Research Article

Cancer Epidemiology, Biomarkers & Prevention

Long-term Effects of Moderate versus High Durations of Aerobic Exercise on Biomarkers of Breast Cancer Risk: Follow-up to a Randomized Controlled Trial

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Abstract

Background: The optimal lifestyle for breast cancer prevention over the long term is unclear. We aimed to determine whether or not the amount of exercise prescribed in a year-long exercise intervention influences breast cancer biomarker levels 1 year later.

Methods: We conducted a 24-month follow-up study (2012–2014) to the Breast Cancer and Exercise Trial in Alberta (BETA), a 12-month, two-armed (1:1), two-center randomized controlled trial of exercise in 400 cancer-free, postmenopausal women. The exercise prescription was moderate–vigorous aerobic exercise, 5 days/week (3 days/week supervised) for 30 minutes/session (MODERATE) or 60 minutes/session (HIGH). Participants were asked not to change their usual diet. We used linear mixed models to compare biomarker concentrations (C-reactive protein, insulin, glucose, HOMA-IR, estrone, sex hormone binding globulin, total estradiol, and free estradiol) over time (0, 12, and 24 months) by group (MODERATE, HIGH), using group–time interactions.

Results: After 12 months of no intervention, 24-month fasting blood samples were available for 84.0% and 82.5% of MODERATE and HIGH groups, respectively ($n = 333/400$). We found no evidence that 0 to 24– or 12 to 24–month biomarker changes differed significantly between randomized groups (HIGH:MODERATE ratio of mean biomarker change ranged from 0.97 to 1.06, P values >0.05 for all). We found more favorable biomarker profiles among participants who experienced greater than the median fat loss during the trial.

Conclusions: Prescribing aerobic exercise for 300 versus 150 minutes/week for 12 months to inactive, postmenopausal women had no effects on longer-term biomarkers.

Impact: Exercise may lead to larger improvements in breast cancer biomarkers after intervention among women who also experience fat loss with exercise.

Introduction

Breast cancer is the most common female cancer in Canada (1) and worldwide (2). In 2017, an estimated 26,300 Canadian women were expected to be diagnosed with breast cancer, and approximately 5,000 breast cancer deaths were projected. More than 80% of these cases were women over age 50 (1). Although physical inactivity is one of the few known modifiable risk factors for breast cancer (3), the optimal amount of physical activity that should be recommended for postmenopausal breast cancer prevention over the long term is unclear.

The American Cancer Society (ACS) and the American Institute for Cancer Research/World Cancer Research Fund (AICR/WCRF) publish physical activity guidelines for cancer prevention. For adults, the ACS guidelines recommend 150 minutes/week of moderate activity or 75 minutes/week of vigorous activity throughout the week (4). The AICR/WCRF recommends 30 minutes/day of moderate activity increasing to 60 minutes/day of moderate activity or 30 minutes/day of vigorous activity as fitness improves (5). Yet questions remain about the applicability of these guidelines with respect to reductions in breast cancer risk. Furthermore, even if an exercise intervention achieves these targets, it is unknown what the long-term implications are for postmenopausal breast cancer risk.

Previous randomized controlled trials (RCT; refs. 6–13) have elucidated several plausible biological mechanisms whereby physical activity can reduce postmenopausal breast cancer risk. These intervention trials have found evidence that physical activity reduces levels of adiposity, endogenous sex and metabolic hormones, and inflammatory markers. Of these trials that have examined how a year-long exercise intervention can alter these biomarkers, three (12–14) have investigated the long-term effects of exercise after the completion of the

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intervention. The NEW Trial, which included one arm that was exercise-only, did examine long-term weight loss maintenance, and changes in sex steroid hormones and sex hormone binding globulin (SHBG; ref. 13).

To address these concerns, we conducted the Breast Cancer and Exercise Trial in Alberta (BETA) in 400 cancer-free, postmenopausal women. BETA was a two-center RCT in Alberta, Canada, that compared the effect of 12 months of moderate–vigorous, aerobic exercise for 300 minutes/week (HIGH) or 150 minutes/week (MODERATE) on biomarkers of postmenopausal breast cancer risk. Exercise adherence on average was 254 and 137 minutes/week at full prescription in the two groups, respectively, for 384 women. Total body fat change (kg) measured using dual X-ray absorptiometry (DXA) was our primary outcome (6). On average ($n = 382$ to $n = 386$) for insulin and glucose (15), high sensitivity C-reactive protein (hs-CRP; ref. 16), estradiol, estrone, and SHBG (17), there were no statistically significant dose effects (i.e., no difference between the 0–12-month biomarker changes in the MODERATE vs. HIGH groups). We have previously reported on the exercise dose effects on body fat at 24 months after randomization (14).

A related question is whether or not higher exercise durations during an intervention will result in longer-lasting benefits for breast cancer prevention. We hypothesized that a high exercise duration maintained for 1 year (300 minutes/week), exceeding the standard recommendation (150 minutes/week), may be superior for improving breast cancer biomarkers over the long-term (0–24 months).

Materials and Methods

The BETA protocol and methods are published elsewhere (6, 18). Briefly, BETA was a two-armed, two-center randomized (1:1) controlled exercise trial that we conducted between June, 2010 and June, 2013. Participants were cancer-free, postmenopausal women in Calgary or Edmonton, Canada. Besides dropouts, no intervention stopped early. The BETA 24-month follow-up study started in June, 2012 and ended in May, 2014.

Participants

We recruited participants through invitation letters from the Alberta Breast Screening Program and media campaigns. Participants who met prescreening criteria and agreed to participate were assessed for eligibility over the telephone by a Study Coordinator. Inclusion criteria were: age 50–74 years, postmenopausal, inactive ($\leq$ 90 minutes/week moderate–vigorous activity), no previous cancer or major comorbidity, body mass index (BMI) of 22–40 kg/m^2, nonsmoker, nonexcessive alcohol, nonhormone therapy user, and physician clearance for unrestricted physical activity. We invited all potentially eligible women to an information session which informed them about the 24-month follow-up. All participants provided written-informed consent to participate. The study protocol (6) was approved by the Alberta Cancer Research Ethics Committee and Conjoint Health Research Ethics Board at University of Calgary, and the Health Research Ethics Board at University of Alberta.

Study coordinators assigned participants according to a randomization scheme, stratified by study center and BMI with stratum-specific block sizes of four or six, to 150 or 300 minutes/week aerobic activity for 12 months. A Statistical Associate generated the random allocation sequence using R (version 3.0, R Foundation for Statistical Computing, Vienna, Austria) and user-defined functions. Staff unrelated to the study prepared numbered envelopes containing allocations.

Intervention

The intervention was previously described (6, 18). In brief, the intervention started with a 12-week ramp-up period leading to 150 (MODERATE) or 300 (HIGH) minutes/week for the remaining 9 months (weeks 13–52). We asked participants to exercise at a fitness facility with our Exercise Trainers for 3 days/week plus 2 days/week on their own. The exercise facilities were the Westside Recreation Centre in Calgary and the Behavioural Medicine Fitness Centre, University of Alberta in Edmonton. Participants wore heart rate monitors (Polar FT4, Polar Electro) in all exercise sessions. Exercise trainers recorded the duration, intensity, perceived exertion, and activity types in weekly exercise logs. At 12 months, we invited participants to a social event when we offered advice for maintaining exercise after intervention. Between 12 and 24 months, there was no intervention and no contact with participants.

Measurements

Details of all measurements are published elsewhere (18). At baseline, 12 months, and 24 months, participants self-administered the validated and reliable Past Year Total Physical Activity Questionnaire which includes all types of physical activity (occupational, household, recreational, and walking/bicycling to/from work; ref. 19). We assigned metabolic equivalent (MET) values to each activity using the Compendium of Physical Activities (20) and calculated moderate–vigorous activity (MET-h/week) as the sum of MET-hours/week for all activities with a MET $\geq$ 3.

We measured physical activity and sedentary behavior objectively at baseline, 6, 12, and 24 months with the ActiGraph GT3X+ accelerometer (ActiGraph, LLC) and the activPAL3 inclinometer (PAL Technologies Ltd.), respectively, as previously described (21).

Research staff obtained measures of physical fitness and body fat 4 times for each participant: at baseline, 6, 12, and 24 months. We studied physical fitness as estimated VO_{2max} using a multistage, modified Balke submaximal cardiorespiratory treadmill test (22). We measured body fat in several ways including anthropometric measurements, full-body DXA scans, and CT scans (14). The primary assessment of body fat used in this study was the full-body DXA measurements.

All biomarkers were measured in blood serum. To measure hs-CRP, insulin, and SHBG concentrations, lab analysts used a solid-phase chemiluminescent immunometric assay on an Immulite 2000 analyzer (Siemens Healthcare Diagnostics Inc.). To measure glucose, analysts used a standard procedure on the Vitros Chemistry System. Lab analysts measured estradiol and estrone by radioimmunoassay with preceding organic solvent extraction and Celite column partition chromatography steps. The sensitivity of each assay and the intra- and interbatch coefficients of variation (% CV) are reported as Supplementary Data (Supplementary Table S1). The acceptability of the assays was assessed using blinded samples as well as low-, medium-, and high-level quality control samples. All quality control sample values were within $\pm$ two SDs of the mean values established from quality control sample analysis performed in multiple assays over time in the participating laboratory. We

calculated HOMA-IR as: fasting glucose (mmol/L) x fasting insulin (μIU/mL)/22.5 (23). We calculated free estradiol using a validated algorithm based on total estradiol and SHBG concentrations and an assumed albumin concentration of 43 g/L (24, 25).

Statistical analysis

The statistical analysis included all randomized participants regardless of adherence level during the trial, excluding nonparticipants of the 24-month follow-up. In univariate analyses, we examined average biomarker concentrations at each time point according to the randomized group assignment. We also compared participants' baseline characteristics and indicators of exercise performance during BETA between the randomized groups. To assess possible selection bias (from excluding nonparticipants), we also compared these characteristics between participants and nonparticipants of the 24-month follow-up.

The primary analysis used linear mixed models with the logarithm of blood biomarker concentrations as the dependent variable (baseline, 12 months, and 24 months; ref. 26). Independent variables (fixed effects) were time of blood sampling randomization group (HIGH or MODERATE) and time–group interaction. Subject was included in the model as a random effect. Our primary interest was the time–group interaction on biomarker change between 0 and 24 months. Biomarker changes between 12 and 24 months were of secondary interest. The least squares means of the fixed effects were used to estimate and assess changes between time points. In all analyses, biomarker changes over time were expressed as ratios of the geometric mean of logarithm-transformed biomarker levels (24:0 months and 24:12 months), and group effects were expressed as ratios of these biomarker changes over time (HIGH:MODERATE).

In secondary exploratory analyses, we examined "time" effects (0–24 and 12–24 months) for the study population overall. We also explored whether or not 0–24-month biomarker changes varied by exercise performance during BETA. Therefore, we stratified the exploratory models by: (1) exercise adherence level during BETA, (2) change in physical fitness level during BETA, (3) change in total body fat during BETA, and (4) change in lean mass during BETA. We hypothesized that 0–24-month biomarker changes would be more favorable (i.e., consistent with lower breast cancer risk) in subgroups of women who exercised more and/or experienced more favorable body composition changes during the core intervention. All analyses and graphics were done using SAS (version 9.2, SAS Institute Inc.). Statistical tests were two-sided with a 0.05 significance level.

Results

Of 400 participants randomized in BETA, we obtained 24-month blood biomarker measurements for 333 women (84.0% and 82.5% of the MODERATE and HIGH groups, respectively). The reasons for no or incomplete follow-up at 24 months are shown in Fig. 1. To assess possible selection bias, we compared characteristics of participants who were included in the 24-month follow-up ($n = 333$) versus those lost to follow-up ($n = 53$; Supplementary Table S2). The proportion lost to follow-up (14%) was identical in the HIGH and MODERATE groups. Women included in the 24-month follow-up study, on average, were somewhat older and had more favorable biomarker profiles than women lost to follow-up (i.e., lower baseline hs-CRP, HOMA-IR, estrogens, and higher SHBG). In addition, women included in the follow-up study were more adherent during the intervention than women lost to follow-up (exercise duration, weeks 13–52: 182 minutes/week vs. 128 minutes/week, respectively) and experienced greater decreases in total body fat (-2.1 kg vs. -0.8 kg) during the core intervention.

Descriptive characteristics for 24-month follow-up participants are shown in Table 1 according to randomized group. Women randomized to the HIGH exercise group, on average, had more favorable biomarker profiles at baseline than the MODERATE group (i.e., slightly lower baseline BMI, hs-CRP, HOMA-IR, sex hormones, and higher SHBG). In examining biomarker changes over time, the trajectories for the HIGH and MODERATE exercise groups were similar (Fig. 2). Although there was no objective measurement of physical activity between 12 and 24 months, besides the 1-week accelerometer data at each time point, both groups self-reported approximately180 minutes/week moderate–vigorous physical activity over the past year ($n = 334$, data not shown; ref. 14).

Table 2 shows our primary results, comparing 0–24-month biomarker changes between the randomized groups using linear mixed models. For all biomarkers, there was no evidence to suggest that 0–24-month biomarker changes differed significantly between the HIGH versus MODERATE groups. All ratios (HIGH: MODERATE) were close to 1.0, and none were statistically significant (P value > 0.05 for all biomarkers). Similarly, there was no evidence that 12–24-month biomarker changes differed significantly between the HIGH and MODERATE groups (P value > 0.05 for all biomarkers). Within each of the HIGH and MODERATE arms, there were statistically significant "time" effects that reflected trends in Fig. 2.

As there was no "group" effect between HIGH and MODERATE intervention groups, a *post hoc* analysis was made to assess long-term biomarker changes with the two groups combined (Supplementary Table S3). Linear mixed models showed statistically significant decreases in insulin, glucose, HOMA-IR, estrone, and SHBG levels between baseline and 24 months. Total and free estradiol levels as well as hs-CRP levels increased significantly between these two time points.

Because many of the biomarkers associated with breast cancer are also related to adiposity, an additional analysis was conducted to compare changes in biomarker concentrations by loss of fat mass between baseline and 12 months. Table 3 shows the results of this analysis comparing women who lost more than the median amount of fat mass (>1.8 kg) with those who lost the median amount or less. The results of this analysis showed that women who lost more than the median amount of fat mass at 12 months had more favorable trends for several biomarkers. Concentrations of hs-CRP were lower at 24 months ($P = 0.05$) among these women, whereas women who lost less fat had increased concentrations at 24 months compared with levels at 12 months and baseline ($P < 0.001$). Similarly for insulin, women with greater fat loss had significantly reduced levels at 24 months compared with baseline ($P < 0.001$), and women who lost less had increased levels at 24 months ($P = 0.002$). Levels of HOMA-IR were also decreased for those who lost more fat mass ($P < 0.001$) and those with smaller losses had slightly increased levels at 24 months, though it was not significant ($P = 0.31$). Finally, levels of SHBG continued on a favorable trend for those with greater losses ($P = 0.59$), whereas women who lost less had significantly decreased SHBG levels at 24 months ($P < 0.001$).

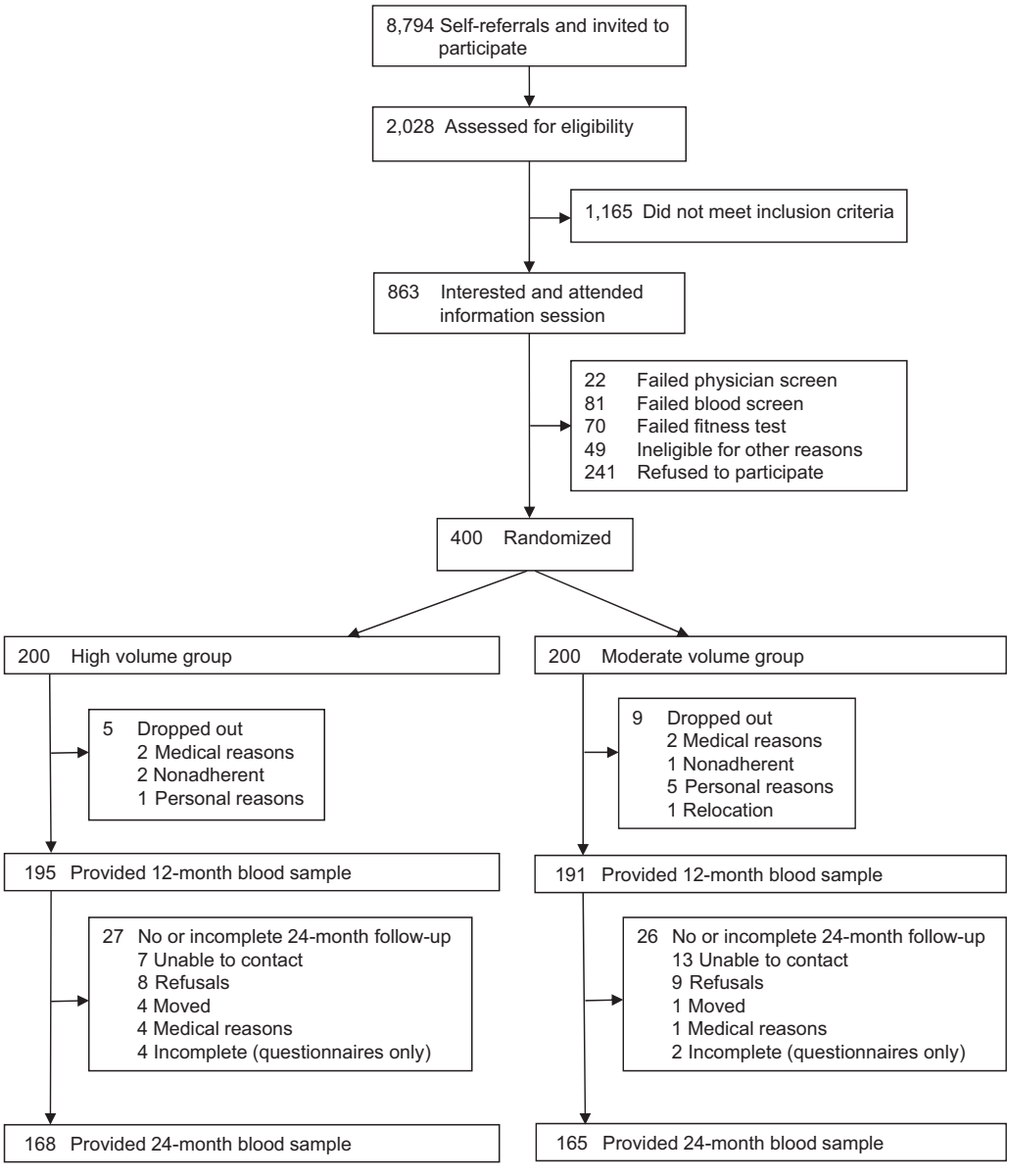

Figure 1.
Flow of participants through BETA (0–12 months) and the 24-month follow-up study (12–24 months), Alberta, Canada, 2010–2014.

Exploratory analyses were conducted on other factors that may affect biomarker concentrations including adherence to the exercise intervention (Supplementary Table S4), physical fitness level (Supplementary Table S5), and loss of lean mass (Supplementary Table S6). A statistically significant decrease in insulin and HOMA-IR levels was found between 0 and 24 months, specifically for women with higher adherence and limited loss of lean mass during the trial. Although SHBG levels decreased and free estradiol increased between 0 and 24 months in nearly all strata, these changes only reached statistical significance for women with lower adherence, lower fitness improvements, and higher changes in lean mass (estradiol was also statistically significantly higher for lower changes in lean mass).

Discussion

In a 24-month follow-up of 333 participants from the BETA trial ($N = 400$), we found no evidence to suggest that biomarker changes between 0 and 24 months or 12 and 24 months differed significantly between the HIGH and MODERATE groups. Overall, between 0 and 24 months, insulin, glucose, estrone, and HOMA-IR levels decreased significantly, which were favorable trends. Yet estradiol and hs-CRP concentrations increased significantly and SHBG decreased significantly between 0 and 24 months, consistent with higher breast cancer risk. Because there was no evidence of a group effect (MODERATE vs. HIGH) immediately after the intervention (15–17), it was not surprising to find no group effect on biomarker concentration changes over the long term.

Table 1. Baseline characteristics[a] of randomized BETA participants with 24-month follow-up data for blood biomarkers; Alberta, Canada, $N = 333$

Baseline characteristics [a]	HIGH Mean ± SD	MODERATE Mean ± SD
N	168	165
Age (years)	59.7 ± 5.0	59.8 ± 5.1
BMI (kg/m^2)	28.9 ± 4.4	29.4 ± 4.4
Maximal oxygen consumption (mL/kg/min)	26.6 ± 5.4	27.0 ± 4.9
Age at menopause (years)	48.9 ± 5.6	49.8 ± 4.6
	Median (Quartiles 1–3)	**Median (Quartiles 1–3)**
Total physical activity (MET-hrs/wk)[b]	90.1 (62.9–115.8)	87.1 (58.8–122.0)
Recreational activity (MET-hrs/wk)[b]	7.8 (3.0–13.9)	7.9 (2.6–12.9)
Moderate–vigorous activity (hrs/wk)[b]	6.9 (2.3–13.3)	5.6 (2.7–13.7)
Total energy intake (kcal/day), past year [b]	1357 (980–1,762)	1414 (1,120–1,773)
Alcohol intake (g/day), past year [b]	3.1 (1.0–7.1)	2.4 (0.7–6.6)
Biomarker concentrations		
hs-CRP (mg/L)[c]	1.52 (0.77–3.38)	1.88 (0.93–4.12)
Insulin (μIU/mL)	8.1 (4.9–12.2)	8.7 (5.8–12.9)
Glucose (mg/dL)[c]	89 (85–95)	92 (86–98)
HOMA-IR	1.8 (1.0–2.8)	2.0 (1.3–3.1)
Estrone (pg/mL)	36.4 (29.7–43.6)	38.6 (30.6–47.8)
Total estradiol (pg/mL)	9.2 (7.4–12.0)	9.5 (7.6–12.8)
SHBG (nmol/L)[c]	47.8 (34.8–66.4)	41.5 (32.5–59.0)
Free estradiol (pg/mL)	0.21 (0.15–0.28)	0.22 (0.17–0.31)
	***N* (%)**	***N* (%)**
Full-time employment	57 (34)	50 (30)
Education		
High school or less	33 (20)	37 (22)
Educated beyond high school	135 (80)	128 (78)
Marital status		
Married/Common law	119 (71)	113 (68)
Other	49 (29)	52 (30)
Race/ethnicity		
White	144 (86)	152 (92)
Other	24 (14)	13 (8)
Medication use		
Past-year anticholesterol	18 (11)	23 (14)
Past-year anti-inflammatory	16 (10)	20 (12)

[a]Detailed results showing baseline characteristics (i.e., start of BETA) and 0 to 12–month changes for the entire study population were reported in Friedenreich and colleagues (5–7). The results in this table reflect 333 participants with blood biomarker data at baseline and 24-month follow-up.
[b]Past-year behavior, derived from self-report (9, 10).
[c]There were no statistically significant differences at baseline between high and moderate exercise groups except for SHBG, P value = 0.02; hs-CRP, P value = 0.03; and glucose, P value = 0.01.

Exploratory analyses found a possible subgroup effect of exercise adherence during the BETA trial, and secondary analyses by fat mass loss between 0 and 12 months revealed the importance of fat mass loss in breast cancer risk reduction. In our previous analysis of adiposity changes at 24 months in these participants, we found that both study arms maintained their fat loss at 24 months and that the body fat loss was greater for the HIGH versus MODERATE group (14). To some extent, all of the blood biomarkers in the present report are influenced by body fatness in postmenopausal women (27). This observation is further supported by our analysis by fat loss, which found that women with higher fat loss had more favorable trends for many of the biomarkers measured including hs-CRP, insulin, and HOMA-IR. However, the unfavorable trends observed in SHBG and estradiol concentrations are more difficult to explain. Although SHBG levels were increased at the 24-month follow-up for women in both groups, these levels decreased by 24 months to levels below baseline. Speculatively, this result may be because women reduced their physical activity between 12 and 24 months and may have regained fat mass during this period. Because abdominal fat inhibits the production of SHBG (28), this explanation is plausible. The increased level of estradiol in both groups is also surprising, with concentrations at 24 months higher than baseline for both intervention groups. This finding may also be a result of the regained fat mass between 12 and 24 months, which was not captured in this analysis. We examined the adiposity and sex hormone changes in a previous paper (17) and noted that the amount of fat loss may have been insufficient to observe significant changes in some sex hormones—particularly changes that would persist 12 months following the intervention. If this explanation is correct, it is unclear why SHBG and estradiol specifically could not be maintained after intervention while other biomarkers had favorable trends.

We are unaware of any previous studies that examined breast cancer biomarker changes after an exercise-only (i.e., no dietary guidelines) dose–response intervention among women with no history of breast cancer. One previous four-armed trial that examined the long-term effects (at 30 months) of weight loss found no changes in SHBG or sex steroid hormones among participants who were randomized to the exercise-only arm (13). Some studies examined biomarkers only among breast cancer survivors, and others have been in the context of diabetes. In a systematic review of exercise-only interventions among breast cancer survivors, inconsistent effects were reported for insulin and C-reactive protein (29). Of nine RCTs included in the review, few consistent results were found for biomarker concentrations. Insulin levels were found to be significantly different from control groups in three RCTs by either remaining stable or decreasing, whereas two RCTs reported no differences between groups (29). There was no consistency found for IGF concentrations, and none of the studies found any evidence of a role for inflammatory markers. However, due to the vast heterogeneity of the included trials, no meta-analysis could be conducted.

An RCT of a year-long, structured educational physical activity intervention included a 12-month follow-up of glucose regulation among 98 overweight/obese adults with impaired glucose tolerance (30). Among 73 completers who received an education program plus pedometer, the level of 2-hour glucose (but not fasting glucose) was significantly reduced at 24 months versus the control group. Lifestyle intervention trials more commonly added a long-term follow-up period in relation to diabetes prevention (31–34) or weight loss (35) when exercise and diet were targeted concurrently. Individuals assigned to lifestyle intervention groups have been shown to have significantly lower cardiovascular and all-cause mortality risks than controls up to 17 years after the intervention (31) and significantly lower risk of diabetes relative to controls 13 years after the intervention (33). In addition, the LOOK AHEAD study (34) of 5,145 overweight/obese adults with type 2 diabetes added a 7-year maintenance intervention to their year-long core lifestyle intervention. A meta-analysis of 44 Diabetes Prevention Program intervention studies in the United States ($n = 8,955$; 3–15 months duration) examined fasting glucose levels approximately 1 year after intervention and found no benefit from the intervention unless a lower intensity "maintenance" intervention (i.e., intermittent in-person

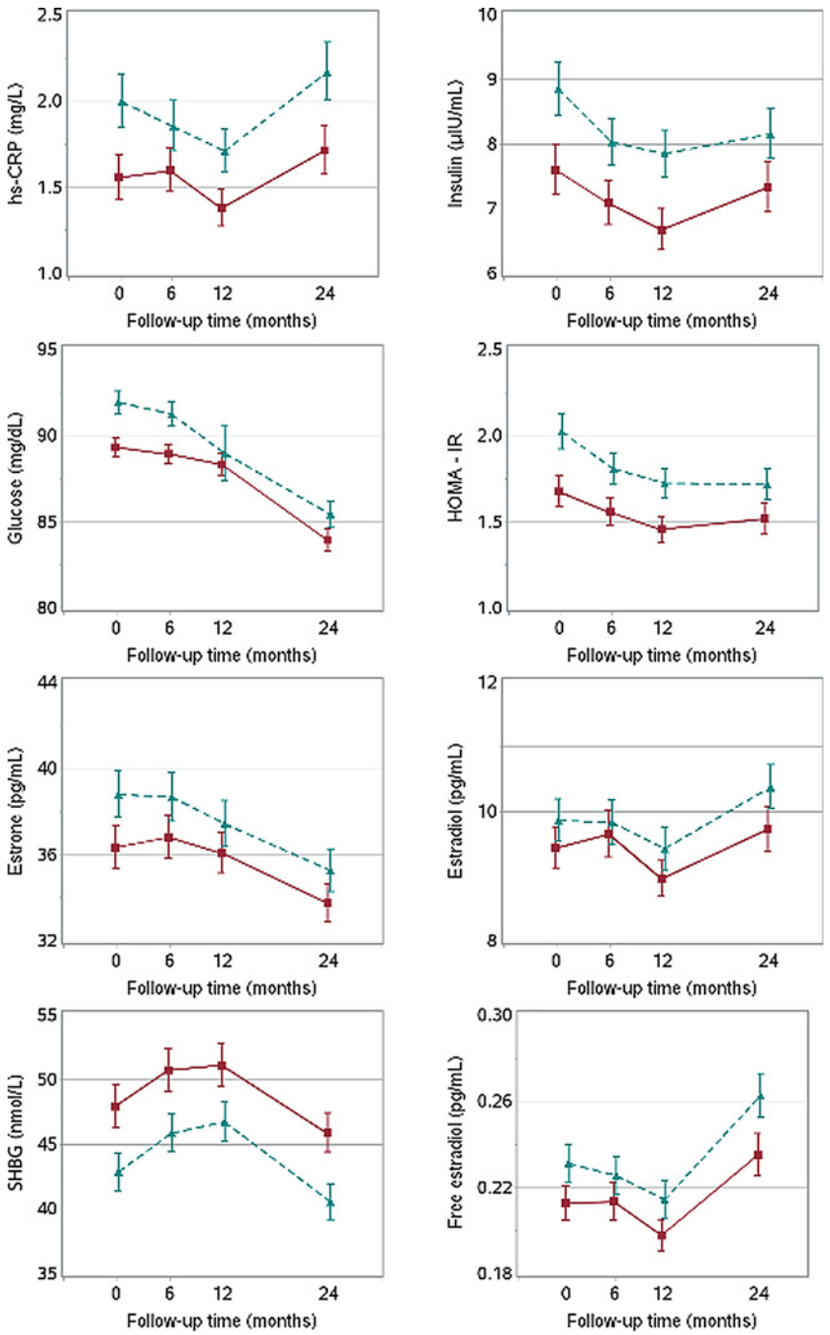

Figure 2.
Geometric mean values (± SE) for circulating biomarker concentrations over time, by randomized group assignment, for BETA participants with complete data at all time points, Alberta, Canada, $N = 333$. The sample size was $n = 168$ in the moderate-duration exercise group, for all biomarkers. The sample size in the high-duration exercise group was $n = 165$ for hs-CRP and insulin; $n = 164$ for glucose, HOMA-IR, estrone, SHBG; and $n = 163$ for total estradiol, free estradiol. The solid line represents the HIGH group, and the dashed line is the MODERATE group results.

sessions or emails) was added to the core intervention (33). Overall, these studies suggest that maintenance is possible, particularly when the lifestyle intervention is long (i.e., >1 year) or includes a maintenance intervention (35).

It is unclear from our study how much of the 0–24-month improvements in insulin, glucose, HOMA-IR, and estrone concentrations overall were attributable to residual effects from the intervention, or to lifestyle behaviors after BETA. At 24 months, both groups self-reported approximately 180 minutes/week of moderate–vigorous physical activity (14), which indicates that the HIGH group decreased their exercise levels following the trial, as seen in other RCTs of postmenopausal women (36–39). Yet at 24 months, both groups reported higher activity levels than at baseline, possibly explaining the improved HOMA-IR concentrations between 0 and 24 months (Supplementary Table S3).

A "detraining" study would help to delineate the cause of biomarker changes by removing exercise completely as a causal factor. Detraining studies have shown that only 2 weeks (40, 41)

Table 2. Biomarker levels and changes over time by randomized group assignment in BETA participants with 24-month follow-up data; Alberta, Canada, $N = 333$

				0–24 months (24 months: baseline)		0–24 months (HIGH:MOD)		12–24 months (24:12 months)		12–24 months (HIGH:MOD)	
Blood biomarker	Baseline mean[a]	12 month Mean[a]	24 month Mean[a]	Time effect[b]	*P* value	Group effect[b]	*P* value	Time effect[b]	*P* value	Group effect[b]	*P* value
hs-CRP (mg/L)											
Moderate ($n = 165$)	2.0	1.7	2.2	1.09	0.13	(Ref.)	-	1.27	<0.001	(Ref.)	-
High ($n = 168$)	1.6	1.4	1.7	1.10	0.08	1.01	0.86	1.24	<0.001	0.98	0.78
Insulin (μIU/mL)											
Moderate ($n = 165$)	8.9	7.9	8.2	0.92	0.014	(Ref.)	-	1.04	0.26	(Ref.)	-
High ($n = 168$)	7.6	6.7	7.3	0.96	0.27	1.05	0.33	1.10	0.005	1.06	0.24
Glucose (mg/dL)											
Moderate ($n = 164$)	91.9	89.0	85.4	0.93	<0.001	(Ref.)	-	0.96	0.001	(Ref.)	-
High ($n = 168$)	89.3	88.4	84.0	0.94	<0.001	1.01	0.52	0.95	<0.001	0.99	0.56
HOMA-IR [c]											
Moderate ($n = 164$)	2.0	1.7	1.7	0.85	<0.001	(Ref.)	-	1.00	0.94	(Ref.)	-
High ($n = 168$)	1.7	1.5	1.5	0.91	0.007	1.06	0.25	1.04	0.25	1.05	0.38
Estrone (pg/mL)											
Moderate ($n = 164$)	38.8	37.4	35.3	0.91	<0.001	(Ref.)	-	0.94	0.002	(Ref.)	-
High ($n = 168$)	36.3	36.1	33.8	0.93	<0.001	1.02	0.42	0.94	0.001	0.99	0.81
Total estradiol (pg/mL)											
Moderate ($n = 163$)	9.9	9.4	10.4	1.05	0.07	(Ref.)	-	1.10	<0.001	(Ref.)	-
High ($n = 168$)	9.4	9.0	9.7	1.03	0.25	0.98	0.62	1.09	0.002	0.99	0.70
SHBG (nmol/L)											
Moderate ($n = 164$)	42.9	46.7	40.6	0.95	<0.001	(Ref.)	-	0.87	<0.001	(Ref.)	-
High ($n = 168$)	48.0	51.2	45.9	0.96	0.005	1.01	0.60	0.90	<0.001	1.03	0.13
Free estradiol (pg/mL)											
Moderate ($n = 163$)	0.23	0.21	0.26	1.14	<0.001	(Ref.)	-	1.23	<0.001	(Ref.)	-
High ($n = 168$)	0.21	0.20	0.24	1.11	<0.001	0.97	0.51	1.19	<0.001	0.97	0.43

Abbreviation: Ref., referent category.

[a]Geometric mean values.

[b]A ratio of geometric means (by time or group assignment) derived from linear mixed models with blood biomarker level as the dependent variable. Independent variables were time of blood sampling (baseline, 12 months, 24 months), treatment group (high duration, moderate duration), and time–group interaction.

[c]HOMA-IR, fasting glucose (mmol/L) x fasting insulin (μIU/mL)/22.5.

or 4 weeks (42) after exercise, biomarker improvements may be lost partially (40) or completely (41, 42) for insulin resistance indicators (40, 42), fasting glucose, and inflammatory markers (41). Yet other detraining studies showed that improvement in insulin-stimulated glucose uptake per unit plasma unit (M/I; ref. 43) and CRP reductions (44) were maintained after 2 weeks (43) or 1 month (44) of detraining. These studies are difficult to extrapolate to BETA given that our follow-up period was 12 months and there is a lack of detraining evidence for estrogens. They do show, however, that biomarker improvements are reversed relatively quickly once exercise has stopped.

The clinical significance of the overall biomarker changes we observed is unclear. First, although our results show trends in the direction of biomarker changes over time, we cannot attribute the absolute biomarker changes entirely to our intervention because we lacked a "no-exercise" control group. Second, we acknowledge that the blood biomarkers in our study are imperfect markers of breast cancer risk and that other biomarkers have been hypothesized to be relevant (45). The choice of biomarkers in our study is justified by RCT evidence of their exercise-responsiveness in postmenopausal women (7, 46, 47), biologic plausibility (48), and a relatively large body of epidemiologic evidence relating each biomarker to breast cancer incidence. Epidemiologic evidence overall is convincing for circulating estrogens (49, 50) and compelling (though somewhat inconsistent) for insulin resistance indicators (51–53) and inflammatory biomarkers (54–56).

Our results are most generalizable to women who are postmenopausal, cancer-free, nonsmokers, not using exogenous hormones, and nondiabetic. There was some evidence of selection bias in our follow-up study because participants who were available for follow-up were somewhat healthier at baseline than nonparticipants and had higher exercise adherence during BETA. Consequently, the absolute biomarkers changes in this report are likely more pronounced than if all BETA participants were included. That is, assuming the most adherent women experienced stronger biomarker changes and greater potential for "rebound" after the trial.

A possible limitation of our study is insufficient statistical power to detect group effects on all biomarker changes, particularly because the 12-month follow-up was an ancillary study to BETA. A second limitation may be some imprecision in estimating 0–24-month biomarker changes because the lab assays occurred on two different days: on one occasion for the baseline and 12-month blood samples, and a second occasion for the 24-month samples. We minimized imprecision by using the same laboratory for all assays; a reputable lab with established protocols and excellent performance metrics on all assays (Supplementary Table S1). Although we did not have objective measures of physical activity between 12 and 24 months, and cannot assess exactly what our participants did during the 1-year long follow-up, we did have objective measures of physical activity at 24 months so could classify the activity levels of our participants accurately for that time point.

A major strength of our study is its novel design. To our knowledge, no other exercise-only trial measured endogenous estrogen levels after the trial to assess persistent or delayed effects. Because higher estrogen exposure is a widely accepted risk factor for breast and endometrial (57) and is also informative for bone health and menopausal symptoms, our findings have important

Table 3. Exploratory analysis of biomarker changes stratified by 0 to 12–month total body fat change (kg)[a] in BETA participants with 24-month follow-up data, Alberta, Canada, $N = 333$

Blood biomarker	0–12-month change, total body fat (kg)	*n*	Baseline Mean[a]	12 month Mean[a]	24 month Mean[a]	0–24 months (24 months: baseline) Time effect[b]	0–24 months (24 months: baseline) *P* value	12–24 months (24:12 months) Time effect[b]	12–24 months (24:12 months) *P* value
hs-CRP (mg/L)	↑Loss[d]	164	1.8	1.3	1.6	0.90	0.05	1.27	<0.001
	Loss/Gain[d]	165	1.7	1.8	2.3	1.31	<0.001	1.24	<0.001
Insulin (μIU/mL)	↑Loss	164	8.7	6.7	7.0	0.81	<0.001	1.05	0.16
	Loss/Gain	165	7.7	7.8	8.5	1.10	0.002	1.09	0.004
Glucose (mg/dL)	↑Loss	164	91.2	88.7	84.1	0.92	<0.001	0.95	<0.001
	Loss/Gain	165	90.2	88.8	85.4	0.95	0.001	0.96	0.015
HOMA-IR[c]	↑Loss	164	2.0	1.5	1.5	0.74	<0.001	1.00	0.90
	Loss/Gain	165	1.7	1.7	1.8	1.04	0.31	1.05	0.17
Estrone (pg/mL)	↑Loss	164	37.7	36.4	33.8	0.90	<0.001	0.93	<0.001
	Loss/Gain	165	37.5	37.2	35.3	0.94	0.003	0.95	0.009
Total estradiol (pg/mL)	↑Loss	164	9.5	8.8	9.6	1.00	0.86	1.08	0.002
	Loss/Gain	165	9.8	9.6	10.6	1.08	0.006	1.10	<0.001
SHBG (nmol/L)	↑Loss	163	45.1	51.1	44.7	0.99	0.59	0.87	<0.001
	Loss/Gain	165	45.6	46.9	42.2	0.93	<0.001	0.90	<0.001
Free estradiol (pg/mL)	↑Loss	164	0.22	0.19	0.23	1.07	0.019	1.21	<0.001
	Loss/Gain	165	0.23	0.22	0.26	1.17	<0.001	1.21	<0.001

[a]Geometric mean values.
[b]A ratio of geometric means (by time) derived from linear mixed models with blood biomarker level as the dependent variable. Independent variables were time of blood sampling (baseline, 12 months, 24 months), treatment group (high duration, moderate duration), and time–group interaction.
[c]HOMA-IR, fasting glucose (mmol/L) x fasting insulin (μIU/mL)/22.5.
[d]Stratified by the median change in total body fat during BETA ($N = 333$) combining the two randomized groups. The median change in total body fat was -1.8 kg. "↑Loss" indicates fat loss ≥1.8 kg, and "Loss/Gain" indicates fat gain, no change, or fat loss < 1.8 kg.

implications beyond breast cancer. Furthermore, participants in BETA were randomized to their exercise prescriptions, providing the highest level of evidence for exercise dose effects. An additional strength is the relatively large sample size attributable to a high retention rate (>80%) at the 12-month follow-up.

Although a year-long, supervised exercise intervention may improve breast cancer risk factors in the short term for physically inactive menopausal women, the long-term impact is unknown. Although our findings do not definitively conclude that prescribing more exercise, beyond 150 minutes/week, leads to larger improvements in breast cancer biomarkers, the results suggest that greater fat loss does lead to more favorable biomarker trends. In the analysis by intervention group, most biomarker changes during the trial (hs-CRP, insulin, SHBG, estradiol) rebounded after the trial had ended. However, for women who lost more than the median amount of fat, the only biomarkers to rebound significantly were SHBG and estradiol. This finding lends support to the hypothesis that fat loss has an independent effect on concentrations of breast cancer biomarkers. Future interventions should combine other strategies with exercise prescription that will increase fat loss and include components that will ensure maintenance beyond the intervention period.

Disclosure of Potential Conflicts of Interest

F.Z. Stanczyk is a consultant/advisory board member for Agile Therapeutics, Therapeutics MD, Dr. Reddy's Laboratories, and Mithra Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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