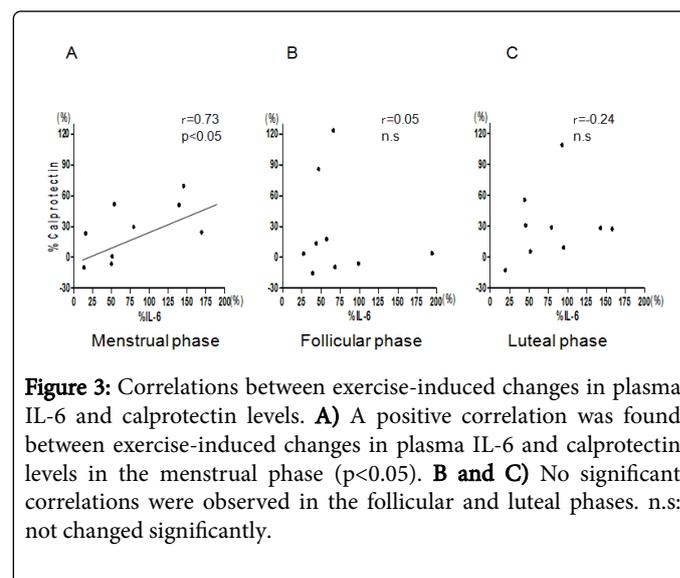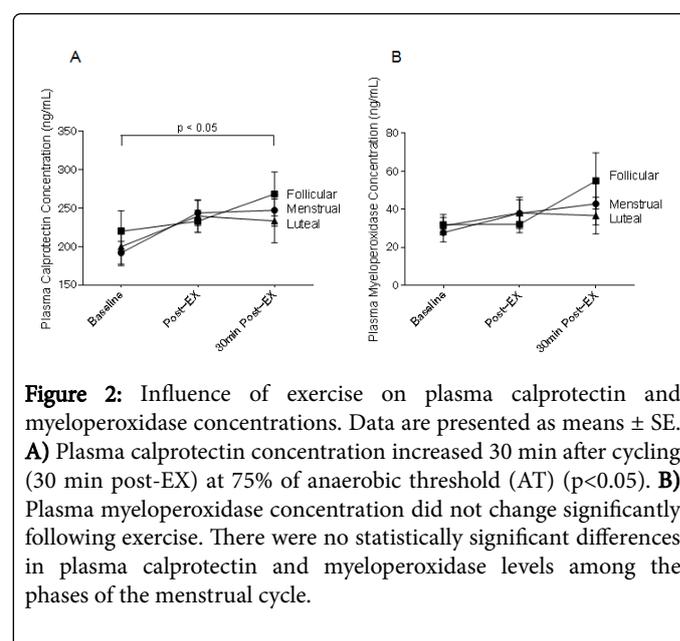
Plasma IL-6 concentration was significantly elevated post-exercise and 30 min post-exercise in all phases of menstrual cycle (Figure 1).

No significant change in plasma IL-8 concentration was found after any of the trials (Figure 1). There were no statistically significant differences in plasma IL-6 and IL-8 levels between the phases of menstrual cycle.

Plasma calprotectin concentration increased significantly 30 min post-exercise in all phases of menstrual cycle (Figure 2). Plasma myeloperoxidase concentration did not change significantly following any of the trials (Figure 2). There were no statistically significant differences in plasma calprotectin and myeloperoxidase levels among the phases of the menstrual cycle.

A positive correlation was found between exercise-induced changes in plasma IL-6 and calprotectin levels in the menstrual phase (r=0.73, $p < 0.05$), whereas no significant correlations were observed in the follicular and luteal phases (Figure 3).

Figure 2: Influence of exercise on plasma calprotectin and myeloperoxidase concentrations. Data are presented as means ± SE. **A)** Plasma calprotectin concentration increased 30 min after cycling (30 min post-EX) at 75% of anaerobic threshold (AT) ($p < 0.05$). **B)** Plasma myeloperoxidase concentration did not change significantly following exercise. There were no statistically significant differences in plasma calprotectin and myeloperoxidase levels among the phases of the menstrual cycle.

Figure 3: Correlations between exercise-induced changes in plasma IL-6 and calprotectin levels. **A)** A positive correlation was found between exercise-induced changes in plasma IL-6 and calprotectin levels in the menstrual phase ($p < 0.05$). **B and C)** No significant correlations were observed in the follicular and luteal phases. n.s: not changed significantly.

Discussion

The aim of this study was to compare inflammatory responses to moderate intensity exercise in the different phases of the menstrual cycle (menstrual, follicular and luteal). In all the 3 menstrual cycle phases, plasma IL-6 concentration increased significantly following exercise, and plasma calprotectin concentration increased significantly 30 min post-exercise. A positive correlation was found between exercise-induced changes in plasma IL-6 and calprotectin levels in the menstrual phase only.

Gender-related differences in immune response to exercise have received growing attention, but in-depth investigations on the influences of menstrual cycles on exercise-induced immune responses are rare. It has been shown that plasma concentrations of calprotectin and myeloperoxidase were increased following prolonged endurance exercise in studies using males [9,10,17] although myeloperoxidase did not change significantly in our study. Since the plasma concentration of myeloperoxidase is influenced by exercise intensity and thermal stress [9,17], it is possible that the 75% of AT in our study was not high enough to induce a significant increase in myeloperoxidase response. Our evidence suggests that calprotectin could be a more sensitive marker of exercise-induced inflammatory response in women.

Calprotectin (otherwise known as S100A8/A9) is a known marker for inflammation and is present in the cytoplasm of neutrophils and surface of monocytes [12]. Upon neutrophil activation or monocyte adhesion to the endothelium, calprotectin is released and may provide not only bacteriostatic, but also cytokine-like effects in the local environment [12]. Neutrophil infiltration is observed in the endometrium in the menstrual phase [5]. Salamonsen et al. [18] reported that during most part of the menstrual cycle, neutrophils are barely detectable in normal endometrium but the number of neutrophils rises perimenstrually, making up 6-15% of the total cell number in the tissue at this time. Recent research also indicated that calprotectin was elevated in plasma of women with endometrial carcinoma [19] and morbid obesity [20]. These findings suggest that the inflammatory condition of the endometrium might be reflected in the plasma calprotectin.

In the present study, resting plasma IL-6 levels were not different throughout the menstrual cycle, and the same exercise-induced changes in plasma IL-6 concentration were observed in all phases. Another study also reported that the resting and the exercise-induced increases in IL-6 were not different in the follicular and the luteal phases [3]. A positive correlation was observed between exercise-induced changes in plasma IL-6 and calprotectin levels only in the menstrual phase, which may be explained by the clinical research findings that IL-6 enhanced neutrophil degranulation when inflammation co-exists in the body [21].

Therefore, strenuous exercise, higher than 75% AT, increases IL-6 and may accentuate stress and inflammatory responses, especially in the menstrual phase. Mortensen et al. [22] found that calprotectin increased following endurance exercise for 3 h and caused a net release of calprotectin from the working leg in male subjects. Further studies will be required to examine the implications of these results in women during different phases of the menstrual cycle.

Previous studies have examined exercise-induced inflammation in female subjects [3,4,7,23-25]. However, studies that have examined inflammatory reaction following exercise in the menstrual phase are limited. In an unpublished study, we investigated the effects of exercise intensity during the menstrual phase and found a marked increase in inflammatory response at 100% AT compared with 75% AT (unpublished data). These findings suggest that exercise intensity may exert a greater effect on inflammatory response than does the effects of the menstrual phase itself. Considering the increasing number of women who exercise to improve their physical fitness, it is important to continue to develop the most appropriate measures to advise and manage the influence of menstrual cycle on exercise-induced inflammation and stress. The present results suggest that calprotectin may be a good marker of exercise-induced inflammation.

Conclusion

In summary, exercising at 75% AT significantly increased the plasma concentration of IL-6 and calprotectin, but did not significantly influence plasma IL-8 and myeloperoxidase concentrations. Calprotectin may be useful as a marker of exercise-induced inflammatory reaction during the menstrual cycle. In addition, because a correlation was observed between plasma IL-6 and calprotectin responses in the menstrual phase, strenuous endurance exercise at an intensity of higher than 75% AT has the potential to promote an inflammatory reaction, especially in the menstrual phase of sedentary females.

Acknowledgements

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