Influence of gender, menstrual phase, and oral contraceptive use on immunological changes in response to prolonged cycling

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Timmons, Brian W., Mazen J. Hamadeh, Michaela C. Devries, and Mark A. Tarnopolsky. Influence of gender, menstrual phase, and oral contraceptive (OC) use on immunological changes in response to prolonged cycling. J Appl Physiol 99: 979–985, 2005. First published May 5, 2005; doi:10.1152/japplphysiol.00171.2005.—This study determined the influence of gender, menstrual phase (MP), and oral contraceptive (OC) use on immunological changes in response to endurance exercise. Twelve women and 11 men similar in age, aerobic power, and activity level cycled for 90 min at 65% maximal aerobic power. Women were OC users (n = 6) or nonusers (NOC) and cycled during the follicular (Fol) and the luteal (Lut) phases. Venous blood was collected before and after exercise to determine leukocyte counts, IL-6 concentrations, and cortisol. Higher resting levels of neutrophils (~1.5-fold) and cortisol (~2.5-fold) were found in OC vs. NOC and men. Exercise-induced immune cell count and IL-6 changes were similar between men and NOC, except for an ~38% greater lymphocyte response in NOC vs. men (P = 0.07). Neutrophil, monocyte, and lymphocyte responses to exercise during Lut in OC were greater than during Fol and also greater than the responses in men (P = 0.003). Changes in immune cell counts were consistently greater during Lut in OC vs. NOC, regardless of MP, but only neutrophil responses reached statistical significance (P = 0.01). The exercise-induced change in IL-6 was ~80% greater in NOC vs. OC during Fol (P = 0.06), but it was similar between these groups during Lut. Cortisol changes with exercise were not different between groups or MP. These results highlight the necessity to control for gender, and in particular OC use, when designing studies evaluating exercise and immunology.

interleukin-6; lymphocytes

THE INFLUENCE OF GENDER ON physiologival responses to exercise has received much attention in the last decade. The presence of, and fluctuations in, sex hormones appear to be important in regulating substrate utilization (52), muscle fatigue (22), temperature regulation (25), and endocrine responses (13) during exercise in humans. Gender differences in exercise responses have clear implications for understanding gender-specific adaptations to exercise for athletic performance and overall health. The impact of exercise on immune function has also received considerable attention in recent years (44), but to what extent gender and fluctuations in sex hormones influence immunological responses to exercise is unclear.

Sex hormones play important roles in the immune system under nonexercise conditions, and several gender-related differences in immune function have been identified. For example, women tend to have greater responsiveness to immunization (35), higher serum concentrations of some immunoglobulins (Ig) (19), a higher absolute number of T-helper lymphocytes (1), and a differential regulation of cytokine production (19, 29). Moreover, compared with the follicular phase (Fol), the luteal phase (Lut) of the menstrual cycle is associated with increased concentrations of leukocyte and lymphocyte subsets (8, 17), a greater capacity of immune cells to produce cytokines (8, 17, 27), greater plasma cytokine activity (29), but a variable effect on plasma cytokine levels (2, 10, 27). In contrast, other studies associate Fol with greater cytokine production from immune cells (29, 47) and higher serum IL-6 levels (2). Considering these differences in resting immune function between men and women and between phases of the menstrual cycle, we were interested in possible gender and menstrual phase (MP) effects on immunological changes in response to exercise.

Notwithstanding a wealth of exercise immunology literature (32, 44), relatively few studies have compared exercise-induced immune changes between men and women or between phases of the menstrual cycle. A number of publications have reported that exercise-induced changes in cell counts and function (3, 33, 37–39, 57) and plasma cytokine levels (33, 56) were not different between men and women. However, it appears that these studies did not control for the menstrual status of the women at the time of testing. Most of these studies were also unclear on how subjects were matched or what, if any, dietary controls were implemented. In a recent paper (42), no differences in immune cell counts or cytokine levels were reported between 12 women and 84 men competing in a marathon. The average age of the entire subject pool was ~42 yr, and there was no mention as to the age of the women, whether they maintained a regular menstrual cycle, at what phase of the menstrual cycle testing occurred, or whether they used oral contraceptives (OC). In other studies, no effect of menstrual cycle was reported on cytokine responses to walking exercise in the cold (20) or on resting salivary IgA levels during a period of training (9). In contrast to studies reporting no differences, other investigations have reported gender differences in immune-related responses to cycling (14) and eccentric exercise (30, 51). In at least one of these studies (51), the phase of the menstrual cycle in which women were tested and OC use were standardized. Thus it appears that the effects of gender and MP on immunological responses to prolonged exercise in humans are unclear due to a lack of systematic, well-controlled investigations.

Therefore, this study was designed, in part, to clarify the influence of gender and MP on immunological changes in...
response to endurance exercise. We recruited women who were users of OC and nonusers (NOC) to elucidate possible influences of OC use on immunological changes in response to exercise, in light of previous research documenting clear effects of OC use on the immune system (41, 59) and associated hormones [e.g., cortisol (34)] under resting conditions.

METHODS

Subjects. Twelve healthy young women and 11 healthy young men volunteered to participate in this study approved by the McMaster University Research Ethics Review Board. All subjects provided written, informed consent before participating. Six of the 12 women were OC users, and all used a triphasic OC type of estrogen and progesterone; 4 used Tricyclen 28, 1 used Tricyclen 21, and 1 used Triphasil 28. Women in the NOC group maintained regular menstrual cycles. Physical and fitness characteristics of the three groups are given in Table 1. Age and maximal aerobic power ($V_{\text{O2 max}}$) expressed relative to fat-free mass were not different between groups, and they engaged in similar amounts of self-assessed recreational activity as prospectively recorded in physical activity logs. All subjects were free of disease and infection and were not taking medications.

Study design. In a preliminary session, $V_{\text{O2 max}}$ was assessed and anthropometric data were collected for each subject. At least 7 days after this initial visit, subjects completed an experimental session that consisted of cycling for 90 min at 65% of their previously determined $V_{\text{O2 max}}$ with venous blood samples collected before (Pre) and immediately after (Post) exercise. Women completed one experimental session during Fol (day 2 ± 1) and one during Lut (day 20 ± 2). There were no differences between the OC and NOC groups as to what day of the menstrual cycle testing occurred. In addition, ovulation was confirmed in NOC women with an ovulation kit (Clear Plan Easy Ovulation Test, Novartis Canada, Mississauga, ON, Canada). To counterbalance the order in which women completed the experimental sessions, six started during Fol and six during Lut. This design required some women to complete all testing within one menstrual cycle, and some required testing to carry over into their next cycle.

Preliminary session. The preliminary session was primarily for the purpose of determining individual $V_{\text{O2 max}}$ values using a progressive, continuous protocol conducted on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands) with a computerized open-circuit gas-collection system (Moxus Modulator oxygen uptake system with O2 analyzer S-3A/I and CO2 analyzer CD-3A, AEI Technologies, IL). All subjects completed 2 min of cycling at 75 W followed by 2 min at 150 W and then 2 min at 200 W. Power output was then increased by 25-W increments every minute until pedaling rate could not be maintained over 60 rpm despite strong encouragement. $V_{\text{O2 max}}$ was taken as the highest 15-s value during the test and confirmed by an apparent plateau in oxygen uptake and/or a respira-

tory exchange ratio >1.12. Power output corresponding to 65% of oxygen uptake was then estimated from the linear relationship between power output and oxygen uptake, and this work rate was confirmed by measuring oxygen uptake at the estimated work intensity for each subject ~30 min after the maximal test.

Experimental session. To avoid the possible influence of prior diet on the effects of exercise on immunological responses, all subjects completed prospective diet records for a minimum of 2 weekdays and 1 weekend day before the first trial, and the diet preceding the subsequent trial (for the women) was consistent for each subject. Subjects were also instructed to maintain their regular exercise routines throughout the study, but no strenuous exercise was allowed during the 2 days before each testing session.

After an overnight fast (~10 h), subjects arrived at the laboratory at the same time early in the morning (either 0700 or 0900) to ensure that timing of exercise and blood collection was consistent across all subjects and all sessions. Because this study was a small part of the subjects’ participation in a larger experiment related to energy metabolism, they were not allowed to eat until testing was completed, but water was provided to maintain body hydration during the testing day. After supine rest, fat-free mass was estimated by bioelectric impedance analysis (model BIA-101A, RJL Systems, Mt. Clemens, MI). A 20-gauge plastic catheter (Becton Dickinson, Franklin Lakes, NJ) was then placed into the antecubital vein of the right arm for blood collection. Blood samples were drawn into one EDTA vacutainer, one heparin vacutainer, and one vacutainer without anticoagulant (Becton Dickinson) at Pre and Post of the cycling task. To eliminate the possibility that ex vivo IL-6 production and secretion from circulating immune cells contributed to plasma levels, the heparin vacutainer was pretreated with brefeldin A (at a final concentration of 10 μg/ml whole blood) to prevent release of intracellular IL-6. Blood in the anticoagulant-free vacutainer was allowed to clot at room temperature, and the heparinized vacutainer was placed on ice until both tubes were centrifuged at 1,750 g at 4°C for 10 min. Plasma and serum were then separated and stored at −50°C until analyzed.

Blood analyses. EDTA-treated blood samples were well mixed and left at room temperature before being delivered to the McMaster University Medical Centre Core Laboratory for subsequent analysis of total leukocytes, neutrophils, lymphocytes, and monocytes using an automated Coulter counter. This blood sample was also used to determine hemoglobin and hematocrit so that blood and plasma volume changes could be estimated according to Dill and Costill (16). All immune cell counts were adjusted for exercise-induced changes in blood volume.

Determination of testosterone, estradiol, progesterone, and cortisol was performed in duplicate on Pre serum samples using solid-phase radioimmunoassays (catalog no. TKTT1, TKE21, TKPG1, and TKCO1, respectively, from Diagnostic Products, Los Angeles, CA). Cortisol was also determined on Post samples. The intra- and interassay coefficient of variation are <7% for testosterone, ≤8% for estradiol, ≤9% for progesterone, and ≤8% for cortisol. Postexercise cortisol levels were adjusted for changes in plasma volume.

A commercially available “high-sensitivity” ELISA kit (catalog no. HS600B, R&D Systems, Minneapolis, MN) was used to measure plasma levels of IL-6. According to the manufacturer, the sensitivity of this assay is 0.039 pg/ml, and in our hands the intra- and interassay coefficient of variation are ≤9%, respectively. Postexercise IL-6 concentrations were adjusted for changes in plasma volume.

Statistical analyses. All values are given as means ± SD. Subject characteristics (Table 1) were analyzed by one-way ANOVA. Given that we were interested in the recruitment of immune components into the peripheral circulation, we calculated the change in values (i.e., Post–Pre) before being submitted to statistical analyses. To determine the effects of OC and MP on resting concentrations of hormone, immune cell counts, and IL-6 and the immune and hormone changes with exercise, women were analyzed separately from men with a two-way repeated-measures ANOVA (group × phase). The corresponding

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men</th>
<th>NOC</th>
<th>OC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>11</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Age, yr</td>
<td>21±1</td>
<td>21±2</td>
<td>22±3</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.8±0.1</td>
<td>1.7±0.0†</td>
<td>1.6±0.0†</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>77±11</td>
<td>66±10</td>
<td>58±8.8</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>18±4</td>
<td>31±4†</td>
<td>25±3†‡</td>
</tr>
<tr>
<td>$V_{\text{O2 max}}$, ml·kg·BM$^{-1}$·min$^{-1}$</td>
<td>45±5</td>
<td>37±9†</td>
<td>40±6</td>
</tr>
<tr>
<td>$V_{\text{O2 max}}$, ml·kg·FFM$^{-1}$·min$^{-1}$</td>
<td>56±6</td>
<td>54±11</td>
<td>54±9</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects; NOC, non-oral contraceptive users; OC, oral contraceptive users; $V_{\text{O2 max}}$, maximal aerobic power; BM, body mass; FFM, fat-free mass. †Significantly different from men, $P ≤ 0.05$. ‡Significantly different from men, $P ≤ 0.0125$. ‡Significantly different from NOC, $P ≤ 0.005$. 

Downloaded from http://jap.physiology.org/ by 10.220.33.1 on September 22, 2017.
resting values and exercise-induced changes for the men were then compared with each of the women’s values (i.e., NOC Fol and Lut, OC Fol and Lut) by a Student’s independent t-test with a Bonferroni correction such that a P value of ≤0.0125 was taken as significant and P values of >0.0125 and ≤0.05 were considered as trends. When ANOVAs were used, significance was set at P ≤ 0.05, and where appropriate, a Tukey’s honestly significant difference post hoc test was applied to determine the significance among means. Resting concentrations of immune cell counts and IL-6 and their respective changes with exercise were tested for a normal distribution, which was confirmed. STATISTICA for Windows 5.0 (StatSoft, Tulsa, OK) software was used to perform ANOVAs and to test for normality. Microsoft Office Excel 2003 (Redmond, WA) software was used to perform t-tests. To assess the association between selected changes in immune parameters and resting sex hormone concentrations, Pearson correlations were calculated. In all cases, correlations were performed with GraphPad Prism 3.0 (GraphPad Software, San Diego, CA) and considered significant when P ≤ 0.05.

RESULTS

Hormones. Table 2 provides resting hormone concentrations for the three groups. Estradiol levels in men were higher than in NOC during both Fol (P = 0.018) and Lut (P = 0.002) but lower than in NOC during Lut only (P = 0.015). Progesterone levels in men were higher but not statistically different from OC during Fol (P = 0.031) and Lut (P = 0.036) and higher than NOC during Fol (P = 0.017), but they were lower than NOC during Lut (P = 0.009). Testosterone was higher in men than either group of women (P < 0.001 for all comparisons).

Estradiol levels were lower in OC compared with NOC (main effect group, P = 0.007), but they did not fluctuate significantly across MP (main effect phase, P = 0.38). Progesterone levels were similar in OC across phases. In NOC, progesterone was higher during Lut than Fol (P = 0.02). Consequently, progesterone during Lut in NOC was greater than during Lut in OC (P = 0.02). Testosterone levels in OC were lower than NOC (main effect group, P = 0.01).

Resting cortisol concentrations in OC were ~2.5-fold higher than in NOC (main effect group, P = 0.058) and men (P < 0.01 for Fol and Lut comparisons). Only cortisol was measured after exercise, and the absolute change in concentration was similar between OC and NOC (308 ± 201 vs. 430 ± 306 nmol/l; P = 0.51), Fol and Lut (485 ± 316 vs. 277 ± 351 nmol/l; P = 0.18), and men and women (223 ± 317 vs. 485 ± 316 nmol/l; P = 0.24).

Immune cell counts. Table 3 provides Pre and Post values of immune cell counts and IL-6 for the three groups. Compared with men, OC had higher but not statistically different total leukocyte and neutrophil counts during Fol (P = 0.022 and P = 0.027, respectively) and Lut (P = 0.029 and P = 0.05, respectively). There were no differences between men and NOC in any of the measured immune cells at rest. The total leukocyte count was higher but not statistically different in OC

Table 2. Resting hormone concentrations in men and in women during two phases of the menstrual cycle

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Men</th>
<th>Fol</th>
<th>Lut</th>
<th>NOC</th>
<th>Fol</th>
<th>Lut</th>
<th>OC</th>
<th>Fol</th>
<th>Lut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol, pmol/l</td>
<td>134±70</td>
<td>195±188</td>
<td>361±263*</td>
<td>42±41bc</td>
<td>14±11bc</td>
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<tr>
<td>Progesterone, nmol/l</td>
<td>2.9±1.0</td>
<td>1.8±0.4*</td>
<td>10.3±8.3bc</td>
<td>1.9±0.6bc</td>
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<tr>
<td>Testosterone, nmol/l</td>
<td>19.9±3.7</td>
<td>1.2±0.2b</td>
<td>1.0±0.4b</td>
<td>0.7±0.2bc</td>
<td>0.7±0.1bc</td>
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<tr>
<td>Cortisol, nmol/l</td>
<td>570±145</td>
<td>527±433</td>
<td>535±540</td>
<td>1,364±772bd</td>
<td>1,355±818bd</td>
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</tbody>
</table>

Values are means ± SD. Fol, midfollicular phase; LUT, midluteal phase. *Significantly different from men, P ≤ 0.05. †Significantly different from men, P ≤ 0.0125. ‡Main effect for group (OC < NOC), P ≤ 0.05. ‡Main effect for group (OC > NOC), P = 0.058. †Main effect for phase (Lut > Fol), P ≤ 0.05. †Interaction effect, significantly different from LUT in NOC, P = 0.02. ‡Interaction effect, significantly different from Fol within NOC, P = 0.02.

Table 3. Immune cell counts and IL-6 at rest and immediately after exercise in men and in women during two phases of the menstrual cycle

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Men</th>
<th>Fol</th>
<th>Lut</th>
<th>NOC</th>
<th>Fol</th>
<th>Lut</th>
<th>OC</th>
<th>Fol</th>
<th>Lut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes, cells × 10^9/l</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>5.6±1.5</td>
<td>5.9±1.3</td>
<td>6.4±1.0</td>
<td>8.4±3.0†</td>
<td>7.6±1.9†</td>
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<tr>
<td>Post</td>
<td>8.5±2.3</td>
<td>8.7±1.1</td>
<td>9.6±1.2</td>
<td>11.9±3.7</td>
<td>14.4±0.8</td>
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<tr>
<td>Neutrophils, cells × 10^9/l</td>
<td>3.2±1.3</td>
<td>3.6±1.4</td>
<td>4.1±1.2</td>
<td>5.7±3.0*</td>
<td>4.8±1.9*</td>
<td></td>
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<tr>
<td>Pre</td>
<td>5.0±1.8</td>
<td>5.2±1.0</td>
<td>5.8±1.3</td>
<td>8.2±3.5</td>
<td>9.6±0.9</td>
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<tr>
<td>Post</td>
<td>5.0±0.1</td>
<td>0.4±0.1</td>
<td>0.5±0.1</td>
<td>0.5±0.2</td>
<td>0.5±0.2</td>
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<tr>
<td>Monocytes, cells × 10^9/l</td>
<td>0.7±0.2</td>
<td>0.7±0.1</td>
<td>0.8±0.2</td>
<td>0.7±0.2</td>
<td>1.0±0.2</td>
<td></td>
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</tr>
<tr>
<td>Lymphocytes, cells × 10^9/l</td>
<td>1.7±0.3</td>
<td>1.5±0.4</td>
<td>1.6±0.4‡</td>
<td>1.8±0.3</td>
<td>2.0±0.3‡</td>
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</tr>
<tr>
<td>Pre</td>
<td>2.5±0.4</td>
<td>2.6±0.4</td>
<td>2.7±0.4</td>
<td>2.7±0.5</td>
<td>3.4±0.7</td>
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<td></td>
</tr>
<tr>
<td>Post</td>
<td>2.0±1.1</td>
<td>2.0±0.8</td>
<td>2.0±1.4</td>
<td>2.4±1.5</td>
<td>2.0±0.5</td>
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</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>6.7±2.8</td>
<td>7.7±2.4</td>
<td>6.3±0.8</td>
<td>5.5±2.1</td>
<td>6.8±1.0</td>
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</tbody>
</table>

Values are means ± SD. Pre, preexercise; Post, immediately after exercise. *Significantly different from men, P ≤ 0.05. †Main effect for group (OC > NOC), P = 0.08. ‡Main effect for phase (Lut > Fol), P = 0.03.
vs. NOC (main effect group, \( P = 0.08 \)), whereas the lymphocyte count was higher during Lut than Fol (main effect phase, \( P < 0.001 \)).

Figure 1 provides the exercise-induced changes in immune cell counts for the three groups. The observed changes in total leukocytes, neutrophils, lymphocytes, and monocytes in OC during Lut were greater than in men (\( P < 0.001 \) for all comparisons). Because there were no differences in immune cell responses between MP in NOC, these data were pooled before being compared with men. Total leukocyte, neutrophil, and monocyte changes were not different between men and NOC, but the change in lymphocytes in NOC was greater but not statistically different from in men (\( P = 0.07 \)).

The exercise-induced change in total leukocytes in OC was greater during Lut than Fol (\( P = 0.02 \)) and greater than in NOC during Fol (\( P = 0.005 \)) and Lut (\( P = 0.01 \)). A similar statistical pattern was evident for neutrophils. The change in monocytes in OC was greater during Lut compared with Fol (\( P = 0.04 \)). Similarly, the change in lymphocytes in OC during Lut was higher but not statistically different from during Fol (\( P = 0.08 \)).

**IL-6.** Resting IL-6 levels were not different between OC and NOC or MP, and women’s values were not different from those of the men. The exercise-induced change in IL-6 (Fig. 2) was not different between MP or between men and women. The change in IL-6 during Fol, however, was greater but not statistically different in NOC vs. OC (\( P = 0.06 \)).

**Correlations.** Resting levels of sex hormones were not correlated with the exercise-induced increase in any immune variable (\( P = 0.20 \) for all correlations). Despite significant percent body fat differences between the groups, it was not associated with any immunological measures.

**DISCUSSION**

This study determined the influence of gender, MP, and OC use on immunological changes in response to exercise. The results indicate that OC use increases the magnitude of change in commonly reported aspects of the cellular immune system during Lut, but not Fol. The exercise-induced increase in lymphocytes but not in total leukocytes, neutrophils, or monocytes, averaged across MP, were greater in NOC vs. men. In addition, the IL-6 response to exercise during Fol was greater in NOC vs. OC. Finally, our observations at rest confirm previous findings of elevated cortisol in OC users (34), compared with NOC and men.

Although investigations into immunological responses to exercise have grown exponentially in the last two decades, our understanding of differences or similarities in the responses among men and women from earlier studies has been weakened by several methodological limitations. We specifically designed our experiment to control for fitness and activity levels and diet of the subjects. Furthermore, each subject exercised at an identical intensity, relative to their individual

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Fig. 1. Changes (\( \Delta \)) in total leukocytes (A), neutrophils (B), monocytes (C), and lymphocytes (D) after endurance exercise in men and in women during 2 phases of the menstrual cycle. Values are means ± SD. Fol, midfollicular phase; Lut, mid-luteal phase; NOC, non-oral contraceptive users; OC, oral contraceptive users. *OC Lut different from OC Fol (\( P = 0.02 \)), NOC Lut (\( P = 0.01 \)), NOC Fol (\( P = 0.005 \)), and men (\( P < 0.001 \)). **OC Lut different from OC Fol (\( P = 0.07 \)), NOC Lut (\( P = 0.01 \)), NOC Fol (\( P = 0.01 \)), and men (\( P < 0.001 \)). ***OC Lut different from OC Fol (\( P = 0.04 \)) and men (\( P < 0.002 \)). ****OC Lut different from NOC Fol (\( P = 0.08 \)) and men (\( P < 0.003 \)). †NOC, averaged across menstrual phases, different from men (\( P = 0.07 \)).

Fig. 2. Changes in IL-6 after endurance exercise in men and in women during 2 phases of the menstrual cycle. Values are means ± SD. *OC Fol different from NOC Fol (\( P = 0.06 \)).
maximal capacity. With this experimental design, our comparison of men and women not using OC is consistent with previous reports by showing no significant effect of gender on changes in total leukocytes, neutrophils, and monocytes in response to exercise (3, 33, 38, 57). In contrast, our data show that the lymphocyte response in women not taking OC is greater than in men. This result is consistent with the findings of De Lanne et al. (14), who reported an ~82% greater lymphocyte increase in women vs. men after 30 min of cycling. In the present study, the increase in lymphocytes was ~38% greater in NOC vs. men.

The reasons for this apparent gender difference in the lymphocyte response to exercise are unclear, but because there was no difference in responses between MP, fluctuations in sex hormones may not be the primary mechanism of action. The lymphocyte response to exercise is composed of cumulative changes in lymphocyte subsets, but it is driven by natural killer cells, which are the most responsive cell type to exercise (44) because of their high surface density of β2-adrenergic receptors (28). Exercise-induced lymphocytosis is mediated by an exercise-induced increase in epinephrine (31, 55). Paradoxically, epinephrine responses to exercise are generally smaller in women than in men (13, 24), but women show greater lymphocyte β2-adrenergic receptor density (58) and post-receptor activity (21, 36) compared with men. Thus the catecholamine-induced downregulation of adhesion molecule expression (40) leading to lymphocyte mobilization during exercise may be greater in women than in men. Because NK cell activity of whole blood is less in women than in men (5, 59), an overall greater lymphocytosis in response to exercise in women may be a compensatory mechanism to maintain functional immune status in the face of physiological stress.

Another main finding in this study is that the leukocytosis of exercise fluctuated across the menstrual cycle in OC but not in NOC. Specifically, exercise-induced changes in total leukocytes, neutrophils, monocytes and lymphocytes during Lut were greater than during Fol in OC. These changes during Lut in OC were always greater than the exercise-induced changes in men. Similarly, the changes in all immune cell counts were consistently greater in OC during Lut than in NOC, regardless of MP. However, statistical significance was reached for the changes in total leukocytes and neutrophils only. The greater neutrophil response in OC vs. NOC and men may be due to a greater growth hormone (GH) response in the former group. It has been shown that GH infusion increases circulating neutrophil levels (26) and that GH responses to exercise are greater in OC users (4, 6). The high cortisol levels in OC may have also influenced neutrophil trafficking in response to exercise by inhibiting cell adhesion to the endothelium (12). However, cortisol levels cannot explain why neutrophil changes were different between MP within OC because this hormone remained constant across MP. Alternatively, exercise-induced changes in epinephrine may also account for differences in neutrophil responses because epinephrine infusion also increases circulating neutrophil levels (55). However, the available literature does not support differences between OC users and nonusers in epinephrine responses to exercise (6, 43). Regardless of the mechanism(s) mediating the observed effects of OC use, it seems clear that the presence of synthetic hormones has a greater influence on exercise-induced changes in immune cell counts than normal fluctuations in endogenous sex hormones. It is also important to note that hormone replacement therapy (HRT) in postmenopausal women has only a minor impact on resting concentrations of immune cells (45). However, whether exercise-induced changes in immune cell counts are different in women taking vs. not taking HRT is unclear.

Although IL-6 levels at rest were virtually identical among groups, the exercise-induced increase in IL-6 during Fol was ~80% greater in NOC vs. OC. That resting IL-6 levels were not different between NOC and OC is consistent with a lack of HRT effect on serum IL-6 levels in postmenopausal women (45). During Fol, resting estradiol levels were greater in NOC vs. OC but were not correlated with the change in IL-6, and it is unlikely that exercise-induced increases in estradiol or progesterone were greater in NOC vs. OC (7). Alternatively, changes in systemic IL-6 levels during exercise may be related to metabolic responses insofar as release of IL-6 from skeletal muscle is enhanced when muscle glycogen is low (48), potentially to induce an increase in liver glucose output (50). In our women, however, postexercise glycogen content (15) and glucose rate of appearance (M. C. Devries, M. J. Hamadeh, and M. A. Tarnopolsky, unpublished observations) were not different between NOC and OC during Fol. Therefore, future work should examine whether OC use influences IL-6 release from contracting muscle (50) and/or uptake by the liver (18) during exercise.

In this study, the exercise-induced increase in neutrophils was greatest during Lut in OC when estradiol levels were the lowest compared with the other groups. Estrogen has been shown to attenuate inflammatory responses to endotoxin in humans (46) and may attenuate inflammatory responses to exercise (53). In ovariectomized rats, estrogen supplementation reduces neutrophil infiltration into skeletal muscle (54). In the present study, we did not find a correlation between resting estradiol levels and changes in neutrophil counts. It may be, however, that the increase in estrogen due to exercise was less during Lut in OC users as has been shown previously (7). A lower estrogen response to exercise in OC may allow a greater inflammatory response to occur in the contracting muscle, leading to greater neutrophil mobilization. Although our laboratory has reported that higher circulating neutrophil counts occur concomitantly with greater muscle inflammation after eccentric exercise in men vs. women (51), the impact of estrogen on these changes remains to be determined.

Our findings of elevated cortisol and neutrophil levels at rest in OC users are consistent with an anti-inflammatory “environment.” Previous work has shown that cortisol levels are higher in OC users vs. nonusers and are also increased during healthy pregnancy (34). In addition, in vitro exposure to synthetic estrogen and progesterone reduces leukocyte transmigration through endothelial cells (23). Thus the higher resting neutrophil counts observed in OC users may be a result of higher circulating cortisol concentrations (12) or a direct effect of synthetic hormones inhibiting their exit from the peripheral circulation. In line with the ~36% overall greater neutrophil levels in OC vs. NOC, Porter et al. (45) reported a smaller nonsignificant 23% higher resting neutrophil count in postmenopausal women taking HRT vs. those not taking HRT. Given the older age of Porter et al.’s subjects (~62 yr), a possible interaction of age and synthetic sex hormone effects on the immune system could explain the smaller differences in
neutrophil counts, and it would be an important area for future research.

Recently (49), it was proposed that IL-6 stimulates cortisol release leading to a neutrocytosis because IL-6 infusion into healthy humans resulted in increased levels of these factors. In the present study, resting levels of cortisol and neutrophil counts were elevated in OC users in the absence of higher resting IL-6 concentrations. Similarly, the greater exercise-induced increase in neutrophils in OC was not accompanied by a greater IL-6 response, and IL-6 is not known to be elevated during healthy pregnancy (11). Our results, therefore, suggest that factors other than IL-6 may be more related to changes in neutrophils and cortisol. However, we did not measure IL-6, neutrophils, or cortisol during recovery from exercise when concentrations of these factors may have continued to change.

In summary, the lymphocytosis of exercise is greater in OC users during Lut and in nonusers compared with men. The presence of synthetic sex hormones influences leukocyte changes during endurance exercise. The effect of exercise on IL-6 is greater in NOC vs. OC during Fol, but not Lut. Our observations at rest (e.g., increased neutrophils and cortisol) confirm an anti-inflammatory environment in OC users. Collectively, these findings highlight the necessity to account for gender when designing studies to evaluate exercise and immunology. In particular, the effects of OC use on lymphocyte subsets and cytokine changes in response to exercise, and their recovery, should be further investigated.

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