Exercise Effect on Oxidative Stress Is Independent of Change in Estrogen Metabolism

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Abstract

Purpose: The effect of exercise training on lipid peroxidation and endogenous estrogens is not well understood in premenopausal women. Exercise effects on these variables could mediate observed associations of exercise with hormonally related cancers, including breast cancer. The purpose of the study is to determine the effect of 15 weeks of aerobic exercise on lipid peroxidation, endogenous estrogens, and body composition in young, healthy eumenorrheic women.

Methods: Fifteen sedentary premenopausal women (18-25 years) participated. Pre- and post-exercise training urine collection (three 24-h samples) started 48 h after most recent exercise session for analysis of a marker of lipid peroxidation (F2-isoprostane) and endogenous estrogens, including 2-hydroxyestrogens, 4-hydroxyestrogens, 16-α-hydroxyestrone, and ratios of these metabolites (2:16, 2:4). Body composition was measured by dual-energy X-ray absorptiometry, and F2-isoprostanes and estrogens were measured by gas chromatography-mass spectrometry.

Results: Aerobic exercise resulted in a 34% decrease in F2-isoprostane (P = 0.02), a 10% increase in fitness (P = 0.004), a 1.2 kg decrease in body mass (P = 0.007), and a 1.8 kg decrease in fat mass (P = 0.94). No significant changes were noted in estrogens.

Conclusions: The effect of exercise training on oxidative stress may be relevant to risk for hormonally related cancers. (Cancer Epidemiol Biomarkers Prev 2008; 17(1):220–3)

Introduction

Exercise is associated with reduced risk for several types of cancer (1-4). Hypothesized mechanisms include changing estrogens, estrogen metabolism, and metabolic factors, such as reduction of excess body weight or, more specifically, excess body fat (5). However, evidence that exercise is associated with reduced risk for cancers unassociated with estrogens (lung and prostate) opens the possibility that the chemopreventive effects of exercise must extend beyond estrogens. We propose that exercise influences oxidative stress in a manner consistent with reduced cancer risk. F2-isoprostanes are a measure of oxidative stress relevant to the types of oxidative DNA damage observed in studies of cancer etiology. A recent case-control study observed a significant inverse association of this oxidative stress marker with risk for breast cancer (6). F2-isoprostane forms through lipid peroxidation reactions with hydroxyl radicals, the same radicals that form 8-hydroxydeoxyguanosine (8-OhdG) adducts when reacted with DNA. Furthermore, recent work shows that urinary levels of F2-isoprostane and 8-OhdG are correlated (7). Two noncontrolled exercise intervention studies in men have noted decreases in oxidative stress as measured by F2-isoprostanes (8, 9). The purpose of this study was to determine the effect of ~15 weeks (three to four menstrual cycles) of aerobic exercise training on oxidative stress (F2-isoprostane), endogenous estrogen metabolites, and body composition in 15 young, healthy eumenorrheic women.

Materials and Methods

Study Design, Recruitment, and Eligibility. This study used a pre-post design with a three to four menstrual cycles intervention. Eligible women were 18 to 25 years old, sedentary (up to two moderate intensity exercise sessions per week for past 6 months), had a self-reported menstrual cycle length between 25 and 32 days, were nulliparous, had intact ovaries and uterus and a body mass index (BMI) between 18 and 40 kg/m2, and drank less than seven alcoholic beverages weekly. Exclusion criteria included hormonal contraception use in the past year, gynecologic problems (e.g. fibroids,
endometriosis, and polycystic ovary syndrome), pregnancy within past 6 months, planning a pregnancy during the study, medical conditions that would prohibit vigorous exercise or would negatively affect our ability to test our primary aims, uncontrolled hypertension, cigarette smoking within past month, or plans to move away from the area during the study.

Initially, 825 women responded to study advertising. Those eligible based on self-reported BMI and age were contacted via telephone ($n = 332$ or $40\%$ of respondents); 177 women chose to complete the eligibility survey. The primary reason for ineligibility ($n = 113$) was current or recent use of exogenous estrogens. Thirty-three women (52% of phone-screened eligible) chose to consent and 5 were determined ineligible at a clinic visit. Twenty-eight (52% of phone-screened eligible) chose to consent and 15 completed exercise and all measurements ($54\%$ of those who started the study) and were therefore included in the analysis. Most common reason for loss to follow-up was time/schedule conflicts ($n = 11$). Access to an exercise facility was provided during the study. Six participants were compensated $15 weekly after observing 11 dropouts among 21 participants ($52\%$ dropout rate). After instituting subject payment, the dropout rate was 9%.

The study was conducted within the guidelines of the University of Minnesota Institutional Review Board. Written informed consent was obtained.

**Measurements.** Clinic visits at baseline and post-intervention took place on the seventh day of the menstrual cycle (follicular phase) after 12 h fast and 48 h after exercise.

**Body Composition.** Body composition was measured by dual-energy X-ray absorptiometry with a Lunar Prodigy DEXA apparatus (Lunar Radiation). Height and weight were measured on a wall-mounted stadiometer and a calibrated digital scale, respectively. Waist circumference was measured in duplicate with a standard measuring tape.

**Fitness Assessment.** Participants walked on a treadmill at 2.5 mph, then 3.0 mph for minutes 1 and 2, then 3.5 mph for the remainder of the test. Starting in minute 3, treadmill incline increased 2% every 2 min until participants reached 80% of their age-predicted maximum heart rate (max HR = $220 -$ age). This workload was converted into metabolic equivalents ($10$).

**Surveys.** Total kilocaloric intake per day and antioxidants from both supplements and foods were assessed with the Diet History Questionnaire ($11$). Demographics were assessed by survey.

**Urine Collection, Processing, and Storage.** Urine was collected for three consecutive 24-h periods (seventh to ninth days of the menstrual cycle) in collection bottles using previously reported methods ($12$). Urine was kept cold and sodium azide was added to 0.1% before aliquoting and storing in a $-70\text{o} C$ freezer until analysis. The three 24-h collections were pooled; 72-h aliquots were analyzed as a single sample. All assay results were expressed as pmol/mg creatinine and baseline and follow-up samples were run in the same batch.

**Laboratory Analyses of Endogenous Estrogens.** Urinary estrogen metabolites were analyzed by modified gas chromatography-mass spectrometry ($12$, $13$). Deuterated internal standards for estrogen metabolites were used. The urinary estrogens analyzed included estrone ($E_1$), estradiol ($E_2$), estriol ($E_3$), 2-hydroxyestrone (2-OHE1), 2-hydroxyestradiol (2-OHE2), 4-hydroxyestrone (4-OHE1), 4-hydroxyestradiol (4-OHE2), and 16α-hydroxyestrone (16α-OHE1). A quality control urine sample was included in duplicate in each assay. The intraassay and interassay variability for this study were $<10\%$ and $12\%$, respectively.

**Laboratory Analysis of F2-isoprostane.** The urinary metabolite of the F2-isoprostane 15-F$_2$-isoprostane, 2,3-dinor-8-iso-prostaglandin F$_2\alpha$ was measured by a gas chromatography-mass spectrometry–based method ($14$). The assay was done by gas chromatography-mass spectrometry analysis on a Hewlett-Packard 5971 MSD quadrupole instrument, using selective ion monitoring, after solid-phase chromatography, thin-layer separations, and derivatization. Control pools were analyzed with every sample batch, and stringent quality control rules were applied. Intraassay and interassay variation were $<5\%$ and $10\%$, respectively. Intraindividual variation was $<15\%$.

**Intervention Protocol.** All study participants were asked to avoid diet or weight changes during the study. All participants began exercising on the 11th day of their menstrual cycle and stopped on the fifth day of their menstrual cycle three to four menstrual cycles later. The five weekly exercise training sessions included 30 min of treadmill or elliptical exercise as well as a brief warm-up, cool-down, and stretching. Exercise intensity was 70% to 75%, 75% to 80%, and 80% to 85% of max HR for weeks 1 to 5, weeks 6 to 10, and week 11 to end of intervention, respectively. Participants recorded time and average heart rate from a Polar Heart Rate monitor on logs checked regularly by intervention staff. Face-to-face contact of participants with staff occurred at least once per week.

**Statistical Analysis.** Means and SDs are presented for baseline, follow-up, and percent change between two time points. Changes were assessed by paired Student’s $t$ test. Predicted change in oxidative stress after statistically accounting for changes in body composition and diet in these regression models was assessed. $t$ tests using the adjusted, predicted change values did not differ from the simpler, more interpretable $t$ tests, which are presented in the table. All statistical tests were two-sided; $P = 0.05$ signified statistical significance. SAS version 8.2 was used for all analyses (SAS Institute).

**Results**

**Study Sample.** The participants were $20.9 \pm 2.4$ years old on average (range, 18-25). All participants were unmarried and either current college students or recent college graduates. Thirteen of the participants self-reported Caucasian ethnicity. Approximately half reported they consumed alcohol (average of 1.9 drinks/wk).

**Exercise Adherence.** Average attendance among the 15 who completed the study and provided post-training data was 87.4% (range, 80-100%). During the exercise sessions, the participants were adherent to the target heart rate range 95.3% of the time.
Table 1. Differences in oxidative stress estrogen metabolites after 15 wk of aerobic exercise in premenopausal women (mean ± SD)

<table>
<thead>
<tr>
<th>Baseline (mean ± SD)</th>
<th>Follow-up (mean ± SD)</th>
<th>% Change</th>
<th>P for the difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2-isoprostanes</td>
<td>78.79 ± 52.13</td>
<td>52.19 ± 19.17</td>
<td>-33.77</td>
</tr>
<tr>
<td>Estrone</td>
<td>44.30 ± 10.49</td>
<td>41.48 ± 18.16</td>
<td>-6.37</td>
</tr>
<tr>
<td>Estradiol</td>
<td>9.35 ± 4.83</td>
<td>9.42 ± 4.31</td>
<td>+0.75</td>
</tr>
<tr>
<td>16-OHE1</td>
<td>19.38 ± 14.42</td>
<td>18.74 ± 16.11</td>
<td>-3.30</td>
</tr>
<tr>
<td>Estradiol</td>
<td>35.83 ± 13.91</td>
<td>32.98 ± 10.66</td>
<td>-7.95</td>
</tr>
<tr>
<td>2-OHE1/2</td>
<td>16.87 ± 9.46</td>
<td>17.82 ± 12.96</td>
<td>+5.63</td>
</tr>
<tr>
<td>4-OHE1/2</td>
<td>2.25 ± 1.09</td>
<td>2.05 ± 1.18</td>
<td>-8.44</td>
</tr>
<tr>
<td>2-OHE1/2/16-OHE1</td>
<td>0.93 ± 0.62</td>
<td>1.02 ± 0.87</td>
<td>+9.68</td>
</tr>
<tr>
<td>2-OHE1/2/4-OHE1/2</td>
<td>0.20 ± 0.25</td>
<td>0.17 ± 0.12</td>
<td>-15.0</td>
</tr>
</tbody>
</table>

NOTE: Units are pmol/mg creatinine for all variables.
*P values are from unadjusted t tests or Wilcoxon rank tests.

Intervention Effects

Oxidative Stress and Endogenous Estrogens. After 15 weeks of aerobic exercise, F2-isoprostanes declined by 33.8% (P = 0.02; Table 1). There were no statistically significant changes in endogenous hormones. Range of menstrual cycle length (26-31 days) did not change due to the intervention.

Fitness, Body Composition, and Diet. The exercise intervention resulted in changes in body composition and fitness variables (Table 2). Submaximal fitness increased significantly and significant decreases in body weight, BMI, and fat mass were observed. A decrease in total self-reported daily kilocalories from 2,206 at baseline to 1,744 at follow-up approached significance (P = 0.07). Intake of each of the antioxidants was lower at follow-up than baseline; none of these changes were significant.

Discussion

In the present study, F2-isoprostanes were significantly decreased among premenopausal women who completed 15 weeks of aerobic exercise training. There was a marginally significant decrease in total energy intake, so it is not possible to rule out that these changes could be due to a combined dietary and exercise effect. The finding that F2-isoprostanes were reduced by 34% may have relevance for carcinogenesis; some of the radicals that lead to the formation of F2-isoprostanes may damage DNA (15), including the formation of the oxidation product 8-OhdG formed by the interaction of a hydroxyl radical with guanine at its C-8 position.

Most prior studies that have examined effects of exercise training on oxidative stress have used measures that are less sensitive, stable, and specific than F2-isoprostanes. For example, electron spin resonance measurement of free radicals is extremely difficult in vivo due to the high dielectric absorption of microwave energy in aqueous solutions (like urine or blood) and the fact that the sample preparation itself can produce free radicals. Thiobarbituric acid reactive substances and malondialdehyde are both considerably less stable than F2-isoprostanes and are less specific as well. Both can be produced through alternate pathways that do not involve oxidative stress.

We are aware of two prior studies that have examined effects of exercise training specifically on F2-isoprostanes in men (8, 9) that observed decreases of F2-isoprostanes ranging from 24% to 31%. We observed a statistically significant 33.8% decrease in F2-isoprostanes in 15 young, eumenorrheic women. The findings of decreases ranging from 24% to 33.8% across three separate studies with varying lengths and intensities of interventions indicate that exercise has the potential to significantly alter the formation of free radicals and lipid peroxidation, which may have particular relevance for cancer prevention.

Table 2. Fitness, body composition, and diet before and after 15 wk of aerobic exercise in premenopausal women (n = 15)

<table>
<thead>
<tr>
<th></th>
<th>Baseline (mean ± SD)</th>
<th>Follow-up (mean ± SD)</th>
<th>% Change</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submaximal fitness</td>
<td>7.7 ± 1.7</td>
<td>8.5 ± 2.0</td>
<td>10.6</td>
<td>0.004</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>72.5 ± 22.1</td>
<td>71.3 ± 21.2</td>
<td>-1.7</td>
<td>0.007</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.3 ± 7.4</td>
<td>25.6 ± 7.1</td>
<td>-2.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>27.0 ± 16.8</td>
<td>25.2 ± 16.6</td>
<td>-6.5</td>
<td>0.04</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>42.2 ± 5.7</td>
<td>42.9 ± 5.2</td>
<td>1.5</td>
<td>0.74</td>
</tr>
<tr>
<td>% Body fat</td>
<td>35.9 ± 11.8</td>
<td>33.9 ± 12.2</td>
<td>-5.6</td>
<td>0.13</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>92.3 ± 17.4</td>
<td>90.0 ± 15.4</td>
<td>-2.5</td>
<td>0.23</td>
</tr>
<tr>
<td>Energy intake (kcal/d)</td>
<td>2,206 ± 1,057</td>
<td>1,744 ± 729</td>
<td>-20.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Antioxidant intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>170.3 ± 154.6</td>
<td>119.3 ± 38.5</td>
<td>-29.9</td>
<td>0.19</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>13.9 ± 7.3</td>
<td>12.3 ± 7.3</td>
<td>-11.7</td>
<td>0.18</td>
</tr>
<tr>
<td>α-Tocopherol (mg)</td>
<td>9.0 ± 5.2</td>
<td>7.1 ± 3.8</td>
<td>-21.2</td>
<td>0.10</td>
</tr>
<tr>
<td>Vitamin A (µg RE)</td>
<td>1,959 ± 1,219</td>
<td>1,673 ± 900</td>
<td>18.6</td>
<td>0.19</td>
</tr>
<tr>
<td>(β-Carotene (µg)</td>
<td>4,397 ± 3,845</td>
<td>3,894 ± 2,828</td>
<td>-9.4</td>
<td>0.48</td>
</tr>
<tr>
<td>Selenium (µg)</td>
<td>103.9 ± 41.8</td>
<td>90.8 ± 42.1</td>
<td>-12.6</td>
<td>0.24</td>
</tr>
</tbody>
</table>
No statistically significant changes were noted in any of the endogenous estrogens measured. Significant changes in estrone, estradiol, or estriol would only have been expected if the level of energy deficiency produced by the exercise training program were sufficient to result in the level of weight loss that has been noted to produce anovulation or at least luteal phase defect (16). However, it has been hypothesized that exercise may alter estrogen metabolism in a manner that could alter cancer etiology (17). We are aware of several prior studies that have examined the effects of exercise training on estrogen metabolism among premenopausal women (18-21) and one in postmenopausal women (17). Of these, only one (22) has observed an intervention effect on the 2:16 ratio in a subset of participants. Ours is the first to examine the effect on the 2:4 ratio. Three publications reported on the observed effects of 5 days of intensive aerobic exercise training on estrogen metabolites (19-21). None of these studies noted significant changes in metabolites during the follicular phase, but significant decreases were noted for 2-hydroxyestrogens after 5 days of intensive training during the luteal phase (20). This suggests that future exercise interventions may need to examine luteal phase changes in addition to, or instead of, follicular phase effects on estrogen metabolites.

Strengths of the current study include the long intervention period and gold-standard approach to measuring oxidative stress and estrogen metabolites. Further, women with menstrual irregularities were excluded. Limitations include the high dropout rate prior to initiating subject payment and lack of a control group. Despite limited statistical power, the pattern of results is useful because of the paucity of data in this area. The study cohort met stringent eligibility criteria; it is unclear if results can be extended beyond this study group.

In summary, a 15-week exercise training intervention that followed the current public health recommendations for sufficient exercise to promote health (23) resulted in a significant decrease in a highly specific marker of lipid peroxidation and improved body composition. No changes were noted in endogenous estrogens. Future controlled trials on the effects of exercise on oxidative stress are warranted.

References
Correction

Correction: Article on Reproductive Factors and Mortality after Breast Cancer

In the article on reproductive factors and mortality after breast cancer in the June 2009 issue, a footnote about the authors was inadvertently deleted (1). Kelly-Anne Phillips and Roger L. Milne, who are listed as the first and second authors, contributed equally to this work.

Reference


Published OnlineFirst 7/14/09.
Copyright © 2009 American Association for Cancer Research.
doi:10.1158/1055-9965.EPI-18-8-COR
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