Research Article

Cancer Epidemiology, Biomarkers & Prevention

The Effects of Aerobic Exercise on Estrogen Metabolism in Healthy Premenopausal Women

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Abstract

Background: It is well accepted that exercise can decrease breast cancer risk. Limited clinical evidence suggests that this risk could be mediated through changes in estrogen metabolism in premenopausal women. Our objective was to investigate the effects of exercise on premenopausal estrogen metabolism pertinent to breast cancer risk.

Methods: Sedentary, healthy, young eumenorrheic women were randomized into an intervention of 30 minutes of moderate-to-vigorous aerobic exercise five times a week for approximately 16 weeks ($n = 212$), or into a usual-lifestyle sedentary control group ($n = 179$). Urinary levels of estrogens [estrone [E_1], estradiol, and estriol] and nine estrogen metabolites were measured at baseline and at study end by liquid chromatography/tandem mass spectrometry. The ratios of 2-hydroxyestrone to 16α-hydroxyestrone (2-OHE_1/16α-OHE_1) and 2-OHE_1 to 4-hydroxyestrone (2- OHE_1/4-OHE_1) were also calculated.

Results: The exercise intervention resulted in significant increases in aerobic fitness and lean body mass and a significant decrease in percent body fat. For exercisers who completed the study ($n = 165$), 2-OHE_1/16α-OHE_1 increased significantly ($P = 0.043$), whereas E_1 decreased significantly ($P = 0.030$) in control participants ($n = 153$). The change from baseline in 2-OHE_1/16α-OHE_1 was significantly different between groups ($P = 0.045$), even after adjustment for baseline values.

Conclusions: The exercise intervention resulted in a significant increase in the 2-OHE_1/16α-OHE_1 ratio but no differences in other estrogen metabolites or ratios.

Impact: Our results suggest that changes in premenopausal estrogen metabolism may be a mechanism by which increased physical activity lowers breast cancer risk. *Cancer Epidemiol Biomarkers Prev; 22(5); 756–64. ©2013 AACR.*

Introduction

It is well-accepted that lifetime estrogen exposure increases the risk for breast cancer as a result of cumulative stimulation of epithelial cell division by estrogen (1). It has also been suggested that some metabolites resulting from the biotransformation and inactivation of estrogen can play a significant role in breast carcinogenesis (2). Specifically, the products resulting from the oxidation of estradiol (E_2) and estrone (E_1) known as hydroxyestrogens have been shown to display varying degrees of carcinogenicity. For example, 2-hydroxyestrone (2-OHE_1) partially antagonizes the growth-stimulatory effect of E_2 in cultured human MCF-7 breast cancer cells (3) whereas 2-hydroxyestradiol (2-OHE_2) has little or no carcinogenic activity in Syrian hamsters (4, 5). In cultured mouse mammary epithelial cells, 16α-hydroxyestrone (16α-OHE_1) increases unscheduled DNA synthesis and promoted anchorage-independent growth (6, 7). The metabolite 4-hydroxyestrone (4-OHE_1) is considered genotoxic due to its redox cycling process, which generates reactive oxygen species (ROS) and highly cytotoxic semiquinone/quinone intermediates that react with DNA (2). The 2-hydroxyestrogens also undergo redox cycling but appear to lack carcinogenic activity due to a more rapid clearance *in vivo* (8) associated with a faster rate of inactivation through *O*-methylation (9, 10). Finally, one product resulting from *O*-methylation, namely 2-methoxyestradiol (2-$MeOE_2$), has been shown to be a potent inhibitor of cell proliferation and angiogenesis (11, 12).

Despite evidence suggesting the possible importance of other aspects of estrogen metabolism on breast cancer, human studies have largely focused on the ratio of 2-OHE_1 to 16α-OHE_1 (2-OHE_1/16α-OHE_1). Given the different genotoxic capacity of these metabolites, it has been hypothesized that metabolism favoring the production of 2-OHE_1 over 16α-OHE_1 may be inversely associated with

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breast cancer risk (13). In premenopausal women, the strongest evidence in favor of this hypothesis comes from 2 early prospective studies in which urine specimens were collected several years before diagnosis. In the Guernsey III cohort study, women in the highest tertile of urinary 2-OHE_1/16α-OHE_1 ratio had a nonsignificantly lower OR (0.75) for breast cancer than women in the lowest 2 tertiles (14). Similarly, in a study reported by Muti and colleagues, women in the highest quintile of the urinary 2-OHE_1/16α-OHE_1 ratio had an adjusted OR for breast cancer of 0.58, although again this was not statistically significant (15). In contrast, in a more recent prospective study, a higher 2-OHE_1/16α-OHE_1 ratio was associated with an increase in premenopausal estrogen receptor (ER)-positive breast cancer (16). However, the association was not statistically significant and estrogen metabolites were measured in serum and not in urine as in the 2 previous studies. Significant relationships between premenopausal breast cancer risk and urinary levels of estrogen metabolites and their ratios have been observed in some case–control studies, but findings have been inconsistent. The case–control studies of both Coker and colleagues (17) and Kabat and colleagues (18) found an increased risk in women with an increased 2-OHE_1/16α-OHE_1 ratio, but other studies did not (19–21). As for other measures, in 2 other studies, control women had significantly higher levels of 2-hydroxyestrogens, 4-hydroxyestrogens, 16α-OHE_1 (22), and 2-OHE_1/4-OHE_1 ratio (23) than women with breast cancer.

While the association between estrogen metabolism and breast cancer risk needs further investigation, epidemiologic evidence strongly supports the association between higher levels of aerobic exercise and reduced risk for breast cancer (24). However, whether exercise in premenopausal women results in what may be favorable effects on estrogen metabolism is not clear. For example, in one small study, highly fit women exercising strenuously for 368 min/wk had similar values of 2-OHE_1, 16α-OHE_1, and 2-OHE_1/16α-OHE_1 ratio than those of women exercising recreationally for only 60 min/wk (25). In contrast, in another small study, higher levels of self-reported physical activity were associated with higher urinary concentrations of 2-OHE_1 and a higher 2-OHE_1/16α-OHE_1 ratio (26). Recently, a large study of 603 women from the Nurses' Health Study II found high levels of physical activity not only to be correlated with a higher 2-OHE_1/16α-OHE_1 ratio but also significantly lower levels of E_2 and 16α-OHE_1 (27). In comparison, data from exercise intervention studies have been conflicting. For instance, in interventions lasting 12 weeks (28), 16 weeks (29), or even 6 months (30), moderate-to-vigorous intensity aerobic exercise in premenopausal women did not result in any significant changes in urinary concentrations of E_1, E_2, estriol (E_3), 2-hydroxyestrogens, 4-hydroxyestrogens, 16α-OHE_1, or either 2-OHE_1/16α-OHE_1 or 2-OHE_1/4-OHE_1 ratios. In 2 small exercise interventions coupled with calorie restriction lasting 4 and 6 months, there were significant increases in urinary levels of luteal phase 16α-OHE_1 and 2-OHE_1/16α-OHE_1, respectively (31, 32).

Overall, the data on the effects of aerobic exercise on premenopausal estrogen metabolism are not only conflicting but also narrow in scope. With the exception of 2 studies (27, 29), all published studies to date have focused on a limited number of estrogen metabolites, namely, 2-OHE_1 and 16α-OHE_1, and their ratio. Furthermore, no study has yet investigated the levels of the 2- and 4-methylated catecholestrogens despite their purported role in breast carcinogenesis as suggested by culture and animal studies. The WISER (Women In Steady Exercise Research) study was a large, randomized, exercise-controlled, parallel-arm, clinical study investigating the effects of 16 weeks of moderate-to-vigorous intensity aerobic exercise on several parameters pertinent to breast cancer risk in sedentary, healthy, young eumenorrheic women. Here, we report changes from baseline in urinary levels of estrogens (E_1, E_2, and E_3), 9 estrogen metabolites [2-OHE_1, 2-OHE_2, 16α-OHE_1, 4-OHE_1, 4-hydroxyestradiol [4-OHE_2,], 2-methoxyestrone, [2-$MeOE_1$], 2-$MeOE_2$, 4-methoxyestrone [4-$MeOE_1$], and 4-methoxyestradiol [4-$MeOE_2$]], and 2 estrogen metabolite ratios (2-OHE_1/16α-OHE_1 and 2-OHE_1/4-OHE_1).

Materials and Methods

Study design

The WISER study was a randomized clinical trial investigating the effects of a 16-week aerobic exercise intervention on breast cancer biomarkers of healthy, premenopausal women. All procedures were approved by the Human Subjects Review Committee at the University of Minnesota (Minneapolis, MN; Institutional Review Board; IRB ID#0505M69867). Written informed consent was obtained from each participant before participation. A complete description of the study design including participant recruitment, screening, randomization, and retention has been published (33).

Briefly, WISER study investigators emailed more than 100,000 female residents of the Minneapolis–St.Paul metropolitan area regarding participation. Women who were interested were screened online based on age (18–30 years old), physical activity (2 or less weekly sessions of moderate intensity exercise), smoking status (nonsmoking), body mass index (BMI; 18–40 kg/m^2 inclusive), and self-reported menstrual cycle length (24–35 days). Women who met these criteria were further screened via telephone ($n = 1684$) and excluded on the basis of previous hormonal contraception use (past 3 months or 12 months if depot-medroxyprogesterone acetate), gynecologic problems, metabolic or endocrine-related diseases, current or recent (past 6 months) pregnancy, nonmelanoma cancer in the past 5 years, alcohol consumption (more than 7 servings/wk), and body weight changes (more than 10% over the past year).

Of the 966 women who attended a 2-hour orientation, 391 provided written consent and were enrolled in the study. After baseline measurements, women were

randomized into either an exercise intervention ($n = 212$) or a no-exercise, usual-lifestyle control group ($n = 179$) for approximately 16 weeks. Randomization was stratified on baseline BMI tertiles ($\leq$22.8, 22.8–26.3, $\geq$26.3) based on the 50th and 75th percentiles from NHANES I data and age (18–24 vs. 25–30). Participants who failed to return for follow-up measures were dropped from of the study. In addition, exercisers were subject to study exclusion if they missed 15 or more exercise sessions. Figure 1 shows the recruitment, screening, randomization, retention, and completion of WISER participants.

Exercise intervention

Women randomized to the exercise intervention trained aerobically 5 times a week for 30 minutes on a treadmill, stair-stepper, or elliptical machine, at a specified intensity based on age-predicted maximal heart rate (max HR) for 16 weeks ($\pm$2 weeks). The exercise intensity was initially set at 65% to 70% of the age-predicted max HR and was gradually increased by 5% every 4 weeks until 80%–85% of age-predicted max HR was reached (stage 1 = 65%–70%; stage 2 = 70%–75%; stage 3 = 75%–80%; stage 4 = 80%–85%).

All training sessions took place at the University of Minnesota's Recreation Center. At the first training session, a certified personal trainer provided instruction on the proper use of the exercise machines, heart rate monitor and watch, and completion of an exercise log after each workout. Trainers supervised exercise sessions and reviewed the exercise logs at least once weekly to monitor adherence and safety. When not meeting with a trainer, participants were expected to complete the remaining of the workout sessions unsupervised. Exercise adherence was assessed using the data from the heart rate monitor (Polar Electro Inc.) and exercise logs.

Any physical activity conducted after randomization and outside the prescribed exercise intervention was assessed at the end of the study with a physical activity questionnaire administered by a research staff member. All participants, regardless of randomization outcome, were asked to maintain their baseline body weight. Control participants were asked to not only to maintain their usual level of physical activity but also to not change their eating habits.

Anthropometrics

Body mass was measured to the nearest 0.1 kg using an electronic scale (Scale Tronix) 4 times throughout the study (baseline, intervention weeks 4 and 8, and follow-up). Height was measured without shoes to the nearest 0.1 cm (Scale Tronix) by a stadiometer at baseline. BMI was calculated by dividing body mass in kg by height in meters squared (kg/m^2). Body composition was

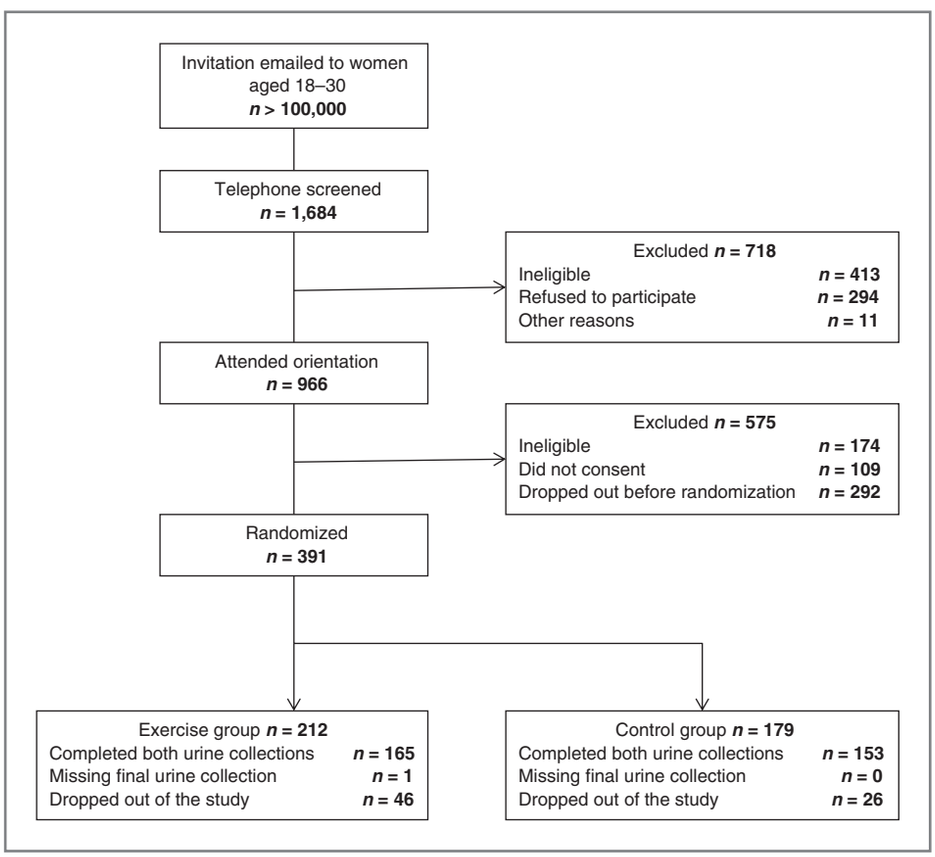

Figure 1. CONSORT diagram showing participant recruitment, screening, randomization, and retention.

assessed at baseline and follow-up by dual-energy X-ray absorptiometry (DXA) using a Lunar Prodigy DXA apparatus (Lunar Radiation Corp.).

Aerobic fitness and physical activity

Aerobic fitness was assessed at baseline and immediately after the intervention with a submaximal treadmill test described previously (33). This workload was then converted to metabolic equivalents (MET) by using a standard conversion formula (34). Self-reported physical activity conducted a year before the study and during the 4-month follow-up period was assessed by a research staff using a modified version of the Modifiable Activity Questionnaire (35). This information was transformed into MET-hours per week (MET-h/wk) using commonly accepted MET values (36).

Dietary intake

Usual dietary intake was assessed through self-reported, 3-day food records completed concomitantly with the urine collections at baseline and follow-up. Nutrient intake was determined using The Food Processor SQL by ESHA Research.

Urine collection

Forty-eight hours before the urine collection, participants were asked to avoid moderate or vigorous exercise and abstain from alcohol. Urine was collected for 3 consecutive 24-hour periods in the midfollicular phase (follicular days 7–9 of baseline menstrual cycle 2 and follow-up menstrual cycle 6). Throughout each day, urine was collected in a 1-L bottle and kept cold with ice packs inside an insulated bag. At the end of each collection day, urine was transferred into a 3-L bottle containing ascorbic acid (1 mg/mL) to prevent oxidation and stored in a home refrigerator or cooler provided by the study. Once the urine collection was completed, collection bottles were retrieved by a research staff member and brought to the General Clinical Research Center at the University of Minnesota for processing. Urine was refrigerated and 0.1% sodium azide was added before the three 24-hour collections were pooled. Aliquots were taken and stored at −20°C until analysis.

Estrogen metabolites

Urinary estrogens (E_1, E_2, and E_3) and their metabolites (2-OHE_1, 2-OHE_2, 16α-OHE_1, 4-OHE_1, 4-OHE_2, 2-$MeOE_1$, 2-$MeOE_2$, 4-$MeOE_1$, and 4-$MeOE_2$) were analyzed in the midfollicular phase of baseline and follow-up cycles by liquid chromatography/tandem mass spectrometry (LC/MS-MS) conducted using a Thermo Electron Quantum Discovery Max Triple Quadrupole LC-MS/MS instrument (37). Quantitative analysis was conducted using Thermo Electron Xcalibur proprietary software.

Samples with nondetectable levels were assigned values of the lowest detectable standard (0.014 ng/mL urine). Concentrations were expressed both as nanomol per day (nmol/d) and nanograms per milligram of creatinine (ng/mg Cr). Urinary creatinine was analyzed at the Fairview University Diagnostic Laboratories.

Samples were run in duplicate and in batches such that each batch contained both baseline and follow-up samples from each participant and an equal number of exercise and control participants. One quality control sample was included in each batch. The mean intra- and interassay coefficient of variations (CV) were 5.1% and 13.4% for E_1; 5.2% and 16.0% for E_2; 5.6% and 11.4% for E_3; 4.2% and 12.3% for 2-OHE_1; 7.7% and 10.8% for 2-OHE_2; 6.2% and 18.7% for 16α-OHE_1; 4.3% and 12.2% for 4-OHE_1; 14.0% and 51.2% for 4-OHE_2; 7.0% and 11.3% for 2-$MeOE_1$; 5.8% and 10.0% for 2-$MeOE_2$; 7.4% and 10.3% for 4-$MeOE_1$; and 6.9% and 7.9% for 4-$MeOE_2$.

Statistical analyses

Unadjusted comparisons of baseline characteristics were conducted using Student t tests for continuous variables and χ^2 tests for categorical variables.

Two estrogen metabolite ratios of interest, namely, the 2-OHE_1/16α-OHE_1 ratio and 2-OHE_1/4-OHE_1 ratio, were calculated by dividing the concentration of 2-OHE_1 by either 16α-OHE_1 or 4-OHE_1, respectively.

Baseline associations between urinary estrogens, their metabolites, and metabolite ratios and measures of body composition, adiposity, fitness, reproductive characteristics, and diet were determined using Spearman correlation coefficients.

The main study analysis assessed the intervention effects using only data from participants who completed the baseline and follow-up urine collection regardless of compliance level. Baseline and follow-up comparisons were conducted using log-transformed values and results are presented as geometric means with 95% confidence intervals (CI). Changes from baseline comparisons were compared on the original scale. All comparisons were adjusted for study design age and BMI strata with a general linear model. When there were significant differences at baseline in an outcome, follow-up and change from baseline comparisons were additionally adjusted for baseline values. Linear models were calculated using SAS software, version 9.2 (SAS institute Inc.). $P < 0.05$ was considered statistically significant.

Estrogen metabolite data were analyzed as both nmol/d and ng/mg Cr. Results from the 2 analyses did not differ significantly, and we report results as nmol/d.

Results

Study participants

Of the 212 and 179 women randomized into the exercise and control groups, 165 (77.8%) and 153 (85.5%), respectively, completed the WISER study. With the exception of education (47% of dropouts had some college education vs. 27% of study completers, $P = 0.002$), dropouts were no different from women who completed the study in any of the baseline demographic characteristic measured (data

not shown). Also, there were no significant differences between exercisers and controls in baseline demographic characteristics (Table 1). In general, women who completed the study were mostly Caucasian (72%), single (82%), nulliparous (93%), had education beyond high school (96%), and had no first-degree relatives with breast cancer (97%).

Baseline estrogen metabolism

With the exception of 2-OHE_1 ($P = 0.084$) and 2-OHE_1/16α-OHE_1 ratio ($P = 0.044$), exercisers had similar levels of urinary estrogens, estrogen metabolites, and 2-OHE_1/4-OHE_1 ratio than control participants at baseline (Table 2). No significant baseline associations between any of the urinary endpoints and measures of body composition, adiposity, fitness, reproductive characteristics, and diet were found.

Overall, the concentration of estrone and its metabolites were higher than their estradiol counterparts, especially for E_1, 2-OHE_1, 4-OHE_1, and 4-$MeOE_1$ as compared with E_2, 2-OHE_2, 4-OHE_2, and 4-$MeOE_2$, respectively. Estrogen hydroxylation showed an isomeric preference for the C-2 position. Specifically, concentrations of 2-OHE_1 were about 14- and 20-fold higher than those of 16α-OHE_1 and 4-OHE_1, respectively, and concentration of 2-OHE_2 about 40-fold those of 4-OHE_2.

Exercise adherence

On average, exercise participants completed 127 min/wk of the assigned 150 minutes of exercise intervention. Details about exercise adherence and compliance can be found elsewhere (38).

Intervention effects

The exercise intervention resulted in significant improvements in body composition and aerobic fitness without changes in body weight. As previously reported, exercisers experienced significant increases in aerobic fitness (0.90 METs reached at 85% of max HR vs. 0.12 METs in controls) and lean body mass (0.55 vs. 0.07 kg), as well as significant decreases in fat mass (0.57 vs. 0.04 kg) and percent body fat (0.95% vs. 0.09%). In contrast, control participants experienced no changes in body composition, aerobic fitness, and body weight despite a significant reduction in daily caloric intake (−224 kcal/d). Exercisers also reduced their food consumption, but only by 18 kcal/d ($P > 0.05$). Details on the effects of this intervention on body composition, body weight, aerobic fitness, and energy intake have been published previously (39).

As previously reported, the exercise intervention resulted in no significant changes in endogenous levels of E_2, estrone sulfate, progesterone, T, or SHBG (38). Exercisers, however, did experience a significant increase in

Table 1. Baseline characteristics of randomized WISER participants ($n = 318$)

	Exercisers ($n = 165$)	Controls ($n = 153$)
Demographics		
Age, y (mean ± SE)	25.4 ± 0.3	25.2 ± 0.3
Not married or partnered, *n* (%)	137 (83%)	124 (81%)
Education beyond high school, *n* (%)	157 (95.2%)	148 (96.7%)
Caucasian, *n* (%)	123 (75%)	107 (70%)
Body composition (mean ± SE)		
Weight, kg	67.5 ± 1.1	67.6 ± 1.2
BMI, kg/m^2	24.8 ± 0.4	24.7 ± 0.4
% body fat	36.4 ± 0.7	36.1 ± 0.7
Fat mass, kg	24.3 ± 0.9	24.1 ± 0.9
Lean mass, kg	39.8 ± 0.4	40.1 ± 0.4
Reproductive characteristics		
Age at menarche, y (mean ± SE)[a]	12.7 ± 0.12	12.7 ± 0.11
Nulliparous, *n* (%)	153 (93%)	144 (94%)
Previous contraceptive use, *n* (%)	84 (51%)	82 (54%)
Family history of breast cancer[b]		
No, *n* (%)	129 (96%)	114 (97%)
Physical activity, fitness, and diet (mean ± SE)		
Moderate exercise (MET-h/wk)	1902 ± 421	1933 ± 525
Aerobic fitness (METs at 85% max HR)	21.9 ± 1.3	21.8 ± 1.4
Total calorie intake,[c] kcal/d	6.9 ± 0.1	7.1 ± 0.1

NOTE: There were no significant differences at baseline between study groups for any of these variables.
[a] $n = 310$.
[b] $n = 251$.
[c] $n = 312$.

Table 2. Effects of 16 weeks of aerobic exercise on estrogen metabolism of WISER participants

	Baseline geometric mean (95% CI)	*P* value for baseline differences	Follow-up geometric mean (95% CI)	*P* value for differences in mean change
Estrone, E_1, nmol/d				
Exercisers	23.1 (19.0–28.2)	0.586	23.0 (18.9–28.0)	**0.042**
Controls	25.0 (20.3–30.8)		23.0 (18.8–28.2)[a]	
Estradiol, E_2, nmol/d				
Exercisers	7.8 (6.8–8.8)	0.786	8.3 (7.2–9.5)	0.725
Controls	8.0 (7.0–9.1)		8.0 (7.2–9.2)	
Estriol, E_3, nmol/d				
Exercisers	21.3 (16.5–27.4)	0.963	18.6 (14.4–24.2)	0.715
Controls	21.1 (16.2–27.5)		21.0 (16.0–27.5)	
16α-OHE_1, nmol/d				
Exercisers	2.9 (2.3–3.7)	0.303	2.6 (2.1–3.4)	0.971
Controls	2.5 (1.9–3.1)		2.5 (1.9–3.2)	
2-OHE_1, nmol/d				
Exercisers	39.3 (33.7–45.8)	0.084	44.2 (38.4–50.8)	0.098
Control	47.6 (40.5–55.8)		45.5 (39.3–52.6)	
4-OHE_1, nmol/d				
Exercisers	2.3 (2.1–2.7)	0.647	2.4 (2.1–2.7)	0.362
Controls	2.4 (2.1–2.8)		2.4 (2.1–2.7)	
2-OHE_2, nmol/d				
Exercisers	3.4 (2.4–5.0)	0.209	3.5 (2.4–5.1)	0.194
Controls	2.5 (1.7–3.6)		2.9 (1.9–4.2)	
4-OHE_2, nmol/d				
Exercisers	0.7 (0.4–1.0)	0.767	0.8 (0.5–1.3)	0.898
Controls	0.7 (0.5–1.2)		0.5 (0.3–0.8)	
2-$MeOE_1$, nmol/d				
Exercisers	8.2 (6.5–10.4)	0.312	9.3 (7.4–11.8)	0406
Controls	6.9 (5.4–8.8)		8.6 (6.8–10.9)	
4-$MeOE_1$, nmol/d				
Exercisers	1.9 (1.4–2.6)	0.562	1.7 (1.2–2.4)	0.488
Controls	2.2 (1.6–3.0)		1.5 (1.1–2.1)	
2-$MeOE_2$, nmol/d				
Exercisers	7.1 (5.4–9.3)	0.269	7.0 (5.4–9.1)	0.556
Controls	5.8 (4.3–7.6)		6.6 (5.1–8.7)	
4-$MeOE_2$, nmol/d				
Exercisers	1.6 (1.2–2.3)	0.254	1.5 (1.0–2.1)	0.552
Controls	1.2 (0.9–1.8)		0.9 (0.7–1.4)	
2-OHE_1/16α-OHE_1				
Exercisers	13.4 (10.4–17.2)	**0.044**	16.8 (13.1–21.4)[b]	**0.045**
Controls	19.3 (14.8–25.1)		18.5 (14.3–24.0)	
2-OHE_1/4-OHE_1				
Exercisers	16.8 (14.8–19.0)	0.104	18.7 (16.8–20.9)	0.123
Controls	19.5 (17.1–22.2)		18.9 (16.9–21.2)	

NOTE: Values are age- and BMI-adjusted geometric means (95% CI). Follow-up and mean change from baseline comparisons in 2-OHE_1 and 2-OHE_1/16α-OHE_1 were additionally adjusted for baseline values.

[a]$P = 0.030$ (within-group differences).

[b]$P = 0.043$ (within-group differences).

urinary 2-OHE_1/16α-OHE_1 ratio ($P = 0.043$), whereas controls had a nonsignificant decrease in 2-OHE_1/16α-OHE_1 ratio. The difference in the change from baseline in 2-OHE_1/16α-OHE_1 ratio between groups was significant ($P = 0.045$), even after adjustment for baseline values. Figure 2 shows that many, but not all, of the participants who experienced an absolute change in 2-OHE_1/16α-OHE_1 ratio greater than 100 had a high baseline

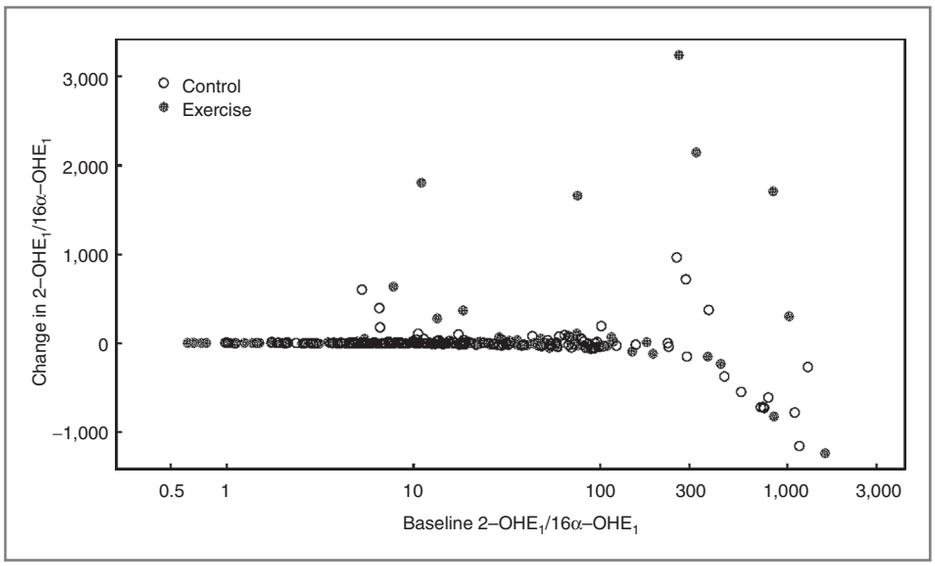

Figure 2. Changes in baseline versus baseline in 2-OHE_1/16α-OHE_1 ratio.

2-OHE_1/16α-OHE_1 ratio. Levels of E_1 remained unchanged in exercisers but decreased in controls resulting in a statistically significant change from baseline between the groups ($P = 0.042$). No significant within-group changes or between-group differences at follow-up were observed for other estrogens, estrogen metabolites, or ratios.

Discussion

We found that in healthy premenopausal women, an exercise regimen of 150 minutes of moderate-to-vigorous aerobic exercise per week for 16 weeks resulted in significant changes in estrogen metabolism in a direction consistent with reduction of breast cancer risk. Specifically, exercise participants experienced a significant increase in urinary levels of 2-OHE_1 and a small nonsignificant decrease in 16α-OHE_1 levels. These changes resulted in a significant increase in the 2-OHE_1/16α-OHE_1 ratio. In contrast, women in the control group had a nonsignificant decrease in 2-OHE_1/16α-OHE_1 ratio largely attributable to those few controls with large baseline ratio having large decreases in the ratio. Controls also had an unexplained significant decrease in E_1. We did not find evidence for exercise resulting in changes in the 4-hydroxylation pathway or other differences that conceivably could have been found.

Overall, our results differ from those of other exercise intervention studies investigating the effects of aerobic exercise on premenopausal estrogen metabolism. For instance, in a small 5-month weight loss clinical trial involving moderate-intensity exercise, both controls and exercisers had significant increases in the urinary 2-OHE_1/16α-OHE_1 ratio, but in contrast to our results, the change in ratio between the groups was not statistically significant (32). In a small pre-post design study, 4 months of moderate exercise coupled with calorie restriction resulted in nonsignificantly higher urinary levels of 2-OHE_1 and 16α-OHE_1 and the ratio (31). In our study, both 2-OHE_1 and the 2-OHE_1/16α-OHE_1 ratio increased significantly whereas 16α-OHE_1 decreased nonsignificantly. Both of these studies differed from our study in that aerobic exercise was coupled with significant calorie restriction making it impossible to discern whether the changes reported were the result of the exercise or diet. When compared with exercise-only interventions, our study remains the only one to report significant changes in estrogen metabolism. For example, in a moderate-to-vigorous aerobic exercise intervention lasting 12 weeks (30–45 minutes, 4 d/wk), Campbell and colleagues reported no significant changes in urinary premenopausal levels of 2-OHE_1, 16α-OHE_1, or 2-OHE_1/16α-OHE_1 ratio (28). Similarly, in a pilot study conducted by our research group, total levels of 2-OHE (2-OHE_1 + 2-OHE_2), 4-OHE (4-OHE_1 + 4-OHE_2), and the 2-OHE_1/16α-OHE_1 ratio remained unchanged in 15 young women exercising aerobically for 30 min/d, 5 times a week for 16 weeks (29). Similarly, Robles-Gil and colleagues did not find significant changes in E_1, E_2, or E_3 in 20 premenopausal women after 6 months of 60 minutes of moderate-intensity exercise 3 ds/wk (30).

A possible explanation for the disparity between the results reported by these exercise intervention studies, and our study may be found in the choice of study design and methodology. The WISER study had many methodologic advantages over previously published research. First, the sample size in our study ($n = 318$) was an order of magnitude or more large than those of the other three studies. Second, our study design used randomized controls (only Campbell and colleagues study was randomized). Third, unlike the studies of Campbell and colleagues and Robles-Gil and colleagues, in which first morning urine samples were used, WISER participants collected three 24-hour urine collections allowing for a more robust and representative analysis of the chronic

effect of aerobic exercise on estrogen metabolism. Finally, our study provides the most comprehensive analysis on the effects of exercise on estrogen metabolism to date. We have analyzed urinary levels of the major parent estrogens (E_1, E_2, and E_3) and 9 of their estrogen metabolites by LC/MS-MS. This newer methodology is considered to be superior not only to the ELISA methods used by these studies but also to the current gold standard gas chromatography/mass spectroscopy (GC/MS) due to its increased sensitivity and sample throughput (37, 40). Unlike Xu and colleagues, we were able to quantify and report 4-OHE_2, concentrations, although its lack of detection in 53.4% of our samples resulted in a higher-than-expected interassay CV.

Altogether, the findings of the WISER study are significant because they provide the first clinical evidence that aerobic exercise can significantly change estrogen metabolism in premenopausal women. Specifically, our results show that such an exercise intervention can lead to increases in 2-OHE_1 and possible decreases in 16α-OHE_1 ultimately resulting in significant increases in the 2-OHE_1/16α-OHE_1 ratio. Importantly, increases in this ratio have been associated with a significant reduction in breast cancer risk. From a clinical point of view, the assessment of urinary 2-OHE_1/16α-OHE_1 ratio is also relevant as it has been found to be a good approximation to the 2-OHE_1/16α-OHE_1 ratio of breast tissue (41). Perhaps one mechanism by which exercise mediates estrogen metabolism is through the regulation of P450 cytochrome enzymes responsible for controlling estrogen hydroxylation and catecholestrogen methylation. Given the implication these results have for breast cancer prevention efforts, future studies should not only attempt to corroborate our results but also investigate the exact mechanisms by which exercise leads to these favorable estrogen metabolism changes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A.J. Smith, W.R. Phipps, K.H. Schmitz, M.S. Kurzer
Development of methodology: A.J. Smith, M.S. Kurzer
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.J. Smith, M.S. Kurzer
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.J. Smith, W.R. Phipps, W. Thomas, M.S. Kurzer
Writing, review, and/or revision of the manuscript: A.J. Smith, W.R. Phipps, W. Thomas, K.H. Schmitz, M.S. Kurzer
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.J. Smith, M.S. Kurzer
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References

1. McTiernan A. Mechanisms linking physical activity with cancer. Nat Rev Cancer 2008;8:205–11.
2. Yager JD. Endogenous estrogens as carcinogens through metabolic activation. J Natl Cancer Inst Monogr 2000;27:67–73.
3. Schneider J, Huh MM, Bradlow HL, Fishman J. Antiestrogen action of 2-hydroxyestrone on MCF-7 human breast cancer cells. J Biol Chem 1984;259:4840–5.
4. Li JJ, Li SA. Estrogen carcinogenesis in Syrian hamster tissues: role of metabolism. Fed Proc 1987;46:1858–63.
5. Liehr JG, Fang WF, Sirbasku DA, Ari-Ulubelen A. Carcinogenicity of catechol estrogens in Syrian hamsters. J Steroid Biochem 1986;24:353–6.
6. Suto A, Bradlow HL, Wong GY, Osborne MP, Telang NT. Experimental down-regulation of intermediate biomarkers of carcinogenesis in mouse mammary epithelial cells. Breast Cancer Res Treat 1993;27:193–202.
7. Telang NT, Suto A, Wong GY, Osborne MP, Bradlow HL. Induction by estrogen metabolite 16 alpha-hydroxyestrone of genotoxic damage and aberrant proliferation in mouse mammary epithelial cells. J Natl Cancer Inst 1992;84:634–8.
8. Lipsett MB, Merriam GR, Kono S, Brandon DD, Pfeiffer DG, Loriaux DL. Catechol estrogens. New York, NY: Raven Press; 1983.
9. Li SA, Purdy RH, Li JJ. Variations in catechol O-methyltransferase activity in rodent tissues: possible role in estrogen carcinogenicity. Carcinogenesis 1989;10:63–7.
10. Roy D, Weisz J, Liehr JG. The O-methylation of 4-hydroxyestradiol is inhibited by 2-hydroxyestradiol: implications for estrogen-induced carcinogenesis. Carcinogenesis 1990;11:459–62.
11. Fotsis T, Zhang Y, Pepper MS, Adlercreutz H, Montesano R, Nawroth PP, et al. The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. Nature 1994;368:237–9.
12. Klauber N, Parangi S, Flynn E, Hamel E, D'Amato RJ. Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and taxol. Cancer Res 1997;57:81–6.
13. Bradlow HL, Hershcopf RJ, Martucci CP, Fishman J. Estradiol 16 alpha-hydroxylation in the mouse correlates with mammary tumor incidence and presence of murine mammary tumor virus: a possible model for the hormonal etiology of breast cancer in humans. Proc Natl Acad Sci U S A 1985;82:6295–9.
14. Meilahn EN, De Stavola B, Allen DS, Fentiman I, Bradlow HL, Sepkovic DW, et al. Do urinary oestrogen metabolites predict breast cancer? Guernsey III cohort follow-up. Br J Cancer 1998;78:1250–5.
15. Muti P, Bradlow HL, Micheli A, Krogh V, Freudenheim JL, Schünemann HJ, et al. Estrogen metabolism and risk of breast cancer: a prospective study of the 2:16alpha-hydroxyestrone ratio in premenopausal and postmenopausal women. Epidemiology 2000;11:635–40.
16. Arslan AA, Shore RE, Afanasyeva Y, Koenig KL, Toniolo P, Zeleniuch-Jacquotte A. Circulating estrogen metabolites and risk for breast

cancer in premenopausal women. Cancer Epidemiol Biomarkers Prev 2009;18:2273–9.
17. Coker AL, Crane MM, Sticca RP, Sepkovic DW. Re: Ethnic differences in estrogen metabolism in healthy women. J Natl Cancer Inst 1997;89:89–90.
18. Kabat GC, O'Leary ES, Gammon MD, Sepkovic DW, Teitelbaum SL, Britton JA, et al. Estrogen metabolism and breast cancer. Epidemiology 2006;17:80–8.
19. Fowke JH, Qi D, Bradlow HL, Shu XO, Gao YT, Cheng JR, et al. Urinary estrogen metabolites and breast cancer: differential pattern of risk found with pre- versus post-treatment collection. Steroids 2003;68:65–72.
20. Kabat GC, Chang CJ, Sparano JA, Sepkovie DW, Hu XP, Khalil A, et al. Urinary estrogen metabolites and breast cancer: a case-control study. Cancer Epidemiol Biomarkers Prev 1997;6:505–9.
21. Ursin G, London S, Yang D, Tseng CC, Pike MC, Bernstein L, et al. Urinary 2-hydroxyestrone/16alpha-hydroxyestrone ratio and family history of breast cancer in premenopausal women. Breast Cancer Res Treat 2002;72:139–43.
22. Adlercreutz H, Fotsis T, Höckerstedt K, Hämäläinen E, Bannwart C, Bloigu S, et al. Diet and urinary estrogen profile in premenopausal omnivorous and vegetarian women and in premenopausal women with breast cancer. J Steroid Biochem 1989;34:527–30.
23. Gaikwad NW, Yang L, Muti P, Meza JL, Pruthi S, Ingle JN, et al. The molecular etiology of breast cancer: evidence from biomarkers of risk. Int J Cancer 2008;122:1949–57.
24. Friedenreich CM, Cust AE. Physical activity and breast cancer risk: impact of timing, type and dose of activity and population subgroup effects. Br J Sports Med 2008;42:636–47.
25. Campbell KL, Westerlind KC, Harber VJ, Friedenreich CM, Courneya KS. Associations between aerobic fitness and estrogen metabolites in premenopausal women. Med Sci Sports Exerc 2005;37:585–92.
26. Bentz AT, Schneider CM, Westerlind KC. The relationship between physical activity and 2-hydroxyestrone, 16alpha-hydroxyestrone, and the 2/16 ratio in premenopausal women (United States). Cancer Causes Control 2005;16:455–61.
27. Matthews CE, Fortner RT, Xu X, Hankinson SE, Eliassen AH, Ziegler RG. Association between physical activity and urinary estrogens and estrogen metabolites in premenopausal women. J Clin Endocrinol Metab 2012;97:3724–33.
28. Campbell KL, Westerlind KC, Harber VJ, Bell GJ, Mackey JR, Courneya KS. Effects of aerobic exercise training on estrogen metabolism in premenopausal women: a randomized controlled trial. Cancer Epidemiol Biomarkers Prev 2007;16:731–9.
29. Schmitz KH, Warren M, Rundle AG, Williams NI, Gross MD, Kurzer MS. Exercise effect on oxidative stress is independent of change in estrogen metabolism. Cancer Epidemiol Biomarkers Prev 2008;17:220–3.
30. Robles Gil MC, Timón R, Toribio AF, Muñoz D, Maynar JI, Caballero MJ, et al. Effects of aerobic exercise on urinary estrogens and progestagens in pre and postmenopausal women. Eur J Appl Physiol 2012;112:357–64.
31. Westerlind KC, Williams NI. Effect of energy deficiency on estrogen metabolism in premenopausal women. Med Sci Sports Exerc 2007;39:1090–7.
32. Pasagian-Macaulay A, Meilahn EN, Bradlow HL, Sepkovic DW, Buhari AM, Simkin-Silverman L, et al. Urinary markers of estrogen metabolism 2- and 16 alpha-hydroxylation in premenopausal women. Steroids 1996;61:461–7.
33. Arikawa AY, O'Dougherty M, Kaufman BC, Smith AJ, Thomas W, Warren M, et al. Women in Steady Exercise Research (WISER): Study design and methods. Contemp Clin Trials 2010;31:457–65.
34. American College of Sports Medicine. Guidelines for exercise testing and prescription. 6th ed. Philadelphia, PA: Lippincott, Williams & Wilkins; 2000.
35. Kriska AM, Knowler WC, LaPorte RE, Drash AL, Wing RR, Blair SN, et al. Development of questionnaire to examine relationship of physical activity and diabetes in Pima Indians. Diabetes Care 1990;13:401–11.
36. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc 2000;32(9 Suppl):S498–504.
37. Xu X, Veenstra TD, Fox SD, Roman JM, Issaq HJ, Falk R, et al. Measuring fifteen endogenous estrogens simultaneously in human urine by high-performance liquid chromatography-mass spectrometry. Anal Chem 2005;77:6646–54.
38. Arikawa AY, O'Dougherty M, Kaufman BC, Schmitz KH, Kurzer MS. Attrition and adherence of young women to aerobic exercise: lessons from the WISER study. Contemp Clin Trials 2012;33:298–301.
39. Smith AJ, Phipps WR, Arikawa AY, O'Dougherty M, Kaufman B, Thomas W, et al. Effects of aerobic exercise on premenopausal sex hormone levels: results of the WISER study, a randomized clinical trial in healthy, sedentary, eumenorrheic women. Cancer Epidemiol Biomarkers Prev 2011;20:1098–106.
40. Nelson RE, Grebe SK, OKane DJ, Singh RJ. Liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of estradiol and estrone in human plasma. Clin Chem 2004;50:373–84.
41. Taioli E, Im A, Xu X, Veenstra TD, Ahrendt G, Garte S. Comparison of estrogens and estrogen metabolites in human breast tissue and urine. Reprod Biol Endocrinol 2010;8:93.

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