Effects of exercise dose on endogenous estrogens in postmenopausal women: a randomized trial

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Abstract

Exercise dose comparison trials with biomarker outcomes can identify the amount of exercise required to reduce breast cancer risk and also strengthen the causal inference between physical activity and breast cancer. The Breast Cancer and Exercise Trial in Alberta (BETA) tested whether or not greater changes in estradiol (E_2), estrone, and sex hormone-binding globulin (SHBG) concentrations can be achieved in postmenopausal women randomized to 12 months of HIGH (300 min/week) vs MODERATE (150 min/week) volumes of aerobic exercise. BETA included 400 inactive postmenopausal women aged 50–74 years with BMI of 22–40 kg/m². Blood was drawn at baseline and 6 and 12 months. Adiposity, physical fitness, diet, and total physical activity were assessed at baseline and 12 months. Intention-to-treat analyses were performed using linear mixed models. At full prescription, women exercised more in the HIGH vs MODERATE group (median min/week (quartiles 1,3): 253 (157 289) vs 137 (111 150); $P < 0.0001$). Twelve-month changes in estrogens and SHBG were $< 10\%$ on average for both groups. No group differences were found for E_2 , estrone, SHBG or free E_2 changes (treatment effect ratios (95% CI) from linear mixed models: 1.00 (0.96–1.06), 1.02 (0.98–1.05), 0.99 (0.96–1.02), 1.01 (0.95, 1.06), respectively, representing the HIGH:MODERATE ratio of geometric mean biomarker levels over 12 months; $n = 382$). In per-protocol analyses, borderline significantly greater decreases in total and free E_2 occurred in the HIGH group. Overall, no dose effect was observed for women randomized to 300 vs 150 min/week of moderate to vigorous intensity exercise who actually performed a median of 253 vs 137 min/week. For total and free E_2 , the lack of differential effect may be due to modest adherence in the higher dose group.

Key Words

- ▶ exercise
- ▶ estrogens
- ▶ sex hormone-binding globulin
- ▶ postmenopause
- ▶ breast cancer

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Introduction

Inadequate physical activity is one of the few known modifiable risk factors for postmenopausal breast cancer (World Cancer Research Fund and American Institute for Cancer Research 2007, 2010). Minimum physical activity recommendations for cancer prevention include 150 min/week (World Health Organization 2011, Kushi *et al.* 2012) or 210 min/week of moderate-intensity activity (World Cancer Research Fund and American Institute for Cancer Research 2007), 150 min/week of moderate-vigorous activity (Canadian Society for Exercise Physiology 2011), and 75 min/week of vigorous activity (World Health Organization 2011, Kushi *et al.* 2012). Whether or not these recommendations are optimal for postmenopausal breast cancer prevention, however, is unclear (Hastert *et al.* 2013). Recently a meta-analysis estimated a 5% decrease in breast cancer risk with every 2 h/week increment in moderate-vigorous recreational activity (Wu *et al.* 2013). Such analyses of observational data could be misleading, however, because of inconsistent methods for defining physical activity across studies, physical activity measurement error, and confounding by other factors. Alternatively, exercise trials with biomarker endpoints provide important evidence (Rundle 2005).

Endogenous estrogens are convincing biomarkers of postmenopausal breast cancer risk (Key *et al.* 2002, Woolcott *et al.* 2010, James *et al.* 2011, Tworoger *et al.* 2011, 2014, Zhang *et al.* 2013, Endogenous Hormones and Breast Cancer Collaborative Group 2015). One pooled analysis showed an approximately twofold higher risk of postmenopausal breast cancer for women categorized in the highest vs lowest quintiles of circulating estradiol (E_2) and estrone concentrations respectively (Endogenous Hormones and Breast Cancer Collaborative Group 2015). Most randomized controlled trials (RCTs) comparing 3–12 month exercise prescriptions of 150–225 min/week vs no exercise in postmenopausal women have improved circulating concentrations of E_2 , estrone, and sex hormone-binding globulin (SHBG) on average by 2–13% (Figuerola *et al.* 2003, Copeland & Tremblay 2004, McTiernan *et al.* 2004, Orsatti *et al.* 2008, Monninkhof *et al.* 2009, Friedenreich *et al.* 2010, Yoo *et al.* 2010, Campbell *et al.* 2012, Kim & Kim 2012). Additional mechanisms are hypothesized to relate physical activity to breast cancer risk (reviewed in Neilson *et al.* (2014)); however, with the exception of body fat (World Cancer Research Fund and American Institute for Cancer Research 2007, 2010 Suzuki *et al.* 2009), these pathways are not as clearly established as for sex hormones.

Previously in the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) trial, we randomized 320 postmenopausal women to 225 min/week of aerobic exercise or usual inactivity over 12 months. In the exercise group, average reductions in circulating E_2 and free E_2 , and the average increase in SHBG, were significantly greater than controls; there was no significant difference for estrone changes (Friedenreich *et al.* 2010). Furthermore in an exploratory analysis, favorable dose–response trends were found between average E_2 changes (but not estrone) and exercise adherence: <150, 150–225 and >225 min/week. However, the ALPHA trial was not designed to examine dose–response trends. Our dose–response analysis was based on self-selected adherence levels, and we had insufficient statistical power for detecting significant differences across adherence strata.

In the Breast Cancer and Exercise Trial in Alberta (BETA), we aimed to formally test the dose–response effects of exercise by examining whether or not greater changes in E_2 , estrone, and SHBG concentrations would be achieved in postmenopausal women randomized to a HIGH (300 min/week) vs MODERATE (150 min/week) volume aerobic exercise prescription over 12 months.

Materials and methods

The BETA trial

Detailed methods for the BETA trial were published previously (Friedenreich *et al.* 2014). The BETA trial was a two-centre, two-armed randomized controlled exercise intervention trial in Calgary and Edmonton, Alberta, conducted between June 2010 and June 2013. The overall aim of the study was to examine the dose–response effects of aerobic exercise on adiposity and a variety of proposed blood biomarkers of postmenopausal breast cancer risk. We hypothesized that more favorable biomarker improvements would occur in women prescribed a higher vs moderate volume of aerobic exercise. The focus of the current report is on endogenous sex hormones, which were secondary outcomes from the trial. Total body fat change was the primary outcome and is described in a separate report (Friedenreich *et al.* 2015).

Participants

Eligible women were postmenopausal, age 50–74 years, BMI 22–40 kg/m² and moderately inactive (≤ 120 min/week

of self-reported moderate-intensity recreational activity or ≤ 3 days/week for <30 min/session maximum; estimated $\text{VO}_{2\text{max}} \leq 34.5$ ml/kg min or 34.6 ml/kg min $\leq \text{VO}_{2\text{max}} \leq 37.0$ ml/kg min and 7-day accelerometer count $<10\,000$ steps/day), with no previous cancer diagnosis except non-melanoma skin cancer and no major co-morbid condition or recent major reconstructive surgery. Women were able to maintain acceptable heart and lung function in a sub-maximal treadmill test, were non-users of exogenous hormones or drugs affecting estrogen metabolism and non-smokers, consumed less than or equal to two drinks of alcohol/day over the past year, were English-speaking, resided in Calgary or Edmonton with access to our fitness facility, were not intending to be away more than 4 weeks consecutively and 8 weeks total over the next year and not on or planning a weight loss program. The study protocol was approved by the Alberta Cancer Research Ethics Committee and the Conjoint Health Research Ethics Board of the University of Calgary and the Health Research Ethics Board of the University of Alberta. All participants provided written informed consent after full explanation of the purpose of the trial and trial procedures. Trial registration on ClinicalTrials.gov was initiated prior to analyzing any primary or secondary outcomes.

Randomization

Four hundred postmenopausal women were randomly assigned in a 1:1 ratio to either a 12-month aerobic exercise intervention of HIGH (300 min/week) or MODERATE (150 min/week) volume. Randomization was stratified by the study centre and BMI (<28.8 , ≥ 28.8). Blocking was used within strata with block sizes of four or six to ensure balance between the two arms with respect to the number of participants. The random allocation sequence was generated via user-defined functions within R Software (version 2.11) (R Core Team 2010). Allocations were concealed in numbered envelopes that were prepared by a staff member unrelated to the study team.

Measurements

Demographic and baseline health information was collected by a questionnaire when ascertaining eligibility. Past year total physical activity, which was the sum of all occupational, household, and recreational activities as well as walking or bicycling to/from work (Friedenreich et al. 2006), and past year usual diet (Csizmadia et al. 2007) were self-reported at baseline and 12 months. All activities reported in the Past Year Total Physical Activity

Questionnaire were converted into the metabolic equivalent of task (MET) h/week per year using the *Compendium of Physical Activities* (Ainsworth et al. 2011). Additionally at baseline and 12 months, submaximal fitness tests were conducted using the same protocols and equipment at both study centers. In Edmonton, fitness tests took place at the Cross Cancer Institute and the Behavioural Medicine Fitness Centre at the University of Alberta. Calgary participants were assessed at the Tom Baker Cancer Centre and the Faculty of Kinesiology, University of Calgary. Fitness testing followed the multi-stage modified Balke treadmill test protocol (Pollock et al. 1982). Testing was complete once a participant reached 85% of her age-predicted maximum heart rate or volitional exhaustion. Each participant's predicted $\text{VO}_{2\text{max}}$ was estimated using the multistage model and the American College of Sports Medicine metabolic equations for maximum oxygen consumption (American College of Sports Medicine 2000).

Anthropometric measurements were taken at the same time as fitness testing. Standing height and weight were measured by research staff using a balance beam scale and stadiometer. Measures were taken in duplicate and then averaged; if the two measures were discrepant, a third measure was taken. Waist circumference was determined using an anthropometric measuring tape and the National Institutes for Health protocol (National Institutes of Health 1998, Canadian Society for Exercise Physiology 2003). Full body dual-energy x-ray absorptiometry (DXA) scans were taken using a Hologic Discovery A DXA system and Hologic QDR Software (Hologic Inc., Bedford, MA, USA) or a GE Healthcare Lunar Prodigy DXA and GE Healthcare enCORE Software (GE Medical Systems Lunar, Madison, WI, USA) to assess lean mass and total fat mass. Percent body fat was calculated as $100\% \times (\text{fat mass} / (\text{fat mass} + \text{lean mass}))$.

Intervention

The intervention began with a goal to reach full exercise prescription within the first 12 weeks by gradually increasing exercise volume. As previously described (Friedenreich et al. 2014), the goal by week 13 was to attain 5 days/week of aerobic exercise for 30 min (MODERATE) or 60 min (HIGH) per session achieving 60–80% maximum heart rate reserve for at least half of each workout. The individual target intensity ranged from 60–70% to 70–80% heart rate reserve depending on ability level. To confirm this intensity, women were asked to wear Polar FT4 heart rate monitors (Polar Electro, Lachine, QC,

Canada) during supervised and unsupervised sessions. Between weeks 13 and 52, women were prescribed supervised sessions at an exercise facility 3 days/week and unsupervised home-based exercise 2 days/week. Supervised sessions took place at the Westside Recreation Centre (Calgary) and the Behavioural Medicine Fitness Centre, University of Alberta (Edmonton). Home-based sessions were initially prescribed once weekly (weeks 5–8) and later increased to twice weekly. Adherence was monitored using weekly exercise logs maintained by participants, documenting activity types, total exercise duration, exercise ‘time in zone,’ average heart rate, and a Borg Rating of Perceived Exertion (Borg 1998). Exercise trainers kept separate logs of the same exercise variables as well as any absences from supervised sessions. Participants were asked not to change their usual diet.

Endogenous sex hormones

Blood samples were drawn at baseline and 6 and 12 months after a minimum 10 h fast and complete abstinence from exercise and alcohol intake for 24 h. If a woman was <55 years of age with no bilateral oophorectomy or menopausal status was uncertain, a follicle-stimulating hormone test confirmed eligibility. All blood samples were stored at -86°C in the Alberta Cancer Research Biorepository in Calgary. E_2 and estrone were measured by radioimmunoassay with preceding organic solvent extraction and Celite column partition chromatography steps. Assay sensitivities were 2 and 4 pg/ml, respectively, and inter-assay coefficients of variation (CV) were 9–14%. SHBG was measured using solid-phase, two-site chemiluminescent immunometric assays on the Immulite analyzer (Siemens Healthcare Diagnostics, Deerfield, IL, USA); assay sensitivity was 1 nmol/l and the inter-assay CV was <10%. Appropriate quality control samples were used to monitor assay reliability. Each participant’s baseline and 6 and 12 month samples were analyzed in the same batch and each batch included an equal number of MODERATE and HIGH blood samples. Blind duplicates were included in and between batches to estimate CVs. All lab personnel were blinded to the intervention assignment.

Sample size/power considerations

Sample size calculations were based on the standard formula for two-sample mean comparisons (Rosner 2011) with $\alpha=0.05$ (two-sided) for comparing mean 12-month biomarker changes (log-transformed) with no adjustment for baseline values. ALPHA trial results

provided standard deviation estimates and yardstick intervention effects. A sample size of 150 participants per group was initially selected allowing 95% power to detect 3 and 4% group differences in 12-month changes in percent body fat and total fat mass, respectively, which were primary outcomes in the trial. Allowing 10% loss to follow-up, a group size of 165 was planned. However, due to a high volunteer response, the sample size was increased to 200 per group.

Statistical analysis

Group differences in demographic and lifestyle information and biomarker concentrations at baseline were compared using a χ^2 test for categorical data, *t*-test for continuous data and Wilcoxon rank-sum test for non-normally distributed continuous data. Because all biomarker data were non-normally distributed, subsequent analyses of a more analytic (rather than descriptive) nature were performed on log-transformed values. Sex hormone changes in the two arms were compared in an intention-to-treat analysis using linear mixed models that accounted for repeated measures of sex hormone concentrations at 6 and 12 months and adjusted for baseline levels of the biomarker of interest as a covariate. Treatment effect ratios, also referred to as ‘treatment effects’ or ‘dose effects’ in this report, were calculated from these models and represented the HIGH:MODERATE ratio of geometric mean biomarker levels over 12 months (reflecting changes from baseline to 6 and 12 months). A treatment effect ratio <1.0 indicated lower biomarker concentrations in the HIGH exercise group compared with the MODERATE group at 6 and 12 months; a ratio >1.0 indicated lower biomarker concentrations in the MODERATE exercise group; and a ratio equal to 1.0 indicated no difference in biomarker concentrations between exercise groups.

In addition, we repeated our intention-to-treat analysis stratified by *a priori* effect modifiers: baseline BMI and study site (Calgary, Edmonton) and also by baseline physical fitness level in a *post hoc* analysis (given effect modification by this variable in the ALPHA trial (Friedenreich et al. 2011)). Furthermore, a sensitivity analysis was performed to account for possible sex hormone hemodilution that might occur as a result of a larger body surface area in obese women (Yang et al. 2011). In so doing, we repeated the intention-to-treat analysis, with and without stratification by baseline BMI, using sex hormone mass as the unit of analysis. Sex hormone mass was calculated as $(\log(\text{sex hormone concentration}) \times ((\text{weight (kg)}^{0.425} \times \text{height (m)}^{0.725} \times 0.2025) \times 1.395))$ as

described previously (Grubb *et al.* 2009, Yang *et al.* 2011) using a plasma volume (L) formula (i.e., body surface area (m^2) $\times$ 1.395) derived for women (Pearson *et al.* 1995). An additional sensitivity analysis was performed removing non-compliant participants who changed energy intake ≥ 1000 kcal/day between baseline and 12 months according to Diet History questionnaires.

In addition, a per-protocol analysis was conducted for women who were 90–100% adherent in the MODERATE group (135–150 min/week) or $\geq 90\%$ adherent in the HIGH group (≥ 270 min/week) on average between weeks 13 and 52 (after the 12-week ramp-up period) according to exercise logs. Furthermore an exploratory analysis was performed, categorizing women by quintiles of total exercise duration without regard to group randomization. The ratio of geometric means (12 months:baseline) was calculated for each quintile and statistical significance was determined from linear models adjusted for the baseline level of the biomarker of interest. Tests for linear trend treated categories as a continuous variable. Moreover, a per-protocol analysis was carried out stratifying by time in zone (average, weeks 13–52) to explore the possibility of enhanced dose effects when exercise was mainly of high intensity. Finally, Spearman correlation coefficients (r_s) were used to describe relations between 12-month changes in each biomarker and hypothesized mediators of change (changes in $\text{VO}_{2\text{max}}$ and adiposity respectively) (Friedenreich *et al.* 2011). All statistical tests were two-sided with a 0.05 level of significance. Analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC, USA).

Results

A total of 400 women were randomized (Fig. 1) as described previously (Friedenreich *et al.* 2015). Baseline characteristics pertaining to the sex hormones analysis are described in Table 1. At baseline, 22, 40, and 38% of women in the HIGH volume group were $\text{BMI} < 25 \text{ kg/m}^2$, $25 \leq \text{BMI} < 30 \text{ kg/m}^2$ and $\text{BMI} \geq 30 \text{ kg/m}^2$, respectively, and similarly for the MODERATE group (19, 42, and 39% respectively). Participants on average were younger postmenopausal women and overweight; most had post-secondary education, were not employed full-time and self-identified as white. There were no group differences at baseline except for average SHBG levels, which were significantly higher in the HIGH volume group. Fourteen women dropped out; nine from the MODERATE arm and five from the HIGH arm (Fig. 1). An additional four women

Figure 1

CONSORT diagram: Flow of participants through the BETA trial, Alberta, Canada, 2010–2013.

with total $E_2 \geq 66 \text{ pg/ml}$ were suspected as perimenopausal or using estrogen treatment and were therefore excluded. No serious adverse events were reported during the trial.

Some exercise adherence data from BETA have been described previously (Friedenreich *et al.* 2015). For women included in the sex hormones analysis, those in the HIGH vs MODERATE group recorded more exercise on average ($P < 0.0001$, two-sample *t*-test), with 12-month median (quartiles 1,3) adherence values of 228 (156 262) and 129 (106 138) min/week, respectively. Between weeks 13 and 52 (at full prescription after the 12-week ramp-up period), median adherence was 253 (157 289) vs 137 (111 150) min/week, and median time in zone was 128 (66 185) vs 88 (54 115) min/week, respectively, for the HIGH and MODERATE groups ($P < 0.0001$). Based on the Past Year Total Physical Activity Questionnaire (Friedenreich *et al.* 2006), usual recreational activity increased by 4.0 and 2.0 h/week (240 and 120 min/week) for HIGH and MODERATE groups, respectively ($P < 0.0001$; Table 2). Women in the HIGH group on average experienced

Table 1 Baseline characteristics of randomized participants in the BETA trial, Alberta, Canada, 2010–2013

Baseline characteristic	Moderate	High
	<i>n</i> (%)	<i>n</i> (%)
No.	200	200
Full-time employment	59 (30)	71 (36)
Education		
≤ High school	45 (23)	45 (23)
College/Trade	63 (32)	58 (29)
Undergraduate degree	54 (27)	59 (30)
Graduate degree	38 (19)	38 (19)
Marital status		
Married/Common in law	139 (70)	136 (68)
Other	61 (30)	64 (32)
Race/Ethnicity		
White	186 (93)	172 (86)
Other	14 (7)	28 (14)
Family history of breast cancer		
≥ 1 first-degree family member	39 (20)	33 (17)
None	161 (80)	166 (83)
Ever used hormone therapy	55 (28)	62 (31)
History of hysterectomy	36 (18)	44 (22)
	Mean ± s.d.	Mean ± s.d.
Age (years)	59.5 ± 5.1	59.4 ± 4.8
Maximal oxygen consumption (ml/kg/min)	26.8 ± 5.0	26.7 ± 5.0 ± 5.3
BMI (kg/m ²)	29.4 ± 4.4	29.1 ± 4.4
Body weight (kg)	77.4 ± 13.0	77.3 ± 13.0
Percent body fat (%)	40.7 ± 5.9	40.5 ± 5.8
Waist circumference (cm)	98.6 ± 10.8	98.7 ± 11.0
	Median (quartiles 1,3)	Median (quartiles 1,3)
Total energy intake (kcal/day)	1414 (1112, 1789)	1379 (1001, 1797)
Alcohol intake (g/day)	2.4 (0.7, 7.8)	3.0 (0.9, 6.4)
Past year total physical activity (MET-h/week)		
Total activity	90.6 (60.6, 121.9)	89.0 (62.2, 115.8)
Occupational activity	25.9 (4.5, 55.2)	34.2 (2.6, 56.3)
Household activity	41.0 (27.0, 60.6)	40.4 (28.3, 60.1)
Recreational activity	7.6 (2.3, 13.0)	6.9 (2.4, 13.3)
Sex hormone concentration		
Estradiol (pg/ml)	9.7 (7.6, 12.9)	9.4 (7.3, 12.2)
Estrone (pg/ml)	39.0 (30.9, 47.3)	37.0 (30.7, 43.7)
Sex hormone binding globulin (nmol/l) ^a	42.7 (32.8, 59.7)	46.1 (34.7, 65.4)
Free estradiol (pg/ml)	0.22 (0.17, 0.32)	0.21 (0.15, 0.29)
Sex hormone mass ^b		
Estradiol (ng)	24.0 (18.4, 34.4)	23.1 (17.9, 31.3)
Estrone (ng)	97.8 (75.5, 126.5)	92.3 (75.2, 113.8)
Sex hormone-binding globulin (nmol)	106.8 (84.5, 145.5)	122.4 (89.7, 164.8)
Free estradiol (ng)	0.57 (0.43, 0.81)	0.53 (0.39, 0.75)

^aThere were no statistically significant differences at baseline between HIGH and MODERATE groups for these variables except for sex hormone binding globulin, $P=0.04$.

^bEstimated as $(\log(\text{sex hormone concentration}) \times ((\text{weight (kg)}^{0.425} \times \text{height (m)}^{0.725} \times 0.2025) \times 1.395))$ as described previously (Grubb et al. 2009, Yang et al. 2011) using a plasma volume (L) formula (i.e., body surface area (m²) $\times$ 1.395) derived for women (Pearson et al. 1995).

significantly greater reductions in percent body fat compared with the MODERATE group (Friedenreich et al. 2015). On average, total energy intake decreased significantly in the MODERATE group over 12 months, but not significantly more than in the HIGH group (Table 2).

At 6 and 12 months, all biomarker changes were <10% (Table 3) and, unexpectedly, were more favorable for the MODERATE vs HIGH volume group. However,

results from linear mixed models suggested no significant group differences with respect to biomarker changes, with all treatment effect ratios approximately equal to 1.0. Stratifying the results by baseline BMI, study site, or estimated VO_{2max} still resulted in non-significant dose effects for all biomarkers except SHBG, which varied by baseline BMI. Specifically, obese women experienced significantly greater SHBG increases with the HIGH

Table 2 Physical activity^a, fitness, diet, and adiposity changes (12 months – baseline) by group assignment in the BETA trial, Alberta, Canada, 2010–2013

Measure	MODERATE			HIGH			Between-group <i>P</i> ^b
	<i>n</i>	Mean change	95% CI	<i>n</i>	Mean change	95% CI	
Total activity (h/week)	184	2.1	–0.2, 4.4	185	3.2	0.9, 5.5	0.51
Total activity (MET-h/week)	184	16.7	9.4, 23.9	185	26.8	19.4, 34.2	0.05
Occupational activity (h/week)	184	0.8	–0.8, 2.5	185	–0.03	–1.8, 1.7	0.48
Occupational activity (MET-h/week)	184	3.7	–1.2, 8.5	185	2.0	–3.6, 7.7	0.67
Household activity (h/week)	184	–0.7	–2.4, 1.1	185	–0.8	–2.3, 0.8	0.92
Household activity (MET-h/week)	184	–0.8	–6.1, 4.5	185	–1.7	–5.9, 2.5	0.78
Recreational activity (h/week)	184	2.0	1.4, 2.5	185	4.0	3.4, 4.6	<0.0001
Recreational activity (MET-h/week)	184	13.8	11.4, 16.3	185	26.5	23.6, 29.4	<0.0001
Maximal oxygen consumption (ml/kg/min)	175	4.0	3.3, 4.8	180	5.0	4.2, 5.9	0.09
Total energy intake (kcal/day)	184	–79.1	–136.0, –21.9	189	–51.0	–108.0, 5.9	0.49
Alcohol intake (g/day)	184	0.0	–0.9, 1.0	189	0.0	–0.7, 0.6	0.88
Body weight (kg) ^c	183	–1.9	–2.4, –1.3	190	–2.6	–3.2, –1.9	0.11
Percent body fat ^c	185	–1.1	–1.5, –0.7	190	–2.0	–2.5, –1.5	0.003
Waist circumference (cm) ^c	183	–4.7	–5.6, –3.9	190	–6.0	–7.1, –4.9	0.06

MET, metabolic equivalent of task.

^aEstimated using the *Past Year Total Physical Activity Questionnaire* (Friedenreich et al. 2006).^bBased on a two-sample *t*-test.^cDetailed analyses of adiposity change are described elsewhere (Friedenreich et al. 2015).

prescription (12-month increase of 13.1% vs 8.2% for the MODERATE group; between-group *P*=0.03 from linear mixed models), whereas non-obese women experienced significantly greater increases with the MODERATE prescription (12-month change of 10.6% vs 2.4% for the HIGH group; between-group *P*=0.02). Intention-to-treat and BMI-stratified results were essentially unchanged when based on biomarker mass instead of biomarker

concentration. Results were also unchanged when ten women with 12-month changes ≥ 1000 kcal/day in dietary energy intake were excluded.

Scatter plots of individual biomarker changes against exercise adherence (data not shown) revealed non-adherence in some participants such that 24% of the women in the HIGH group (*n*=48) exercised ≤ 150 min/week on average over 12 months; in the

Table 3 Changes in endogenous estrogen and SHBG concentrations over 12 months in the BETA trial, Alberta, Canada, 2010–2013

Sex hormone	Baseline		6 months			12 months			Treatment effect ^a		Between-group <i>P</i> ^b
	Geometric mean	95% CI	Geometric mean	95% CI	Percent change ^c	Geometric mean	95% CI	Percent change ^c	Ratio of HIGH:MOD	95% CI	
Estradiol (pg/ml)											
MODERATE	9.9	9.3, 10.5	9.7	9.1, 10.2	–2.4	9.5	8.9, 10.1	–4.5	1.00	0.96, 1.06	0.90
HIGH	9.4	8.9, 9.9	9.5	9.0, 10.0	0.4	9.1	8.6, 9.6	–3.6			
Estrone (pg/ml)											
MODERATE	38.6	36.7, 40.6	38.2	36.3, 40.2	–1.2	37.4	35.6, 39.3	–3.2	1.02	0.98, 1.05	0.42
HIGH	36.4	34.8, 38.0	36.8	35.1, 38.5	1.1	36.4	34.7, 38.2	0.1			
SHBG (nmol/l)											
MODERATE	43.2	40.6, 46.0	46.2	43.5, 49.0	6.8	47.4	44.6, 50.4	9.6	0.99	0.96, 1.02	0.58
HIGH	47.6	44.7, 50.8	50.2	47.1, 53.4	5.3	50.7	47.7, 53.9	6.4			
Free estradiol (pg/ml)											
MODERATE	0.23	0.22, 0.25	0.22	0.21, 0.24	–4.5	0.21	0.20, 0.23	–7.5	1.01	0.95, 1.06	0.83
HIGH	0.21	0.20, 0.23	0.21	0.20, 0.22	–1.3	0.20	0.19, 0.21	–5.5			

SHBG, sex hormone binding globulin.

^aHIGH:MODERATE ratio of geometric means for biomarker levels over 12 months, adjusted for biomarker level at baseline. A ratio <1.0 indicates lower biomarker concentrations in the HIGH exercise group at 6 and 12 months; a ratio >1.0 indicates lower biomarker concentrations in the MODERATE exercise group; a ratio equal to 1.0 indicates no difference in biomarker concentrations between the HIGH and MODERATE exercise groups. Sample sizes were *n*=189 for MODERATE and *n*=193 for HIGH groups.

^b*P* for testing group difference over 12 months from the linear mixed model, adjusted for biomarker level at baseline.

^cPercent hormone change at 6 or 12 months from baseline for that group.

Table 4 Changes in endogenous estrogen and SHBG concentrations over 12 months in adherent women^a in the BETA trial, Alberta, Canada, 2010–2013

Sex hormone	Baseline		6 months			12 months			Treatment effect ^b		Between-group <i>P</i> ^c
	Geometric mean	95% CI	Geometric mean	95% CI	Percent change ^d	Geometric mean	95% CI	Percent change ^d	Ratio of HIGH:MOD	95% CI	
Estradiol (pg/ml)											
MODERATE	10.2	9.2, 11.4	10.2	9.1, 11.3	−0.76	9.6	8.5, 10.9	−6.2	0.93	0.86, 1.01	0.09
HIGH	8.8	8.2, 9.5	8.7	8.0, 9.4	−0.85	7.9	7.3, 8.5	−10.6			
Estrone (pg/ml)											
MODERATE	38.7	35.3, 42.3	38.6	34.9, 42.7	−0.13	36.9	33.6, 40.7	−4.5	1.01	0.96, 1.07	0.66
HIGH	34.6	32.4, 36.9	35.5	32.9, 38.3	2.6	33.6	31.5, 36.0	−2.8			
SHBG (nmol/l)											
MODERATE	44.9	40.3, 50.0	47.6	42.9, 52.9	6.1	49.4	44.5, 54.9	10.1	1.02	0.96, 1.08	0.45
HIGH	48.3	43.4, 53.8	53.2	47.8, 59.1	10.1	52.6	47.5, 58.3	9.0			
Free estradiol (pg/ml)											
MODERATE	0.24	0.21, 0.27	0.23	0.21, 0.26	−2.7	0.21	0.19, 0.24	−9.2	0.93	0.85, 1.00	0.06
HIGH	0.20	0.18, 0.22	0.19	0.17, 0.21	−4.2	0.17	0.16, 0.19	−13.2			

SHBG, sex hormone binding globulin.

^aIncluded the subgroup of women for whom, across weeks 13–52 (at full prescription), average adherence in the exercise logs was 90–100% in the MODERATE group (135–150 min/week; *n*=57) or ≥90% in the HIGH group (270–350 min/week; *n*=78).^bHIGH:MODERATE ratio of geometric means for biomarker levels over 12 months, adjusted for biomarker level at baseline. A ratio <1.0 indicates lower biomarker concentrations in the HIGH exercise group at 6 and 12 months; a ratio >1.0 indicates lower biomarker concentrations in the MODERATE exercise group; a ratio equal to 1.0 indicates no difference in biomarker concentrations between the HIGH and MODERATE exercise groups.^c*P* for testing group difference over 12 months from the linear mixed model, adjusted for biomarker level at baseline.^dPercent hormone change at 6 or 12 months from baseline for that group.

MODERATE group 3% of the women exercised >150 min/week. Therefore, a per-protocol analysis was done based on exercise minutes per week (Table 4). In the per-protocol sample, all biomarker 12-month changes were <14% and treatment effect ratios were not significantly different from 1.00. However, total and free E₂ reductions were slightly greater for the HIGH vs MODERATE group resulting in borderline significant dose effects (between-group *P*=0.09 and *P*=0.06 respectively). In an exploratory analysis, inverse dose-response trends were found between decreasing total and free E₂ concentrations and increasing quintiles of total exercise time (Table 5). The highest 12-month reductions in total and free E₂ concentrations were −11.0 and −13.7%, respectively, for women with average adherence estimates >245 min/week, although similar reductions were also observed in the second and third quartiles.

In a per-protocol analysis examining exercise intensity (Table 6), women who exercised ≥60% of their prescribed exercise duration in their target heart rate zone (i.e., at least 90 min/week in the MODERATE group or at least 180 min/week in the HIGH group time in zone) experienced stronger dose effects with respect to total and free E₂ changes (11.2–13.5% 12-month decreases in the HIGH group vs 2.3–5.6% in the MODERATE group; *P*=0.03, *P*=0.01 between groups for total and free E₂,

respectively). However, E₂ was also reduced with less time in zone (12-month decreases of 10.1–12.9% for the HIGH group; 14.9–17.2% for MODERATE). Among all participants, 12-month body weight changes correlated significantly with biomarker changes (*r*_s=0.21, *P*<0.0001 for total E₂; *r*_s=0.17, *P*<0.0009 for estrone; *r*_s=−0.44, *P*<0.0001 for SHBG; *r*_s=0.29, *P*<0.0001 for free E₂). Correlations with percent body fat change were somewhat weaker (*r*_s=0.13, *P*=0.01 for total E₂; *r*_s=0.08, *P*=0.12 for estrone; *r*_s=−0.27, *P*<0.01 for SHBG; *r*_s=0.17, *P*<0.01 for free E₂). Change in waist circumference was significantly correlated with SHBG and free E₂ changes (SHBG: *r*_s=−0.27, *P*<0.0001; free E₂: *r*_s=0.14, *P*=0.007) but not total E₂ (*r*_s=0.09, *P*=0.09) or estrone (*r*_s=0.04, *P*=0.49). Similarly, changes in estimated VO_{2max} were weakly correlated with changes in SHBG (*r*_s=0.11, *P*=0.04) and free E₂ (*r*_s=−0.11, *P*=0.04) but not total E₂ (*r*_s=−0.09, *P*=0.08) or estrone (*r*_s=0.02, *P*=0.64).

Discussion

On average, circulating concentrations of total E₂, estrone, SHBG, and free E₂ changed similarly over 12 months for women prescribed 150 or 300 min/week of aerobic exercise. Treatment effect ratios, representing the HIGH:MODERATE ratio of geometric mean biomarker levels over 12 months, were all null, providing no evidence of a dose

Table 5 Hormone concentrations at baseline and 12 months by quintile of exercise adherence^a for MODERATE and HIGH groups combined

Sex hormone	Baseline		12 Months		12 Months:Baseline ^b		Percent change ^c	P value ^d	P-trend ^e
	Geometric mean ^b	95% CI	Geometric mean ^b	95% CI	Geometric mean ratio	95% CI			
Estradiol (pg/ml)									
≤ 110 min/week	10.3	9.3, 11.4	10.8	9.8, 12.0	1.06	0.98, 1.14	5.2	Ref	0.002
110–134 min/week	9.6	8.8, 10.5	8.9	8.2, 9.8	0.93	0.88, 0.99	−7.1	0.002	
134–152 min/week	10.2	9.3, 11.2	9.3	8.4, 10.4	0.92	0.86, 0.99	−8.3	0.003	
152–245 min/week	9.4	8.6, 10.3	9.6	8.9, 10.4	1.02	0.94, 1.1	1.9	0.19	
> 245 min/week	8.8	8.2, 9.5	7.8	7.2, 8.4	0.89	0.84, 0.95	−11.0	<0.0001	
Estrone (pg/ml)									
≤ 110 min/week	40.5	37.5, 43.7	39.2	36.0, 42.7	0.97	0.91, 1.03	−3.1	Ref	0.97
110–134 min/week	37.4	34.5, 40.5	35.8	33.0, 38.8	0.96	0.91, 1.00	−4.4	0.40	
134–152 min/week	38.5	35.6, 41.6	38.6	35.6, 41.8	1.00	0.95, 1.06	0.3	0.51	
152–245 min/week	36.5	33.9, 39.4	37.3	34.7, 40.1	1.02	0.96, 1.09	2.0	0.43	
> 245 min/week	34.6	32.4, 37.0	33.7	31.5, 36.1	0.97	0.93, 1.02	−2.5	0.44	
Sex hormone binding globulin (nmol/l)									
≤ 110 min/week	44.0	39.9, 48.6	47.0	42.4, 52.2	1.07	1.01, 1.13	6.9	Ref	0.37
110–134 min/week	44.6	40.5, 49.2	47.9	43.6, 52.7	1.07	1.02, 1.13	7.4	0.83	
134–152 min/week	42.2	38.4, 46.4	46.7	42.6, 51.2	1.11	1.06, 1.16	10.8	0.36	
152–245 min/week	48.5	43.5, 54.1	51.1	46.3, 56.3	1.05	1.01, 1.09	5.2	0.95	
> 245 min/week	48.1	43.2, 53.5	52.7	47.6, 58.3	1.10	1.05, 1.15	9.5	0.26	
Free estradiol (pg/ml)									
≤ 110 min/week	0.24	0.21, 0.27	0.25	0.22, 0.28	1.03	0.95, 1.12	2.7	Ref	0.002
110–134 min/week	0.22	0.2, 0.25	0.20	0.18, 0.22	0.91	0.86, 0.96	−9.3	0.004	
134–152 min/week	0.24	0.22, 0.27	0.21	0.19, 0.24	0.89	0.83, 0.96	−11.6	0.003	
152–245 min/week	0.21	0.19, 0.23	0.21	0.19, 0.23	1.00	0.93, 1.09	0.5	0.28	
> 245 min/week	0.20	0.18, 0.22	0.17	0.15, 0.19	0.86	0.81, 0.93	−13.7	<0.0001	

^aQuintile split groups with $n=76$, 76 , 78 , 76 , and 76 for five groups based on the exercise adherence levels ≤110, 110–134, 134–152, 152–245, > 245 min/week, respectively, using average adherence from exercise logs, weeks 1–52.

^bValues are unadjusted.

^cPercentage hormone change at 12 months from baseline for that level of adherence.

^dP-values for hormone change at 12 months from baseline between controls and that level of adherence, adjusted for the baseline value. Ref, referent group.

^eTrend analysis for hormone change at 12 months from baseline between controls and three adherence groups, adjusted for the baseline value.

effect on circulating estrogen or SHBG concentrations. Exploratory analyses suggested our null findings may be partly adherence related and, possibly, that dose effects might vary with baseline BMI and when exercise is done mainly with vigorous intensity.

Compared with the ALPHA trial, which tested an exercise prescription of 225 min/week using similar methods and participants (Friedenreich *et al.* 2010), estrogen reductions in BETA were smaller on average (4–8% in BETA vs 12–13% in ALPHA for free and total E₂; 0–3% in BETA vs 5% in ALPHA for estrone) and SHBG increases were slightly larger in BETA (6–10% in BETA vs 3% in ALPHA). Similarly, other exercise trials in postmenopausal women typically improved these biomarker concentrations by 2–13% (Figuerola *et al.* 2003, Copeland & Tremblay 2004, McTiernan *et al.* 2004, Orsatti *et al.* 2008, Monninkhof *et al.* 2009, Friedenreich *et al.* 2010, Yoo *et al.* 2010, Campbell *et al.* 2012, Kim & Kim 2012).

Thus, despite the relatively high exercise prescription in BETA, we could not demonstrate greater estrogen or SHBG changes than reported previously in similar trials. The reasons for this finding are unclear but might relate to modest adherence, particularly in the high volume group.

Epidemiologic research has generally shown that exercise-induced changes in estrogen concentrations are attributable to weight loss in postmenopausal women (Friedenreich *et al.* 2011, Campbell *et al.* 2012, Stolzenberg-Solomon *et al.* 2012, Jones *et al.* 2013). Adipose tissue becomes a major site of estrone and E₂ biosynthesis after menopause via aromatase activity (Folkerd & Dowsett 2013, Kinoshita *et al.* 2014), and consequently, BMI relates positively to circulating E₂ and estrone concentrations (Endogenous Hormones and Breast Cancer Collaborative Group 2015). Furthermore, BMI is inversely related to SHBG concentrations

Table 6 Changes in endogenous estrogen and SHBG concentrations over 12 months in adherent women^a stratified by time at target intensity; the BETA trial, Alberta, Canada, 2010–2013

Biomarker	Prescribed exercise duration	n	6 month percent change from baseline	12 month percent change from baseline	Treatment effect ^b		Between-group P ^c
					Ratio of HIGH:MOD	95% CI	
Proportion of time at high intensity (average min/week) ^d							
Estradiol (pg/ml)							
< 60% prescribed	MODERATE	17	−13.4	−14.9	1.07	0.96, 1.20	0.23
	HIGH	44	−0.8	−10.1			
≥ 60% prescribed	MODERATE	40	5.2	−2.3	0.88	0.79, 0.99	0.03
	HIGH	34	−1.0	−11.2			
Estrone (pg/ml)							
< 60% prescribed	MODERATE	17	−7.5	−8.6	1.09	1.00, 1.18	0.06
	HIGH	44	2.2	−4.0			
≥ 60% prescribed	MODERATE	40	3.2	−2.7	0.99	0.91, 1.07	0.74
	HIGH	34	3.2	−1.1			
SHBG (nmol/l)							
< 60% prescribed	MODERATE	17	10.4	9.5	1.00	0.90, 1.11	0.93
	HIGH	44	10.4	9.0			
≥ 60% prescribed	MODERATE	40	4.3	10.3	1.04	0.97, 1.13	0.27
	HIGH	34	9.7	8.9			
Free estradiol (pg/ml)							
< 60% prescribed	MODERATE	17	−16.0	−17.2	1.07	0.95, 1.21	0.26
	HIGH	44	−4.4	−12.9			
≥ 60% prescribed	MODERATE	40	3.6	−5.6	0.86	0.77, 0.97	0.01
	HIGH	34	−4.0	−13.5			

^aIncluded $n = 135$ women for whom, across weeks 13–52 (at full prescription), average adherence in the exercise logs was 90–100% in the MODERATE group (135–150 min/week; $n = 57$) or $\geq 90\%$ in the HIGH group (≥ 270 min/week; $n = 78$).

^bHIGH:MODERATE ratio of geometric means for biomarker levels over 12 months adjusted for biomarker level at baseline. A ratio < 1.0 indicates lower biomarker concentrations in the HIGH exercise group at 6 and 12 months; a ratio > 1.0 indicates lower biomarker concentrations in the MODERATE exercise group; a ratio equal to 1.0 indicates no difference in biomarker concentrations between the HIGH and MODERATE exercise groups.

^cP for testing the HIGH-MODERATE group difference over 12 months from the linear mixed model, adjusted for biomarker level at baseline.

^dTime at high intensity was defined as time exercising at an intensity of 60–80% heart rate reserve averaged for each participant over 52 weeks. Cut points for the stratified analysis were 60% of the prescribed durations, i.e., 90 min/week in the MODERATE group and 180 min/week in the HIGH group.

(Endogenous Hormones and Breast Cancer Collaborative Group *et al.* 2011, Goto *et al.* 2014), which restrict E_2 bioavailability through SHBG binding (our analysis of free E_2 therefore reflects the bioavailable portion). The significant correlations we observed in BETA between E_2 and body weight changes, and between SHBG and changes in body weight and waist circumference, are consistent with a mediating role for adiposity. For unknown reasons, adiposity correlations were not found with estrone.

It is unclear, then, why we did not find significant dose effects despite greater fat loss with the 300 min/week prescription (Table 2). It is possible that because the magnitude of the difference in fat loss between the two groups was rather low ($\sim 1\%$ difference between groups with respect to total fat loss), there were limited effects on sex hormones. Similarly, there may have been insufficiently large group differences with respect to other mediating factors besides adiposity, e.g., insulin mediating SHBG levels (Pugeat *et al.* 1991). Another explanation

relates to hemodilution of sex hormone concentrations in obesity (Grubb *et al.* 2009, Yang *et al.* 2011). If the body surface area was decreased through weight loss in our trial, then decreased vascular volume would manifest through increased sex hormone concentrations, thereby confounding our primary analysis. However, a sensitivity analysis based on biomarker mass made essentially no difference in our primary results or in our stratified analyses of obese women.

There may also have been effect modification. For instance, our finding of stronger SHBG increases with the 300 min/week prescription in obese women might reflect the stronger reductions in subcutaneous abdominal fat we observed in this subgroup (Friedenreich *et al.* 2015), which could mediate SHBG changes (Liedtke *et al.* 2012). We cannot explain why greater SHBG increases occurred with the 150 min/week prescription in non-obese women, which was unexpected, but this effect was diminished in the per-protocol sample (data not shown). Results stratified by time in zone suggested an enhanced dose effect on

E₂ when most of the prescribed exercise was done at high intensity. However, the dose effect seemed to be due to relatively small E₂ changes in women who exercised for a moderate duration (135–150 min/week) at mainly high intensity (≥ 112 min/week) for reasons that are unclear. Perhaps exercise compensation was more common in this subgroup. With regard to estrone, the modest changes observed in BETA and the lack of dose dependency, as in the ALPHA trial (Friedenreich et al. 2010), overall do not support a dose–response relation between estrone and our exercise prescriptions. An important limitation of our trial was that the statistical power for assessing the efficacy of 300 min/week aerobic exercise was lower than planned because some women exercised below this level. Sensitivity analyses to account for non-adherence indeed revealed slightly stronger dose–response trends for total and free E₂ changes (Tables 4 and 5) suggesting that significant dose effects might have been found if more women assigned to the high volume group had exercised >245 min/week. Still, 12-month E₂ changes even at the highest levels of adherence were modest ($<14\%$). Furthermore, we acknowledge the key limitations of our per-protocol analyses – namely, selection bias and lower statistical power. Finally, the generalizability of our results may be limited to healthy postmenopausal women with BMI 22–40 kg/m², who are non-users of exogenous hormones and physically inactive but highly motivated to exercise.

To our knowledge, BETA is the largest dose–response exercise trial designed to inform postmenopausal breast cancer prevention. In previous dose–response RCTs, exercise prescriptions did not target primary cancer prevention, few breast cancer biomarkers were assessed, and only some (Asikainen et al. 2002, Asikainen et al. 2003, Morss et al. 2004, Dalleck et al. 2009) studied postmenopausal women exclusively. The dose–response to Exercise in Women trial (Morss et al. 2004, Church et al. 2009) was a large RCT statistically powered to compare exercise durations in over 450 postmenopausal women. However, lower volumes of exercise were prescribed than in BETA (50% VO_{2max} for approximately 75, 140, and 190 min/week) and sex hormones were not assessed. BETA was a long-term intervention trial that was tightly controlled (Friedenreich et al. 2014) and study retention was exceptionally good with a 3.5% dropout rate overall. Furthermore, our extraction-based assays for quantifying estrone and E₂ in serum were sensitive and specific (Endogenous Hormones and Breast Cancer Collaborative Group 2015).

In conclusion, our intention-to-treat analysis provided no evidence of a difference, with respect to endogenous estrogen or SHBG changes, in prescribing 300 vs 150 min/week of moderate-vigorous aerobic exercise to inactive postmenopausal women. For some individuals, dietary modification may be more feasible than increasing exercise volume for the purpose of lowering endogenous estrogen concentrations. For instance, one previous RCT in 439 overweight/obese postmenopausal women showed that free E₂ concentrations were decreased by $\sim 5\%$ on average in women prescribed 12 months of aerobic exercise, vs 26% in women prescribed exercise and a reduced-calorie diet (Campbell et al. 2012). Still, higher (vs lower) volumes of aerobic exercise offer important benefits for many other facets of postmenopausal health including physical fitness, cardiovascular and cognitive health, and overall survival (Church et al. 2007, Swift et al. 2012, Anderson et al. 2014). Furthermore, 300 min/week of aerobic exercise may be superior to 150 min/week for lowering breast cancer risk through other biologic pathways besides sex hormones, for example, insulin sensitivity and chronic low-grade inflammation (Rose & Vona-Davis 2014). Future analyses from BETA will address this question. The effects of exercise on myokines (Hojman et al. 2011), immune function, oxidative stress, telomere length and DNA methylation have been understudied in the context of breast cancer risk (Neilson et al. 2014) and could also be examined in future exercise trials.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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