No effect of short-term 17β-estradiol supplementation in healthy men on systemic inflammatory responses to exercise

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Timmons, Brian W., Mazen J. Hamadeh, and Mark A. Tarnopolsky. No effect of short-term 17β-estradiol supplementation in healthy men on systemic inflammatory responses to exercise. Am J Physiol Regul Integr Comp Physiol 291: R285–R290, 2006.—Sex-based differences in inflammatory responses to exercise may be mediated by estrogen through increased muscle membrane stability and/or inhibited cytokine production. In this study, in vivo effects of estrogen on systemic inflammation-related responses to exercise were assessed in healthy men. In a double-blind, placebo-controlled, crossover design, 11 men cycled for 90 min at 65% VO2 max after 8 days of 17β-estradiol supplementation (ES; 2 mg/day) or placebo (PL; glucose polymer). After a 2-wk washout, exercise was repeated after 8 days on the alternate treatment. Blood was collected pre- and postexercise to determine IL-6, soluble intercellular adhesion molecule-1 (sICAM-1), neutrophil counts, and cortisol. Preexercise serum was assayed for sex hormones. ES increased estradiol (133 ± 71 to 840 ± 633 pmol/L, P = 0.005) and reduced testosterone (19.9 ± 3.7 to 16.1 ± 3.9 nmol/L, P = 0.007). Exercise increased cortisol (P = 0.02), IL-6 (P < 0.001) and neutrophil counts (P < 0.001) with no influence on sICAM-1 (P = 0.34) and no effect of ES on these changes. Postexercise IL-6 and neutrophil counts were correlated (r = 0.58, P = 0.005); postexercise IL-6 and cortisol (r = 0.18, P = 0.43) and postexercise cortisol and neutrophil counts (r = 0.06, P = 0.78) were not. Postexercise sICAM-1 was not correlated with the above variables (P ≥ 0.79). In conclusion, 8 days of ES in healthy men did not influence systemic inflammation-related responses to acute exercise. Future studies should investigate 17β-estradiol effects on IL-6 production and neutrophil infiltration within skeletal muscle during and after exercise.

neutrophils; interleukin-6; cycling; humans

ACUTE EXERCISE LEADS TO SIGNIFICANT changes in systemic levels of inflammation-responsive mediators such as IL-6 and neutrophils. The origins of these factors during exercise remain speculative, but evidence suggests that IL-6 is derived from contracting skeletal muscle (16, 28), and increased neutrophil levels are a likely consequence of mobilization from marginated pools, including bone marrow (33, 42). The precise roles of exercise-induced changes in IL-6 and neutrophils, however, are less clear. Given the reported findings of increased IL-6 expression (16) and of neutrophil infiltration (7) within skeletal muscle following exercise, one potential role for these factors is to control inflammatory responses arising from contraction-induced muscle damage. To this end, an anti-inflammatory relationship between systemic levels of IL-6, neutrophils, and cortisol has been proposed to operate during exercise (27).

In both human (13, 30) and animal (26, 35) studies, skeletal muscle-specific inflammatory responses to exercise demonstrate significant sex-based differences. In general, females experience less muscle inflammation (26, 30, 35), suggesting that sex hormones exert strong anti-inflammatory effects. In particular, estrogen is believed to play a significant anti-inflammatory role (34), but the mechanisms by which estrogen exerts this effect during exercise are not well understood. At the level of skeletal muscle, estrogen may serve as a muscle membrane stabilizer to minimize disruption of intracellular calcium homeostasis, leading to less calpain-induced production of neutrophil chemoattractants and, thus, less neutrophil infiltration (34). Indeed estrogen supplementation (ES) attenuates neutrophil infiltration of skeletal muscle after exercise in male (35, 36) and ovariectomized female (37) rats and also attenuates calpain activity in the latter group (37). In line with these observations, our laboratory has previously reported less leukocyte infiltration of skeletal muscle accompanied by lower systemic levels of neutrophils during recovery from eccentric exercise in women vs. men (30). Alternatively, estrogen may reduce neutrophil recruitment by inhibiting their release from bone marrow (12) or by interacting with cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), resulting in reduced soluble ICAM-1 (sICAM-1) levels (40). In postmenopausal women, acute ES lowers the plasma IL-6 response to endotoxin in vivo (21), and estrogen can directly inhibit IL-6 production from human bone cells in vitro (8). In contrast, in vitro IL-6 production from rat smooth muscle cells under inflammatory conditions is not attenuated by estrogen (15). The effects of estrogen on IL-6 responses to acute exercise, however, have not been described, nor have estrogen effects on IL-6 expression within skeletal muscle been evaluated. Considering the above evidence, it is possible that ES in humans may attenuate inflammation-related responses (e.g., IL-6 and neutrophils) to exercise. To date, these in vivo effects have not been studied. Because muscle production of IL-6 can account for elevated plasma concentrations of this cytokine (28) and because bone marrow release of neutrophils contributes to their elevated systemic levels during exercise (33, 42),
we reasoned that an effect of estrogen at the tissue level would result in a blunted systemic IL-6 and neutrophil response.

Therefore, this study was designed, in part, to investigate the effects of ES in healthy men on systemic inflammation-related responses to exercise. We hypothesized that ES in men would lead to an attenuation of exercise-induced increases in IL-6 and neutrophil counts. We also measured sICAM-1 as a possible indicator of alterations in cell adhesion molecule expression, due to ES and exercise, thus contributing to neutrophil mobilization. Given the proposed anti-inflammatory effect of IL-6 during exercise (27), we further examined the relationship between IL-6, neutrophil counts, and cortisol in response to exercise and hypothesized that these factors would be statistically associated.

METHODS

Subjects. Eleven healthy recreationally active men volunteered to participate in this study, which was approved by the McMaster University Research Ethics Review Board. The subjects were informed of the study details, advised of the risks and benefits associated with the study, and provided written consent before participating. Their age and physical and fitness characteristics were (mean ± SD): age, 21 ± 1 yr; height, 1.77 ± 0.05 m; weight, 77 ± 11 kg; % body fat, 18 ± 4%; and maximal aerobic power (\(\text{V}_\text{O}_2\text{max}\)), 45 ± 5 ml·kg·body mass⁻¹·min⁻¹.

Study design. The men in this study were part of a larger project of sex-related effects on immunological and metabolic responses to exercise. In a preliminary session, \(\text{V}_\text{O}_2\text{max}\) was assessed and anthropometric data were collected for each subject. Subjects were then randomly assigned to one of two groups in a double-blind, crossover, placebo (PL)-controlled design. One group received 17β-estradiol (Shire BioChem, St. Laurent, Quebec, Canada) for 8 days, whereas the other group received PL (400 mg/days of a glucose polymer, Abbott Laboratories, Ross Division, St. Laurent, Quebec, Canada).

On day 9, the subjects cycled for 90 min at 65% \(\text{V}_\text{O}_2\text{max}\) with venous blood samples collected before and at 90 min of exercise (referred to as preexercise). This intensity and duration of exercise were established to ensure robust elevations in neutrophil counts and IL-6 and to allow all subjects to complete the cycling bout without mid-exercise failure. On the basis of 8-day physical activity records provided by the subjects, the mode, intensity, and duration of exercise were easily achieved. On the basis of 8-day physical activity records provided by the subjects, the mode, intensity, and duration of exercise were easily achieved. On the basis of 8-day physical activity records provided by the subjects, the mode, intensity, and duration of exercise were easily achieved.

Experimental trials. 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. After supine rest, a 20-gauge plastic catheter (Becton Dickinson, Franklin Lakes, NJ) was placed into the antecubital vein of the right arm for blood collection. Approximately 15 min after the first blood sample, subjects commenced cycling (Excalibur Sport, Lode, Groningen, The Netherlands) at the required intensity. Blood samples were drawn into one EDTA vacutainer, one heparin vacutainer, and one vacutainer without anticoagulant (Becton Dickinson) at each collection time. To eliminate the possibility that the ex vivo IL-6 production and secretion from circulating immune cells contributed to plasma levels, the heparinized evacuated tube 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 tube was allowed to clot at room temperature (RT) and placed on ice until both tubes were centrifuged at 1750 g at 4°C for 10 min. The subjects were not allowed to eat until testing was completed, but water was provided to maintain body hydration during the testing day. Water intake was recorded and provided in equal amounts during their next trial.

Blood analyses. Determination of testosterone, estradiol, and progesterone was performed in duplicate in preexercise serum samples using commercially available radioimmunoassay (RIA) kits (Cat. No. TKT1, TKE1, and TKP1, respectively, from Diagnostic Products, Los Angeles, CA). Cortisol was determined in pre- and postexercise serum samples using an RIA (Cat. No. TCKO1, Diagnostic Products). In our hands, the intra- and inter-assay coefficients of variation (CVs) are <7% for testosterone, <8% for estradiol, <5% for progesterone, and <8% for cortisol. Postexercise cortisol levels were adjusted for changes in plasma volume. Commercially available ELISA kits were used to measure plasma levels of IL-6 (Cat. No. HS600B, R&D Systems, Minneapolis, MN) and sICAM-1 (Cat. No. BBE1B, R&D Systems). According to the manufacturer, the sensitivity of each assay is 0.039 pg/ml for IL-6 and less than 0.35 ng/ml for sICAM-1. In our hands, the intra- and inter-assay CVs are ≈9% for IL-6 and ≤7% for sICAM-1. Postexercise IL-6 and sICAM-1 concentrations were adjusted for changes in plasma volume. EDTA-treated blood samples were well-mixed and left at RT before delivered to the McMaster University Medical Centre. Neutrophils were analyzed as part of a complete blood count using an automated Coulter counter. Hemoglobin and hematocrit were also determined so that blood and plasma volume changes could be estimated according to Dill and Costill (5). Neutrophil counts were adjusted for exercise-induced changes in blood volume.

Statistical analyses. All values are given as means ± SD. IL-6, sICAM-1, neutrophil counts, and cortisol were tested for a normal distribution, which was confirmed in all cases. A Student’s paired t-test was used to determine differences in sex hormones. A two-way repeated-measures ANOVA (trial × time) was used to assess differences in IL-6, sICAM-1, neutrophil counts, and cortisol. Where appropriate, Tukey’s honestly significant difference post hoc test was applied to determine the significance among means. 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 Student’s t-tests. GraphPad Prism 4.03 (GraphPad Software, San Diego, CA) was used.
to determine Pearson correlations between immune parameters at 90 min of exercise and sex hormone concentrations at rest. For all statistical procedures, the threshold for statistical significance was set at $P \leq 0.05$.

**RESULTS**

Sex hormone concentrations are presented in Table 1. ES increased resting levels of estradiol 6.3-fold ($P = 0.005$) and reduced testosterone by 19% ($P = 0.007$) but did not affect resting levels of progesterone. Resting cortisol levels were unaffected by ES, and cortisol increased significantly with exercise during both trials ($P = 0.02$), with no effect of ES ($P = 0.18$) on these changes. Cortisol increased from 585 ± 146 to 828 ± 308 nmol/l during PL and from 503 ± 160 to 1,020 ± 588 nmol/l during ES.

During both trials, exercise significantly increased IL-6 ($P < 0.001$, Fig. 1) and neutrophil counts ($P < 0.001$, Fig. 2), with no effect of ES on these changes. Neither exercise ($P = 0.34$) nor ES ($P = 0.57$) influenced sICAM-1 (Fig. 3). Pearson correlations were then used to investigate intraindividual relationships between sex hormone concentrations at rest and postexercise IL-6, sICAM-1, neutrophil counts, and cortisol. All $P$ values for these correlations were $\geq 0.20$, except for a trend between testosterone and cortisol ($r = -0.42$, $P = 0.053$).

To examine the proposed relationships between IL-6, neutrophil counts, and cortisol during exercise (27), Pearson correlations were performed on values collected at 90 min of exercise (Fig. 4). IL-6 and neutrophil counts were significantly correlated ($r = 0.58$, $P = 0.005$), but IL-6 and cortisol ($r = 0.18$, $P = 0.43$) and cortisol and neutrophil counts ($r = 0.06$, $P = 0.78$) were not. No correlations were present between sICAM-1 and IL-6 ($r = -0.05$, $P = 0.86$), neutrophil counts ($r = 0.04$, $P = 0.88$), or cortisol ($r = -0.07$, $P = 0.79$).

**DISCUSSION**

This study examined in vivo effects of ES in healthy men on systemic inflammatory responses to exercise. On the basis of previous human and animal work and in vitro studies, we hypothesized that ES would attenuate exercise-induced increases in systemic levels of IL-6, neutrophils, and cortisol. Contrary to our hypothesis, however, ES had no effect on IL-6 or neutrophil responses and even slightly, but not significantly, enhanced the cortisol response to a 90-min endurance cycling task. In addition, we examined the relationships between postexercise IL-6, neutrophil counts, and cortisol, because an anti-inflammatory relationship between these systemic factors has been proposed to operate during exercise (27). Our analyses revealed a single significant association between neutrophil counts and IL-6.

Animal work has demonstrated that ES in male (35, 36) and ovariectomized female (37) rats significantly attenuates neutrophil infiltration of skeletal muscle after exercise. Likewise, our laboratory has previously reported that, compared with men, women experience less leukocyte infiltration of skeletal muscle accompanied by ~38% lower systemic neutrophil levels during recovery from eccentric exercise (30). In the current study, we reasoned that a possible attenuating effect of estrogen at the tissue level would be evident in the peripheral circulation as a blunted systemic response. However, postexercise neutrophil counts were not influenced by short-term ES and varied by less than 4% between trials. A post hoc sample size calculation, with $\alpha$ set at 0.05 and $\beta$ set at 0.20 (3), revealed that 697 additional subjects would be required to elicit a statistically significant difference in neutrophil counts. In contrast to these male data, our laboratory recently reported that neutrophil counts were elevated at rest and their response to exercise was greater in women taking oral contraceptives (OC) vs. both men and women not taking OC (38). The greater exercise neutrophilia was observed during the OC-use phase of their menstrual cycle when estradiol levels were lowest (38). On the other hand, hormone replacement therapy (HRT) in postmenopausal women does not appear to influence resting neutrophil counts (18), but their response to acute exercise in this population is unknown. In the current study, we also measured sICAM-1 levels to investigate a possible interaction of ES and exercise on cell adhesion properties, given previous

**Table 1. Sex hormone concentrations with and without estrogen supplementation in men**

<table>
<thead>
<tr>
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<th>PL</th>
<th>ES</th>
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<tbody>
<tr>
<td>Estradiol, pmol/l</td>
<td>133±71</td>
<td>840±633*</td>
</tr>
<tr>
<td>Progesterone, nmol/l</td>
<td>2.9±1.0</td>
<td>2.6±0.8</td>
</tr>
<tr>
<td>Testosterone, nmol/l</td>
<td>19.9±3.7</td>
<td>16.1±3.9*</td>
</tr>
</tbody>
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Values are means ± SD. PL, placebo; ES, estrogen supplementation. $^*$Significantly different from PL, $P < 0.007$.

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Fig. 1. IL-6 concentrations before and immediately after exercise with and without estrogen supplementation in men. Values are means ± SD. PL, placebo; ES, estrogen supplementation; Pre, before exercise; Post, immediately after exercise. Main effect time (Post > Pre), $P < 0.001$.

Fig. 2. Neutrophil counts before and immediately after exercise with and without estrogen supplementation in men. Values are means ± SD. Main effect time (Post > Pre), $P < 0.001$. 

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reports that HRT in postmenopausal women reduces sICAM-1 concentrations (9, 23, 40). Similar to neutrophil counts, however, we found no effect of ES or interaction with exercise on systemic levels of sICAM-1.

Before the current investigation, we were aware of no study that addressed the in vivo effects of estrogen on plasma IL-6 responses to exercise. In postmenopausal women, HRT has been associated with reduced IL-6 concentrations at rest in some (25, 29) but not all (20) studies, and acute ES lowers the in vivo IL-6 response to endotoxin (21). Similar to the neutrophil findings, however, we found no significant effect of short-term ES in healthy men on systemic IL-6 responses, which varied by less than 1% between trials. Interestingly, systemic IL-6 responses to exercise in women tend to be greater when their estrogen levels are lowest (38). However, the overall IL-6 response to exercise is similar between OC users and nonusers despite significantly higher estradiol levels in the OC nonusers (38). Thus it appears that estrogen has little impact on systemic levels of IL-6 in response to endurance-type exercise.

A limitation in the current study is that we did not determine sex hormone concentrations in postexercise samples, suggesting that the acute change in estrogen, or its interaction with other hormones, may be relevant to the acute inflammation-related changes. However, the resting estradiol concentration achieved with supplementation was higher than the expected estrogen response to exercise alone (1, 31). It may also be possible that a longer duration and/or higher dose of ES are required before estrogen effects are observed in men. Nevertheless, Van Baal et al. (40) found that only 4 wk of HRT lowered inflammation-related markers in postmenopausal women, and the IL-6 response to endotoxin in vivo was attenuated with plasma estradiol levels elevated to only half of those achieved in the current study. Although we used a higher dose of 17β-estradiol in a previous investigation (2), plasma levels in that study were supraphysiological (∼2,500 pmol/l), thus reducing physiological relevance. Our aim in the current study was to elicit plasma estradiol levels comparable to those found during the luteal phase of the menstrual cycle, and this was achieved. In addition, the current supplementation protocol was sufficient to alter substrate selection during rest and exercise in the same subjects (10), thus indicating that physiological adjustments to the altered concentrations of sex hormones did occur. Moreover, one of the strengths of this study was its randomized, double-blind, crossover and placebo-controlled design, whereby the subjects served as their own control, hence reducing errors due to an inappropriate sample size (i.e., Type II error). It is therefore likely that we had sufficient statistical power to detect a significant physiological difference, if one existed.

Although the skeletal muscle-specific anti-inflammatory effects of estrogen during exercise have previously been argued (34), we did not detect an effect of estrogen on systemic levels of inflammatory markers. A possible effect of ES on neutrophils, however, may still be present at the level of skeletal muscle. Evidence also supports a direct inhibitory effect of estrogen on IL-6 gene expression in various cell lines (19, 22) and IL-6 production by bone cells (8). Therefore, it remains possible that an effect of ES was present at the level of skeletal muscle, in terms of neutrophil infiltration, or at the level of bone marrow, in terms of IL-6 release, and future research needs to address these possibilities. It is also likely that a more eccentrically biased exercise task would have induced a larger inflammatory response, which might have been more sensitive...
to our ES protocol. This study is now under way in our laboratory.

In this investigation, we confirmed an association between IL-6 and neutrophil counts during exercise. In contrast, we found no statistical association between cortisol and IL-6 or between neutrophil counts and cortisol at the same time points. Steensberg et al. (27) recently proposed that exercise-induced elevations in IL-6 exert an anti-inflammatory effect by increasing cortisol concentrations, which then result in neutrophilia. Importantly, this effect was proposed to occur during exercise, which is why we calculated correlations between values taken at 90 min of exercise. We are aware that changes in IL-6, neutrophil counts, and even cortisol would continue into the recovery period (1–2 h) after exercise, but our objective was to elucidate the relationship between the above-mentioned inflammatory markers during exercise, as proposed (27). Although a cause-effect relationship cannot be assumed, our findings suggest that exercise neutrophilia may be a direct effect of IL-6. Other investigators have reported a statistical association between IL-6 and neutrophil counts during exercise in humans (33, 42), and IL-6 infusion into rats (39) and rabbits (32) induces an immediate increase in systemic levels of neutrophils. It is also noteworthy that no correlation was found between postexercise neutrophil counts and IL-6 in women taking or not taking OC (38). In fact, women taking OC had elevated cortisol levels and neutrophil counts, compared with men and women not taking OC, but their resting IL-6 levels and overall IL-6 response to exercise were similar (38). Given that the experiment of Steensberg et al. (27) was conducted with men, the relationship between exercise-induced changes in anti-inflammatory mediators may be different in men and women, in particular, women taking OC. This possibility is consistent with clinical observations of sex-based differences in the inflammatory response associated with sepsis (14, 24).

On the basis of our finding that ES does not acutely influence systemic inflammatory responses to exercise in healthy young men and that there may be differences in the regulation of anti-inflammatory responses to exercise between men and women, it may be that sex differences in the expression of estrogen receptors contribute to these observations. The expression of ERα mRNA, for example, appears to be lower in men, compared with women, in skeletal muscle (41) and in lung tissue (6), but higher in men than in women in adipocytes (4). In addition, ERα immunoreactivity of inflammatory cells and fibroblasts of synovial tissue is higher in older men than in older women (11). Therefore, the effects of ES may be tissue-specific, thus influencing metabolic and inflammatory responses differently. It is also of interest that compounds structurally related to 17β-estradiol, but that do not activate the estrogen receptor, attenuate the postexercise expression of heat shock proteins in rat skeletal muscle (17). This latter finding suggests that nongenomic hormonal actions of estrogen may also influence inflammatory responses to tissue stress and injury and contribute to differences between the sexes.

In summary, this study did not demonstrate an effect of short-term ES in healthy men on systemic levels of inflammation-responsive mediators during exercise. To clarify the possible interaction of estrogen, inflammation and muscle contraction, future work should investigate responses to eccentrically biased exercise at the skeletal muscle level. These investigations would have relevance to women taking OC or HRT in terms of muscle repair capacity. The current study also demonstrated a significant relationship between postexercise neutrophil counts and IL-6. The lack of association between other inflammatory mediators suggests that IL-6 may have a direct effect on neutrophil recruitment during exercise.

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