Short Communication

Effects of a Moderate Intensity Exercise Intervention on Estrogen Metabolism in Postmenopausal Women

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Abstract

Physical activity has been associated with reduced breast cancer risk, potentially via hormonal pathways, and high urinary excretion of 2-hydroxyestrone (2-OH E1) relative to 16α-hydroxyestrone (16α-OH E1) also has been associated with reduced breast cancer risk. Studies suggest that body composition and exercise can influence estrogen metabolism. We determined the effects of a 12-month moderate intensity aerobic exercise intervention on urinary 2-OH E1, 16α-OH E1, and their ratio in overweight and obese, previously sedentary, postmenopausal women, ages 50–75 years. Women were randomized to a 12-month exercise intervention (n = 87) or stretching control group (n = 86); 170 completed the study. Urinary 2- and 16α-OH E1 were measured in spot urines collected at baseline, 3, and 12 months. Body composition was measured at baseline and 12 months. Differences between exercisers and controls for excretion of estrogen metabolites were determined using general estimating equations. Further analyses assessed change in estrogen metabolites and their ratio by subgroups of change in body composition. Overall, there were no significant effects of the exercise intervention on 2-OH E1, 16α-OH E1, or their ratio (P > 0.05). There appeared to be an effect of change in intra-abdominal fat and adherence to the exercise intervention on change in the estrogen metabolites or their ratio. However, this did not reflect a potentially desirable change in estrogen metabolites associated with the exercise intervention. Thus, this 12-month moderate intensity exercise intervention did not significantly alter urinary excretion of 2-OH E1, 16α-OH E1, or their ratio in this population of women.

Introduction

A large body of evidence implicates estrogens in the etiology of breast cancer (1) and it has been proposed that the determinants of circulating estrogen concentrations, and of estrogen metabolism, are potentially the primary causes of breast cancer (2). Physical activity has been associated with reduced risk of breast cancer (3), potentially via hormonal pathways. Following menopause, the primary source of estrogen is from the conversion of androgens to estrogens by adipose tissue aromatase (4, 5). Because exercise decreases body fat, especially abdominal fat, it has been proposed that an exercise intervention might reduce fat mass and subsequently alter the sex-hormone profile in postmenopausal women to one associated with lower risk for breast cancer (6, 7).

Estrogen metabolism in humans is mediated primarily by cytochrome P450 enzymes (8), and two of the major hydroxylated metabolites of estrone are 2-hydroxyestrone (2-OH E1) and 16α-hydroxyestrone (16α-OH E1) (9, 10). They are produced by competing pathways and have markedly different properties. 2-OH E1 is weakly estrogenic and data from studies in vitro and in animal models have suggested that it is anticarcinogenic (10). In contrast, 16α-OH E1 is more estrogenic, has been shown to form covalent bonds with estrogen receptors, and may be genotoxic (11–13). Therefore, it has been suggested that urinary excretion of these estrogen metabolites can be used as a risk marker for breast cancer (14, 15). In support of this hypothesis, some (16–19), but not all (20), retrospective and prospective studies have reported an increased risk of breast cancer associated with low urinary excretion of 2-OH E1 relative to 16-OH E1.
Numerous factors have been suggested as possible modulators of estrogen metabolism in humans. For example, intakes of Brassica vegetables increase urinary excretion of 2-OH E1 relative to 16α-OH E1, potentially via induction of cytochrome P450 enzymes (21). In relation to body composition, obesity has been inversely associated with 2-hydroxylation (22, 23), and studies among athletes have reported positive associations between leanness and favoring the 2-hydroxylation pathway (24, 25), suggesting that both body composition and exercise (possibly via effects on body composition) can influence estrogen metabolism.

The Physical Activity for Total Health study was a randomized controlled clinical trial comparing the effect of a 12-month moderate intensity aerobic exercise intervention versus stretching control on the sex hormone profile in sedentary women (26). We report the effects of the exercise intervention on the urinary excretion of 2-OH E1, 16α-OH E1, and their ratio.

Materials and Methods

**Study Participants.** Full details of recruitment procedures and study participants have been published elsewhere (26, 27). Briefly, 173 women ages 50–75 years were recruited through mass mailings and media advertising. Eligible study participants had a body mass index (BMI) of 25–40 kg/m² or BMI between 24 and 25 kg/m² and percentage body fat >33%. They were postmenopausal [not experiencing menstrual periods for the previous 12 months, and follicle stimulating hormone (FSH) >30 mIU/ml for women aged 50–55 years] and sedentary (exercising at a level to produce sweating <60 min/week). Other major exclusion criteria included: use of hormone therapy, herbal hormones, tamoxifen, raloxifene, or oral contraceptives within the past 6 months; having medical conditions contraindicating moderate-to-vigorous intensity exercise; having a clinical diagnosis of diabetes or fasting blood glucose levels >140 mg/dl; currently using tobacco; and current moderate to high alcohol intake (more than two drinks per day). Full details of inclusion and exclusion criteria have been published elsewhere (26).

**Data Collection.** Demographic, medical history, reproductive history, and hormone-use history information were collected via a self-administered questionnaire at baseline. A food frequency questionnaire (FFQ) was used to assess dietary intake at baseline, 3, and 12 months (28). Height and weight were measured to the nearest 0.1 kg and 0.1 cm, respectively, at baseline, and after 3 and 12 months, using a balance-beam scale and stadiometer, respectively. Body fat percentage and lean tissue mass were assessed at baseline and after 12 months using a dual-energy X-ray absorptiometry (DEXA) whole-body scanner (Hologic QDR 1500, Hologic Inc., Waltham, MA). Abdominal fat, intra-abdominal fat (at the umbilicus, L4-L5 space; 125 kV and 8 mm thickness), and subcutaneous fat were measured at baseline and 12 months by computed tomography (CT; General Electric model CT 9800 scanner, Waukesha, WI). Maximal oxygen consumption (VO₂max) was measured at baseline and after 12 months; participants completed a maximal-graded treadmill test, with heart rate and oxygen uptake monitored by an automated metabolic cart (Medgraphics, St. Paul, MN). The test began at 3.0 mph and 0% grade. The speed or grade (2%) increments of the treadmill increased every 2 min. Treadmill tests were terminated when the participant reached volitional fatigue. Further details on all measurements have been published elsewhere (29).

**Intervention.** Women were randomly assigned to an exercise intervention group (87 women) or a stretching control group (86 women), stratified by BMI (>27.5 versus ≥27.5 kg/m²). The exercise intervention consisted of at least 45 min of moderate-intensity exercise 5 days/week for 12 months. The aerobic exercise program was divided into two parts: a 3-month intensively monitored facility-based and a 9-month home-based exercise period. During the first 3 months, participants were required to attend three sessions/week at the exercise facility and to exercise 2 days/week at home. During the subsequent 9 months, participants were required to attend one session/week at the facility and to exercise 4 days/week on their own; participants were allowed to exercise additional days at the facility if they wished to do so. The training program began with a target of 40% of maximal heart rate for 16 min per session, and gradually increased to 60–70% of maximal heart rate for 45 min per session by week 8, at which point it was maintained for the duration of the study. Subjects primarily walked on a treadmill or rode a stationary bicycle at the training facility; women were encouraged to do 5–10 min of strength training to reduce risk of injury during the intervention. A variety of home exercises were suggested and encouraged, including walking, aerobics, and bicycling. Women were asked to keep daily logs of all sports or recreational activities they performed.

Participants in the stretching control group attended weekly 45-min stretching classes for 12 months, and were asked not to change other exercise habits during the study.

Both groups (exercisers and controls) were asked not to change their dietary patterns during the study.

**Urine Samples, Estrogen Metabolites, and Creatinine.** Study participants provided a fasting spot urine sample during clinic visits at baseline, and at months 3 and 12, and samples were stored at −70°C before analysis. Urine samples supplemented with vitamin C (62.5 mg per 25 ml of urine) were analyzed for 2-OH E1 and 16α-OH E1, and non-supplemented urine was used for creatinine analysis. Urinary estrogen metabolites were measured using the commercially available Estramet 2/16 enzyme immunoassay (EIA) kits (Immucor Inc., Bethlehem, PA). On receipt of the kits, all components were stored as recommended by the manufacturer. Urinary levels of 2-OH E1 and 16α-OH E1 were quantified as described previously (30, 31). All urine samples, standards, and controls were assayed in triplicate, and all samples from each subject were assayed in the same batch. Samples were batched such that, within each batch, the number of exercise and control subjects was approximately equal, the randomization dates of subjects were similar, and the sample order was random. Laboratory personnel were blinded with regard to subject identity. For quality control, a pool
sample was created from ineligible subjects who were postmenopausal and not taking exogenous estrogens. Two blinded quality control samples and one laboratory quality control (not blinded) were included on each plate. Standard curves (using a log-linear scale) were sigmoidal and only the linear portion of the curve was used for quantitation. A coefficient of variation (CV) was calculated for each sample using data from triplicate measurements. Samples with a CV greater than 15% (primarily samples that were too concentrated or too dilute) were re-assayed using the appropriate dilution, or a mean of two out of the three wells was used, contingent on the CV of the two wells being less than 15%. If a sample needed to be re-assayed, all three time points for that woman were re-assayed on the same plate. On the basis of the blinded quality control sample, intra- and inter-assay CVs were 7.2% and 12.2%, respectively, for 2-OH E1, and 8.0% and 15.3%, respectively, for 16α-OH E1.

Urinary creatinine concentrations were measured using a kinetic modification of the Jaffe reaction using the Roche Reagent for Creatinine (Roche Diagnostic Systems, Nutley, NJ) on a Roche Cobas Mira Plus chemistry analyzer. The assay was linear to 20 mg/dl and the intra- and inter-assay CVs were 1.8% and 1.9%, respectively.

At baseline, 3 and 12 months, respectively, 2-OH E1 data were unavailable for 10, 8, and 9 women, and 16α-OH E1 data were unavailable for 6, 4, and 9 women. Reasons for data being unavailable included urine sample not collected, concentrations of estrogen metabolites that were not in the linear portion of the standard curve/unacceptable CVs that remained so even when duplicates or re-assays were considered, or creatinine data not available.

Statistical Analysis. All statistical analyses were performed using the SAS statistical package version 8.02 (SAS Institute, Cary, NC). The concentrations of 2-OH E1 and 16α-OH E1 were adjusted for creatinine excretion to account for variability in urinary output. Spearman correlation coefficients were calculated for each sample using data from triplicate measurements. The estrogen metabolites and baseline body composition measures, and between change in the estrogen metabolites and change in body composition measures. Brassica vegetable intakes have been associated with levels of 2-OH E1 and 16α-OH E1 (21); therefore, we assessed differences between exercisers and stretchers for mean change (baseline to 12 months) in vegetable and fiber intakes using unpaired t tests. We also investigated differences between treatment groups in total energy intakes at each time point, and change over time, using unpaired t tests. Differences between exercisers and stretchers for changes in estrogen metabolites and their ratio were tested using linear regression via general estimating equations, and all analyses were based on assigned treatment at the time of randomization (i.e., intent-to-treat). Estrogen metabolite data were log transformed (ln) to normalize the data for the general estimating equations analyses. In a secondary analysis, we explored between-group differences for changes in estrogen metabolites from baseline to 12 months, stratifying by change in weight [gained >0.5 kg (reference group), gained or lost ≤0.5 kg, lost 0.5–3 kg, lost >3 kg]; change in percentage body fat [gained >0.5 % (reference group), gained or lost ≤0.5%, lost 0.5–2%, lost >2%]; change in intra-abdominal fat (gained >15 cm² (reference group), gained 0–15 cm², lost up to 15 cm², lost >15 cm²); change in lean body mass [lost >700 g (reference group), lost up to 700 g, gained 0–700 g, or gained >700 g]; and change in VO2max [decrease or no change (reference group), 1–10% increase, or >10% increase]. In addition, for women in the exercise group, duration of exercise [minutes per week of sports or recreational activities of at least three metabolic equivalents, METs (32)] was calculated, and mean change in the estrogen metabolites was compared across tertiles of adherence (<130 min/week; 130–190 min/week; >190 min/week).

Results

Selected baseline characteristics did not differ significantly between the exercise and control groups (Table 1). There were no associations between baseline levels of the estrogen metabolites (2-OH E1, 16α-OH E1, and their ratio) and BMI, percentage body fat, lean body mass (g), abdominal fat (cm²), and subcutaneous fat (cm²) (P > 0.05). Correlations between change in bladder intra-abdominal fat (cm²) and 2-OH E1 and 16α-OH E1 were weak (r = -0.17, P = 0.03; and r = -0.20, P = 0.01, respectively); there was no correlation between baseline intra-abdominal fat and the 2-OH E1:16α-OH E1 ratio (r = -0.02, P = 0.75).

Of the 87 women randomized into the exercise intervention group: 81 (93%) were considered adherent (i.e., met ≥80% of the goal of 225 min/week of moderate-to-vigorous intensity exercise); 87 (100%) returned for the 3-month clinic visit; 84 (97%) returned for the 12-month clinic visit. On average, the women participated in 199 min of moderate-to-vigorous intensity exercise over 4 days/week. Of the 86 women randomized into the stretching control group, all returned for the 3- and 12-month clinic visits. Six controls (7%) increased their exercise to 225 min/week, based on a self-reported questionnaire.

Table 1. Baseline characteristics of randomized participants

<table>
<thead>
<tr>
<th></th>
<th>Exercisers (n = 87)</th>
<th>Controls (n = 86)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>60.7 ± 6.7</td>
<td>60.6 ± 6.8</td>
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<tr>
<td>Weight (kg)</td>
<td>81.4 ± 14.1</td>
<td>81.7 ± 12.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.4 ± 4.1</td>
<td>30.5 ± 3.7</td>
</tr>
<tr>
<td>% Body fat1</td>
<td>47.5 ± 4.8</td>
<td>47.4 ± 4.6</td>
</tr>
<tr>
<td>Lean tissue (kg)</td>
<td>39.9 ± 5.6</td>
<td>39.9 ± 4.9</td>
</tr>
<tr>
<td>Subcutaneous fat (cm²)</td>
<td>385 ± 124</td>
<td>364 ± 107</td>
</tr>
<tr>
<td>Intra-abdominal fat (cm²)</td>
<td>146 ± 60</td>
<td>147 ± 56</td>
</tr>
<tr>
<td>Ethnicity (% Non-Hispanic Whites)</td>
<td>86.0%</td>
<td>87.2%</td>
</tr>
<tr>
<td>Education (% beyond high school)</td>
<td>88.5%</td>
<td>89.5%</td>
</tr>
</tbody>
</table>

1Means ± SD unless indicated otherwise. All differences between treatments were non-significant.

1Measured by dual-energy X-ray absorptiometry.

3Measured by computed tomography (computed tomography data not available for one woman in the exercise group, and data on subcutaneous fat not available for one woman in the control group).

4Data not available for one woman in the exercise group.
From baseline to 12 months, women in the exercise group lost an average of 1.29 kg (SD 3.8 kg), whereas women in the stretching control group gained an average of 0.1 kg (SD 3.0 kg) \((P < 0.01)\). Further details regarding changes in body composition have been reported elsewhere (29). There were no significant differences between exercisers and stretchers for changes in dietary intake of vegetables and fiber from baseline to 3 months, or from baseline to 12 months \((P > 0.1; \text{data not shown})\). In addition, there were no statistically significant differences between exercisers and stretchers in total energy intake at baseline, 3, or 12 months, or in change from baseline to 3 months or baseline to 12 months \((P > 0.1; \text{data not shown})\).

There were weak correlations between change in lean tissue mass and change in 2-OH E\textsubscript{1} among exercisers \((r = 0.16, P = 0.05)\) and between change in lean tissue mass and change in 2-OH E\textsubscript{1};16α-OH E\textsubscript{1} ratio \((r = 0.17, P = 0.04)\). When stratified by intervention group, there were weak correlations between change in lean tissue mass and change in 2-OH E\textsubscript{1} among exercisers \((r = 0.25, P = 0.03)\), and between change in intra-abdominal fat and change in 2-OH E\textsubscript{1} among controls \((r = -0.23, P = 0.05)\). There were no other correlations between changes in body composition measures and changes in the estrogen metabolites and their ratio \((P > 0.05)\).

Overall, there were no differences between the groups for changes in 2-OH E\textsubscript{1}, 16α-OH E\textsubscript{1}, or their ratio \((Table 2)\). When stratified by change in body-composition measures, change in intra-abdominal fat appeared to modify the effect of exercise on changes in 2-OH E\textsubscript{1} and the 2-OH E\textsubscript{1};16α-OH E\textsubscript{1} ratio \((Table 3)\); when compared with the reference group (women who gained >15 cm\textsuperscript{2}), differences between treatment groups were statistically significant for the changes in 2-OH E\textsubscript{1} and the 2-OH E\textsubscript{1};16α-OH E\textsubscript{1} ratio among women who lost up to or

### Table 2. Geometric mean (95% confidence interval) 2-OH E\textsubscript{1}, 16α-OH E\textsubscript{1}, and their ratio at baseline, and at months 3 and 12 for women in the exercise intervention and control groups

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<thead>
<tr>
<th></th>
<th>Exercisers</th>
<th>Controls</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>3 months</td>
<td>12 months</td>
<td>Baseline</td>
<td>3 months</td>
<td>12 months</td>
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<tr>
<td>2-OH E\textsubscript{1} (ng/mg Cr)</td>
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<tr>
<td>Gained &gt;15 cm\textsuperscript{2}</td>
<td>5.54 (3.93, 7.79)</td>
<td>5.69 (4.28, 7.55)</td>
<td>7.10 (5.56, 9.07)</td>
<td>5.95 (4.73, 7.47)</td>
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<tr>
<td>Gained 0–15 cm\textsuperscript{2}</td>
<td>6.84 (4.98, 9.41)</td>
<td>7.11 (5.51, 9.18)</td>
<td>7.66 (6.04, 9.72)</td>
<td>6.29 (5.06, 7.82)</td>
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<tr>
<td>Lost up to 15 cm\textsuperscript{2}</td>
<td>7.25 (5.72, 9.19)</td>
<td>6.25 (5.08, 7.71)</td>
<td>5.38 (4.06, 7.13)</td>
<td>6.92 (5.28, 9.07)</td>
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<tr>
<td>Lost &gt;15 cm\textsuperscript{2}</td>
<td>7.18 (5.48, 9.39)</td>
<td>6.88 (5.53, 8.53)</td>
<td>6.81 (5.34, 8.70)</td>
<td>7.66 (6.13, 9.58)</td>
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<td>16α-OH E\textsubscript{1} (ng/mg Cr)</td>
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<tr>
<td>Gained &gt;15 cm\textsuperscript{2}</td>
<td>5.42 (3.85, 7.63)</td>
<td>5.24 (4.02, 6.82)</td>
<td>5.86 (4.68, 7.32)</td>
<td>5.96 (4.97, 7.15)</td>
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<tr>
<td>Gained 0–15 cm\textsuperscript{2}</td>
<td>6.62 (4.88, 8.99)</td>
<td>6.58 (5.15, 8.42)</td>
<td>5.93 (4.79, 7.34)</td>
<td>5.45 (4.56, 6.51)</td>
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<tr>
<td>Lost up to 15 cm\textsuperscript{2}</td>
<td>5.95 (4.73, 7.47)</td>
<td>5.15 (4.27, 6.20)</td>
<td>4.94 (3.85, 6.34)</td>
<td>5.45 (4.36, 6.81)</td>
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<tr>
<td>Lost &gt;15 cm\textsuperscript{2}</td>
<td>5.81 (4.56, 7.40)</td>
<td>5.74 (4.71, 7.00)</td>
<td>5.95 (4.76, 7.43)</td>
<td>6.09 (5.10, 7.28)</td>
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<tr>
<td>Ratio</td>
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<tr>
<td>Gained &gt;15 cm\textsuperscript{2}</td>
<td>1.00 (0.73, 1.36)</td>
<td>1.08 (0.82, 1.43)</td>
<td>1.18 (0.95, 1.46)</td>
<td>1.00 (0.84, 1.19)</td>
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</tr>
<tr>
<td>Gained 0–15 cm\textsuperscript{2}</td>
<td>1.03 (0.78, 1.36)</td>
<td>1.12 (0.87, 1.46)</td>
<td>1.17 (0.95, 1.43)</td>
<td>1.19 (1.00, 1.41)</td>
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<tr>
<td>Lost up to 15 cm\textsuperscript{2}</td>
<td>1.22 (0.99, 1.50)</td>
<td>1.14 (0.94, 1.39)</td>
<td>1.06 (0.83, 1.35)</td>
<td>1.24 (1.01, 1.52)</td>
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<tr>
<td>Lost &gt;15 cm\textsuperscript{2}</td>
<td>1.24 (0.99, 1.56)</td>
<td>1.21 (0.98, 1.50)</td>
<td>1.17 (0.95, 1.45)</td>
<td>1.26 (1.06, 1.50)</td>
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Note: Cr, creatinine.

*Sample sizes for those who gained >15 cm\textsuperscript{2} (reference group), gained 0–15 cm\textsuperscript{2}, lost up to 15 cm\textsuperscript{2}, or lost >15 cm\textsuperscript{2}, respectively: 2-OH E\textsubscript{1}, exercisers at baseline = 81, 79, and 77; 2-OH E\textsubscript{1}, controls = 79, 82, and 84; 16α-OH E\textsubscript{1}, exercisers = 83, 82, and 79; 16α-OH E\textsubscript{1}, controls = 81, 81, and 82; ratio exercisers = 81, 80, and 78; ratio controls = 84, 83, and 85 (NB—creatinine-adjusted data were not used for the calculation of the ratio; data for women whose creatinine data are unavailable \((n = 4)\) were included, giving rise to a higher sample size for the ratio than for the individual metabolites).

†Difference between exercisers and controls for change from baseline to 3 months.

‡Difference between exercisers and controls for change from baseline to 12 months.

### Table 3. Geometric mean (95% confidence interval) 2-OH E\textsubscript{1}, 16α-OH E\textsubscript{1}, and their ratio at baseline and at 12 months for women in the exercise and control groups, stratified by change in intra-abdominal fat

<table>
<thead>
<tr>
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<th>Exercisers</th>
<th>Controls</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>12 months</td>
<td>Baseline</td>
<td>12 months</td>
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<tr>
<td>2-OH E\textsubscript{1} (ng/mg Cr)</td>
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<td></td>
</tr>
<tr>
<td>Gained &gt;15 cm\textsuperscript{2}</td>
<td>6.89 (6.06, 7.84)</td>
<td>6.45 (5.73, 7.26)</td>
<td>6.81 (5.98, 7.76)</td>
<td>6.66 (5.95, 7.46)</td>
<td>0.41</td>
<td>0.76</td>
</tr>
<tr>
<td>Gained 0–15 cm\textsuperscript{2}</td>
<td>5.98 (5.31, 6.73)</td>
<td>5.61 (5.08, 6.20)</td>
<td>5.78 (5.13, 6.52)</td>
<td>5.76 (5.22, 6.35)</td>
<td>0.21</td>
<td>0.64</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.15 (1.03, 1.29)</td>
<td>1.14 (1.01, 1.26)</td>
<td>1.14 (1.02, 1.27)</td>
<td>1.16 (1.03, 1.28)</td>
<td>0.62</td>
<td>0.57</td>
</tr>
</tbody>
</table>

\(p < 0.05\) for change from baseline to 12 months between exercisers and controls for that level of intra-abdominal fat change versus gained >15 cm\textsuperscript{2} intra-abdominal fat; all other comparisons, \(P > 0.05\).

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greater than 15 cm². When stratified by changes in weight, lean body mass, percentage body fat, and VO2max, there were no statistically significant differences between treatment groups (data not shown).

Among women in the exercise group, there were statistically significant differences between the low (reference) and intermediate adherence groups for the change in 2-OH E1/16α-OH E1 ratio from baseline to 3 months and from baseline to 12 months. However, differences between the low and high adherence groups, and trend tests on the adherence categories, were not statistically significant (Table 4).

One woman in the exercise group had large changes in 2-OH E1 and 16α-OH E1 from baseline to 12 months (−56.9 ng/mg creatinine and −41.3 ng/mg creatinine, respectively); excluding her data from the analyses did not substantially change the results.

### Discussion

To our knowledge, this is the first study to have investigated the effects of a 12-month exercise intervention on the urinary excretion of 2-OH E1, 16α-OH E1, and their ratio, in a population of previously sedentary and overweight postmenopausal women. Overall, we did not see a significant effect of the intervention on 2-OH E1, 16α-OH E1, or their ratio.

Data from a number of cross-sectional studies have suggested that an individual’s body fat content might influence estrogen metabolism. For example, in athletes, the extent of 2-hydroxylation was inversely associated with adiposity (24, 25, 33), and in a comparison of obese and anorexic girls, higher body weight was associated with a reduction in 2-hydroxylation and an increase in 16α-hydroxylation (22). In a study of obese and normal weight individuals, obesity also was associated with significantly lower 2-hydroxylation, but there was no effect on 16α-hydroxylation (23). In contrast with these earlier cross-sectional associations, we did not observe a significant association of BMI, percentage body fat, lean body mass, abdominal fat, or subcutaneous fat with baseline levels of 2-OH E1, 16α-OH E1, or their ratio. However, we selected only overweight or obese women for this study; therefore, the homogeneity of the study population may have limited our ability to detect potential associations. There were weak inverse associations between baseline intra-abdominal body fat and 2-OH E1 and 16α-OH E1. Reasons for this are unclear, but might be due to low levels of aromatase activity in intra-abdominal body fat (34), resulting in reduced levels of estrogens available for conversion to 2-OH E1 and 16α-OH E1.

Our findings are consistent with a study of premenopausal women assigned to an intensive 6-month dietary and lifestyle intervention, which included goals to increase physical activity and to lose weight (35). In that study, the change in 2-OH E1:16α-OH E1 ratio was not significantly different from the change among women assigned to the control group. Change in body fat was not measured and the authors suggested that, even though their study participants lost weight, it may not have been sufficient to bring about the depletion of fat stores necessary for increasing 2-hydroxylation (35). Similarly, in our study, despite the fact that women in the exercise group lost significantly more weight than the women in the control group (29), the reduction in weight was modest. When we stratified our analyses according to weight change, differences between treatment groups remained non-significant, further suggesting that the extent of weight loss in this study may not have been sufficient to have a substantial effect on the estrogen metabolites.

We observed weak positive correlations between change in lean body mass and change in 2-OH E1, which is partially in agreement with studies in athletes and obese individuals, in which adiposity was negatively associated with 2-hydroxylation (22–25, 33). However, stratification by change in lean body mass in the longitudinal (general estimating equations) analysis did not modify the effect of the intervention on change in the estrogen metabolites.

### Table 4. Geometric mean (95% confidence interval) 2-OH E1, 16α-OH E1, and their ratio at baseline, and at months 3 and 12 in exercisers by minutes of exercise per week

<table>
<thead>
<tr>
<th>2-OH E1 (ng/mg Cr)</th>
<th>Baseline</th>
<th>3 months</th>
<th>12 months</th>
<th>P1</th>
<th>P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;130 min/week</td>
<td>6.17 (4.90, 7.78)</td>
<td>5.91 (4.86, 7.19)</td>
<td>6.51 (5.31, 7.99)</td>
<td>0.47</td>
<td>0.11</td>
</tr>
<tr>
<td>130–190 min/week</td>
<td>7.91 (6.16, 10.15)</td>
<td>6.39 (5.18, 7.90)</td>
<td>6.01 (4.88, 7.41)</td>
<td>0.72</td>
<td>0.96</td>
</tr>
<tr>
<td>&gt;190 min/week</td>
<td>6.85 (5.45, 8.60)</td>
<td>6.26 (5.13, 7.64)</td>
<td>6.79 (5.60, 8.24)</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>16α-OH E1 (ng/mg Cr)</td>
<td>5.86 (4.70, 7.31)</td>
<td>4.96 (4.12, 5.97)</td>
<td>5.35 (4.43, 6.46)</td>
<td>0.19</td>
<td>0.37</td>
</tr>
<tr>
<td>130–190 min/week</td>
<td>5.81 (4.60, 7.34)</td>
<td>5.73 (4.73, 6.95)</td>
<td>5.28 (4.35, 6.40)</td>
<td>0.23</td>
<td>0.86</td>
</tr>
<tr>
<td>&gt;190 min/week</td>
<td>6.23 (5.04, 7.72)</td>
<td>5.74 (4.82, 6.85)</td>
<td>6.13 (5.16, 7.29)</td>
<td>0.19</td>
<td>0.37</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.05 (0.87, 1.28)</td>
<td>1.20 (0.99, 1.45)</td>
<td>1.21 (0.99, 1.48)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>130–190 min/week</td>
<td>1.43 (1.16, 1.76)</td>
<td>1.06 (0.87, 1.30)</td>
<td>1.14 (0.93, 1.40)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>&gt;190 min/week</td>
<td>1.06 (0.87, 1.28)</td>
<td>1.12 (0.92, 1.36)</td>
<td>1.07 (0.89, 1.29)</td>
<td>0.36</td>
<td>0.36</td>
</tr>
</tbody>
</table>

*Sample sizes for those who exercised <130 min/week, 130–190 min/week, and >190 min/week, respectively: 2-OH E1, baseline = 28, 24, 29, 3 months = 28, 24, 27, 12 months = 25, 24, 28; 16α-OH E1, baseline = 28, 25, 30, 3 months = 27, 25, 30, 12 months = 25, 24, 30; ratio, baseline = 28, 24, 29, 3 months = 28, 25, 27, 12 months = 25, 24, 29.

P1 value for difference in metabolite change from baseline to 3 months between adherence groups (referent is <130 min/week adherence group), P trend > 0.05.

P1 value for difference in metabolite change from baseline to 12 months between adherence groups (referent is <130 min/week adherence group), P trend > 0.05.
There appeared to be an effect of change in intra-abdominal fat and adherence to the intervention on the change in the estrogen metabolites and their ratio, but it did not, overall, reflect a potentially desirable change in the estrogen metabolites associated with the exercise intervention.

The intensity of physical activity has been positively associated with serum levels of 2-OH E1 in premenopausal women in one (36), but not another (25), study. In our study, the intensity of exercise undertaken by the participants was moderate; therefore, we cannot exclude the possibility that a more vigorous intensity intervention might have a significant effect on estrogen metabolism, potentially via greater changes in weight and/or body composition than were seen in the present study. Alternatively, it is possible that the effects of exercise on these estrogen metabolites might differ between premenopausal and postmenopausal women.

Another factor that could have contributed to the essentially null findings in this study is the timing of the intervention in relation to life events. It is possible that exercise during adolescence or early adulthood, when breast tissue is developing, may be more important in terms of providing protection against breast cancer (37–39), although not all (40–42), studies have shown protective effects of exercise during adolescence or early adulthood on subsequent risk of breast cancer. It would be interesting to determine the effects of an exercise intervention on estrogen metabolism in adolescents or young adults.

To our knowledge, this is one of the largest and longest exercise intervention trials among postmenopausal women to date; however, despite this, a potential limitation of this study is the sample size. A small number of women (7%) in the control group increased their level of physical activity to 225 min/week, but this might have a significant effect on estrogen metabolism, potentially via greater changes in weight and/or body composition than were seen in the present study. Alternatively, it is possible that the effects of exercise on these estrogen metabolites might differ between premenopausal and postmenopausal women.

In conclusion, this 12-month moderate intensity exercise intervention did not significantly alter the urinary excretion of 2-OH E1, 16α-OH E1, or their ratio in this population of postmenopausal women.

Acknowledgments
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Effects of a Moderate Intensity Exercise Intervention on Estrogen Metabolism in Postmenopausal Women


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