

Changes in insulin resistance indicators, IGFs, and adipokines in a year-long trial of aerobic exercise in postmenopausal women

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Abstract

Physical activity is a known modifiable lifestyle means for reducing postmenopausal breast cancer risk, but the biologic mechanisms are not well understood. Metabolic factors may be involved. In this study, we aimed to determine the effects of exercise on insulin resistance (IR) indicators, IGF1, and adipokines in postmenopausal women. The Alberta Physical Activity and Breast Cancer Prevention Trial was a two-armed randomized controlled trial in postmenopausal, inactive, cancer-free women. A year-long aerobic exercise intervention of 225 min/week ($n=160$) was compared with a control group asked to maintain usual activity levels ($n=160$). Baseline, 6- and 12-month serum levels of insulin, glucose, IGF1, IGF-binding protein 3 (IGFBP3), adiponectin, and leptin were assayed, and after data collection, homeostasis model assessment of IR (HOMA-IR) scores were calculated. Intention-to-treat analyses were performed using linear mixed models. The treatment effect ratio (TER) of exercisers to controls was calculated. Data were available on 308 (96.3%) women at 6 months and 310 (96.9%) women at 12 months. Across the study period, statistically significant reductions in insulin (TER=0.87, 95% confidence interval (95% CI)=0.81–0.93), HOMA-IR (TER=0.86, 95% CI=0.80–0.93), and leptin (TER=0.82, 95% CI=0.78–0.87), and an increase in the adiponectin/leptin ratio (TER=1.21, 95% CI=1.13–1.28) were observed in the exercise group compared with the control group. No significant differences were observed for glucose, IGF1, IGFBP3, adiponectin or the IGF1/IGFBP3 ratio. Previously inactive postmenopausal women who engaged in a moderate-to-vigorous intensity exercise program experienced changes in insulin, HOMA-IR, leptin, and adiponectin/leptin that might decrease the risk for postmenopausal breast cancer.

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Introduction

Physical activity reduces the risk of postmenopausal breast cancer (Monninkhof *et al.* 2007, World Cancer Research Fund and the American Institute for Cancer Research 2007, Friedenreich & Cust 2008, Lynch *et al.* 2011), but the biologic mechanisms underlying this association are not well understood. Insulin (Osborne *et al.* 1976, Chappell *et al.* 2001) insulin-like growth factor 1 (IGF1 and its binding proteins; Yu & Rohan 2000, Lann & LeRoith 2008) leptin (Catalano *et al.* 2003, Geisler *et al.* 2007, Cirillo *et al.* 2008), and adiponectin (Brakenhielm *et al.* 2004, Dieudonne *et al.* 2006, Arditi *et al.* 2007) are metabolic factors hypothesized to mediate the association between physical activity and postmenopausal breast cancer risk (Neilson *et al.* 2009).

Differing levels of evidence support roles for these proposed biomarkers in breast cancer, but the greatest pool of epidemiologic data in postmenopausal women exists for insulin (Gunter *et al.* 2009, Kabat *et al.* 2009, Neilson *et al.* 2009) and IGF1 and its binding proteins (Key *et al.* 2010). The link between insulin and breast cancer is now sufficiently convincing that metformin, a pharmacologic agent that improves insulin sensitivity, is being considered for breast cancer prevention and treatment (Gonzalez-Angulo & Meric-Bernstam 2010) with clinical trials underway (Cazzaniga *et al.* 2009, Goodwin *et al.* 2009). An association between high circulating IGF1 and breast cancer risk has been demonstrated fairly consistently among premenopausal, but not postmenopausal, women (Fletcher *et al.* 2005) and associations with IGF-binding protein 3 (IGFBP3) have been inconsistent (Renehan *et al.* 2006). Nevertheless, a recent pooled analysis found a significantly increased risk of breast cancer with elevated IGF1 and IGFBP3 in postmenopausal women (Key *et al.* 2010).

Leptin and adiponectin are adipokines that relate strongly to obesity and insulin resistance (IR; Pittas *et al.* 2004, Dyck *et al.* 2006) but their roles in breast cancer are unclear. While it is biologically plausible that leptin increases postmenopausal breast cancer risk, epidemiological findings have been inconsistent (Neilson *et al.* 2009, Wu *et al.* 2009, Maccio *et al.* 2010). In contrast, most studies in postmenopausal women have shown significant inverse associations between adiponectin and breast cancer risk (Neilson *et al.* 2009). Emerging research suggests that the ratio of adiponectin:leptin (A/L or conversely, L/A) provides a surrogate measure of IR (Finucane *et al.* 2009), and perhaps also acts as an indicator of postmenopausal breast cancer risk, which may be

more sensitive than either marker on its own (Cleary *et al.* 2009, Jarde *et al.* 2009).

Since all of these proposed biomarkers are related to IR and obesity, we hypothesized that their levels could be modified with sustained physical activity. Only a few studies have addressed this question previously via randomized controlled trials (RCTs) on postmenopausal women (Neilson *et al.* 2009), including one RCT that focused on breast cancer (Frank *et al.* 2005). A need for additional tightly controlled, long-term intervention trials to examine the impact of exercise on proposed biomarkers for breast cancer risk has been widely recognized (McTiernan *et al.* 1999, Friedenreich 2001, IARC Working Group 2002, Kushi *et al.* 2006, Physical Activity Guidelines Advisory Committee 2008). In response to this recognized need for more research, the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial was a 12-month exercise intervention trial designed to improve the understanding of the biologic mechanisms mediating the association between physical activity and postmenopausal breast cancer risk. We previously reported that the intervention significantly decreased adiposity (Friedenreich *et al.* 2010b) and circulating estradiol and increased sex hormone-binding globulin (Friedenreich *et al.* 2010a), which were primary outcomes of the trial. Here, we report on metabolic factors as secondary outcomes, namely, insulin, glucose, homeostasis model assessment of IR (HOMA-IR), IGF1, IGFBP3, IGF1/IGFBP3 ratio, leptin, adiponectin, and A/L ratio.

Materials and methods

The design and methods for the ALPHA Trial have been described (Friedenreich *et al.* 2010a). In brief, this study was an efficacy trial designed as a two-centred, two-armed RCT conducted in postmenopausal women living in Calgary and Edmonton, Alberta, Canada. The study and protocol were approved by the Research Ethics Boards at the Alberta Cancer Board and the Universities of Calgary and Alberta. All participants provided written informed consent.

Participants and randomization

We recruited women from the general population through targeted mailings to participants in the Alberta Breast Screening Program, posters and brochures distributed to family physicians, and through media campaigns. Specific eligibility criteria included: age 50–74 years; postmenopausal; no previous cancer diagnosis; no major co-morbidities; acceptable heart

and lung function assessed by a fitness test; inactive (<90 min/week of exercise or, if 90–120 min/week, having a $\text{VO}_{2\text{max}}$ level <34 ml/kg per min); physician clearance for unrestricted physical activity; normal fasting levels of glucose, serum cholesterol, TSH, alanine aminotransferase; body mass index (BMI) 22–40 kg/m²; non-smoker; <14 drinks of alcohol/week; no medications or exogenous hormones that might influence estrogen metabolism; and not currently in a weight loss program or planning to undertake the one.

A stratified (on center and BMI), blocked randomization was achieved using a random number program in S-plus written by the study biostatistician (R F B). Numbered, sealed envelopes were opened by the Study Coordinator at the time of randomization. Outcome assessments were blinded to group assignment.

Intervention

The exercise prescription was aerobic exercise for at least 45 min, 5 days/week for 1 year. At least three sessions/week were facility-based with on-site exercise trainers, and the remaining sessions were home-based. Participants wore heart rate monitors (Polar A3) to maintain workout intensity at the target of 70–80% of heart rate reserve. The prescription ramped up over the first 3 months and was maintained for the final 9 months. Adherence was monitored through weekly participant- and trainer-administered exercise logs. Controls were asked to maintain their inactive lifestyle. All participants were instructed not to change their usual diet.

Covariate information

Demographic characteristics, medical and reproductive history were obtained from a self-administered baseline questionnaire; all other covariate measures were taken at baseline and 12 months. Dietary intake of the previous year was assessed using the National Cancer Institute's Diet History Questionnaire previously adapted for use in Canadian populations (Csizmadi *et al.* 2007) and physical activity was assessed using the past year total physical activity questionnaire (Friedenreich *et al.* 2006). Fitness was assessed using a modified Balke treadmill protocol to estimate maximum oxygen consumption ($\text{VO}_{2\text{max}}$) from submaximal exercise intensities. Duplicate anthropometric measurements were taken using standardized methods. Total body fat was assessed using whole body dual X-ray absorptiometry scans (Friedenreich *et al.* 2010b). Percent body fat was calculated as $100\% \times (\text{fat mass}/(\text{fat mass} + \text{lean mass}))$.

Blood collection and hormone assays

Blood was collected after a minimum 10 h fast at baseline (60 ml), 6 and 12 months (40 ml), and medications taken in the past 24 h were recorded. Participants were asked not to exercise for 24 h pre-blood draw. All blood samples were collected, processed, and stored within 12 h of collection, then shipped and stored in -86°C freezers until assay. Analyses were conducted by the Reproductive Endocrine Research Laboratory at the University of Southern California (FZS), a laboratory with well-established protocols and quality control procedures.

Each participant's samples from the three time points were included in a single batch, but the order was randomized. Each batch had an equal number of samples from exercisers and controls with similar randomization dates and two pooled quality control samples. The quality control serum pool consisted of blood drawn from women who were interested in the study, postmenopausal, and not taking hormone replacement therapy, but deemed ineligible after blood draw for reasons unrelated to the metabolic factors of interest. Additional repeat blood samples from volunteers were included to assess within-subject, between-time variability. Laboratory personnel were blinded to subject and quality control sample identity.

Insulin, IGF1 and IGF1BP3 were measured by direct chemiluminescent immunoassay using the Immulite Analyzer (Siemens Medical Solutions Diagnostics, Malvern, PA, USA); intra-assay coefficients of variation (CV) were 3, 2 and 7%, and inter-assay CV were 3, 4 and 7% respectively. Glucose was measured using the oxygen rate method, employing an oxygen electrode on a Beckman glucose analyzer (Model DT60II, Vitros System, Johnson & Johnson, Rochester, NY, USA); the intra-assay CV was 8% and the inter-assay CV was 13%. A surrogate measure of whole body insulin sensitivity and β -cell function, HOMA-IR, was calculated as: $\text{fasting glucose (mM)} \times \text{fasting insulin } (\mu\text{IU/ml})/22.5$ (Hosker *et al.* 1985, Matthews *et al.* 1985, Bonora *et al.* 1989, Levy *et al.* 1991, Rudenski *et al.* 1991, Turner *et al.* 1993). Leptin and total adiponectin were measured by RIA (Millipore, St Charles, MO, USA); intra-assay and inter-assay CV were 6 and 5%, and 8 and 12% respectively.

Sample size

Sample size calculations were based on the primary estrogen and adiposity outcomes for which power $\geq 80\%$ was available (Friedenreich *et al.* 2010a). For IR outcomes, using the same approach for sample size estimation as for the primary outcomes, namely the normal theory formula for comparing means of two

independent samples with $\alpha=0.05$ (two-sided; Rosner 1986) and s.d. from the Physical Activity for Total Health trial, (Irwin et al. 2003, McTiernan et al. 2004) we had power >95% to detect changes of 10–20%.

Statistical analysis

The primary analysis assessed the intervention effect based on assigned group at randomization regardless of adherence for participants with complete data at baseline and end of study (intent-to-treat). Skewness in these biomarkers was corrected using natural logarithm transformations. Intervention effects were evaluated with general linear models considering IR indicators, IGF1, IGF1BP3, leptin, and adiponectin measures at 6 and 12 months as repeated measures.

Specifically, we used the following general linear model:

$$Y_{ij} = \beta_0 + \beta_1 X_i + \gamma Z_{ij} + \varepsilon_{ij}$$

where, for i th subject, Y_{ij} is a log-transformed biomarker measure at j th time ($j=1$ for 6 months and $j=2$ for 12 months), X_i is an indicator variable for the exercise intervention arm (0 for control and 1 for exercise intervention), Z_{ij} is a vector of adjustment

variables including the baseline biomarker value, time, and change in the total energy intake, and $(\varepsilon_{i1}, \varepsilon_{i2})$ is a potentially correlated error vector that followed a bivariate Gaussian distribution with a mean of 0. Of the parameters β_0 , β_1 , and γ (associated with the intercept, exercise intervention, and adjustment variables respectively), β_1 was our main interest, representing the adjusted difference in the mean log biomarker measure between the exercise intervention group and the control group over the two follow-up time points, adjusting for the baseline biomarker value, time, and change in the total energy intake. We referred to the exponential of β_1 as the treatment effect ratio (TER), as it is a ratio of adjusted geometric means of the biomarker for the exercise intervention group over the control group.

Secondary analyses examined whether or not the effect of exercise on the metabolic factors varied by adherence and body composition change from baseline to 12 months. Spearman's rank correlation coefficients were estimated to relate changes in metabolic factors to changes in percent body fat in exercisers. We classified exercise adherence into three categories pre-defined by public health guidelines: <150, 150–225, and >225 min/week (Warburton et al. 2007, Physical

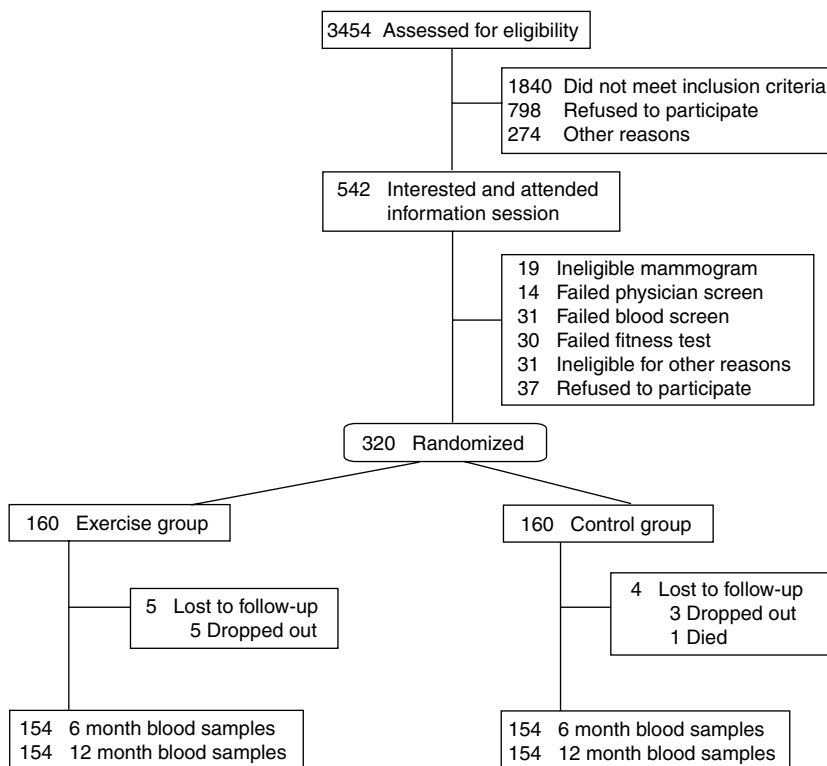


Figure 1 Recruitment, randomization, and follow-up of participants in the Alberta Physical Activity and Breast Cancer Prevention Trial 2003–2006.

Activity Guidelines Advisory Committee 2008). All statistical tests were two-sided with a significance level set at 0.05. Statistical analyses were performed using SAS Software (Version 9.1; SAS Institute, Inc., Cary, NC, USA).

Results

A total of 3454 women were assessed for eligibility; 320 women were randomized and nine were lost to follow-up post-randomization. The primary analysis included 154 exercisers and 154 controls with 6-month blood samples and 154 exercisers and 156 controls with 12-month blood samples (Fig. 1). Recruitment began in May 2003 and was completed in June 2006. Exercisers and controls were similar at baseline with respect to age, body composition, and proposed biomarker concentrations (Table 1). Baseline leptin concentrations were high relative to normal weight women (Mahabir *et al.* 2007) and ~80% of women were overweight or obese (BMI \geq 25).

Table 1 Baseline characteristics of participants, Alberta physical activity, and Breast Cancer Prevention Trial 2003–2006, $n=320^a$

Baseline characteristic	Exercisers ($n=160$)	Controls ($n=160$)
	Mean \pm s.d.	Mean \pm s.d.
Age (years)	61.2 \pm 5.4	60.6 \pm 5.7
Body composition measurements		
Body mass index (kg/m ²)	29.1 \pm 4.5	29.2 \pm 4.3
Intra-abdominal fat area (cm ²)	101.4 \pm 55.4	103.2 \pm 56.0
Total body fat (kg)	30.9 \pm 8.2	31.3 \pm 8.6
Percent body fat	42.2 \pm 4.9	42.4 \pm 5.7
	N (%)	N (%)
Education (> high school)	112 (70)	102 (64)
White race/ethnicity	144 (91)	145 (91)
	Median (IQR)	Median (IQR)
Insulin (μ U/ml)	6.2 (3.8–9.5)	5.7 (3.4–8.9)
Glucose (mM)	5.4 (4.9–6.1)	5.3 (4.9–5.8)
HOMA-IR	1.4 (0.9–2.4)	1.4 (0.8–2.3)
IGF1 (ng/ml)	117 (95–138)	119 (100–141)
IGFBP3 (μ g/ml)	4.0 (3.5–4.5)	3.9 (3.4–4.5)
Leptin (ng/ml)	19.2 (13.5–25.6)	20.9 (12.8–30.1)
Adiponectin (μ g/ml)	11.5 (9.1–17.2)	12.5 (9.6–16.1)
Adiponectin/leptin	0.63 (0.38–1.07)	0.64 (0.37–1.09)
IGF1/IGFBP3	29.5 (25.9–33.6)	30.5 (25.9–40.0)

IQR, interquartile range; HOMA-IR, homeostasis model assessment of insulin resistance = fasting glucose (mM) \times fasting insulin (μ U/ml)/22.5; IGF1, insulin-like growth factor 1; IGFBP3, insulin-like growth factor-binding protein 3.

^aThere were no statistically significant differences at baseline between exercisers and controls for these variables.

Adherence to our intervention has been previously reported (Friedenreich *et al.* 2010a). In brief, exercisers reported a larger 12-month increase in recreational activity than controls (20.2 vs 3.2 metabolic equivalent (MET)-hours/week, $P < 0.001$). Sixteen controls (10%) reported increasing recreational activity levels by 20 MET-hours/week (equivalent to 200 min/week activity \geq 6 MET-level (vigorous)) or more. Physical fitness, measured by VO_{2max} , increased in exercisers versus controls (3.9 vs 0.7 ml/kg per min, $P < 0.001$).

The main analyses showed an inverse effect of exercise on insulin, HOMA-IR, and leptin ($P < 0.001$; Table 2) and a positive effect on the A/L ratio ($P = 0.001$). The greatest changes in exercisers occurred in the first 6 months. Twelve-month percent changes relative to baseline were -10.3% for insulin, -11.4% for HOMA-IR, -18.9% for leptin, and $+24.9\%$ for the A/L ratio. There was no difference between exercisers and controls across 12 months for changes in glucose, IGF1, IGFBP3, IGF1/IGFBP3, or adiponectin.

In secondary analyses, exercise duration was consistently associated with monotonic reductions in insulin, HOMA-IR, and leptin concentrations (Table 3) but not glucose, IGF1, IGFBP3, IGF1/IGFBP3, or adiponectin (data not shown). The A/L ratio increased with increasing weekly duration of exercise. The highest decreases of 16 and 30% were achieved with exercise > 225 min/week for HOMA-IR and leptin, respectively, while the A/L ratio increased by 48% in this subgroup; also glucose decreased significantly (5.4%, $P = 0.013$). No significant changes ($P < 0.05$) occurred with exercise < 150 min/week for any proposed biomarker.

The effects of the intervention on insulin, HOMA-IR, leptin, and the A/L ratio were attenuated after adjustment for adiposity change (Table 4). Adjustment for change in total or percent body fat had a greater impact on TER than changes in body weight or intra-abdominal fat, with effects becoming non-statistically significant for HOMA-IR ($P = 0.073$ and $P = 0.065$) and the A/L ratio ($P = 0.207$ for both adiposity measures). Correlations between percent body fat change and metabolic changes in exercisers (data not shown) were strongest for leptin ($r_s = 0.50$, $P < 0.001$) and the A/L ratio ($r_s = -0.55$, $P < 0.001$).

Discussion

This year-long aerobic exercise intervention among postmenopausal inactive women resulted in reductions in insulin, HOMA-IR, and leptin concentrations and an increased A/L ratio, an emerging novel marker of IR. Changes in glucose, IGF1, IGFBP3, and adiponectin were not observed. This trial has provided

Table 2 Difference between exercisers and controls on concentrations of proposed biomarkers over 6 and 12 months from baseline

	Baseline	6 months	12 months	Treatment effect ratio of exercise/control (95% CI) ^a	Between-group P value
	Geometric mean (95% CI)*	Geometric mean (95% CI)	Geometric mean (95% CI)		
Insulin (μIU/ml)					
Exercisers ^b	6.1 (5.5 to 6.8)	5.4 (4.8 to 5.9)	5.3 (4.8 to 5.9)	0.87 (0.81 to 0.93)	<0.001
Controls ^c	5.7 (5.2 to 6.3)	6.0 (5.4 to 6.7)	5.9 (5.3 to 6.6)		
Glucose (mM)					
Exercisers	5.5 (5.4 to 5.7)	5.5 (5.3 to 5.7)	5.5 (5.3 to 5.6)	0.99 (0.97 to 1.02)	0.581
Controls	5.5 (5.3 to 5.6)	5.5 (5.4 to 5.7)	5.5 (5.3 to 5.6)		
HOMA-IR					
Exercisers	1.5 (1.3 to 1.7)	1.3 (1.2 to 1.5)	1.3 (1.1 to 1.4)	0.86 (0.80 to 0.93)	<0.001
Controls	1.4 (1.2 to 1.5)	1.5 (1.3 to 1.6)	1.4 (1.3 to 1.6)		
IGF1 (ng/ml)					
Exercisers	117 (112 to 123)	114 (109 to 119)	115 (110 to 121)	1.00 (0.98 to 1.02)	0.995
Controls	120 (114 to 126)	116 (110 to 122)	116 (111 to 122)		
IGFBP3 (μg/ml)					
Exercisers	3.9 (3.8 to 4.0)	3.8 (3.7 to 4.0)	3.8 (3.7 to 3.9)	0.99 (0.97 to 1.00)	0.163
Controls	3.9 (3.8 to 4.0)	3.9 (3.7 to 4.0)	3.9 (3.7 to 4.0)		
IGF1/IGFBP3					
Exercisers	29.9 (28.9 to 31.0)	29.6 (28.6 to 30.6)	30.1 (29.1 to 31.2)	1.01 (0.99 to 1.03)	0.377
Controls	30.6 (29.3 to 31.9)	29.9 (28.7 to 31.2)	30.1 (28.9 to 31.3)		
Leptin (ng/ml)					
Exercisers	18.8 (17.3 to 20.4)	14.8 (13.5 to 16.2)	14.9 (13.5 to 16.5)	0.82 (0.78 to 0.87)	<0.001
Controls	19.5 (17.7 to 21.4)	18.5 (16.7 to 20.4)	19.1 (17.4 to 21.1)		
Adiponectin (μg/ml)					
Exercisers	11.8 (10.9 to 12.9)	11.7 (10.7 to 12.7)	12.2 (11.3 to 13.3)	0.99 (0.97 to 1.02)	0.680
Controls	12.2 (11.4 to 13.1)	12.3 (11.4 to 13.2)	12.2 (11.4 to 13.1)		
Adiponectin/leptin					
Exercisers	0.63 (0.56 to 0.71)	0.79 (0.69 to 0.90)	0.82 (0.71 to 0.94)	1.21 (1.13 to 1.28)	<0.001
Controls	0.63 (0.56 to 0.71)	0.67 (0.59 to 0.75)	0.64 (0.56 to 0.72)		

CI, confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance; IGF1, insulin-like growth factor 1; IGFBP3, IGF-binding protein 3.

^aThe treatment effect ratio was calculated from a general linear model for each biomarker outcome, estimating a parameter whose anti-logarithm corresponds to the ratio of adjusted geometric means of the biomarker for the exercise intervention group over the control group: this ratio was assumed to be common at 6 and 12 months post-randomization.

^bExercise group: *n*, baseline=160, 6 month=154, 12 month=154.

^cControl group: *n*, baseline=160, 6 month=154, 12 month=156.

new and strong evidence for the role of these biomarkers in the association between physical activity and breast cancer risk that supports preliminary findings from previous studies and expands that research to other biomarkers.

Only a few large RCTs have examined the effects of a long-term exercise-only intervention on these metabolic factors in older women. Of five previous noteworthy RCTs with similar study populations and/or outcomes of interest (Houmard *et al.* 2004, Frank *et al.* 2005, Giannopoulou *et al.* 2005, Pi-Sunyer *et al.* 2007, Arsenault *et al.* 2009), only one trial lead by McTiernan (Frank *et al.* 2005, McTiernan *et al.* 2005) was of comparable size, duration and scope to the ALPHA Trial. In contrast to the other trials, the ALPHA Trial included healthy, postmenopausal women with a BMI ranging from 22–40 kg/m² rather than women with a BMI > 25 kg/m². Consequently,

these results are more generalizable to the population at risk for postmenopausal breast cancer. The ALPHA Trial also had several other study design features that distinguish it from earlier RCTs, including the largest sample size of healthy women, the most tightly controlled exercise intervention that was supervised for the entire 12-month period, a minimal loss to follow-up, and the novel combination of biomarkers that were examined. In particular, no previous RCT has examined the effect of exercise on the ratio of adiponectin/leptin. Since this ratio appears to estimate IR (Finucane *et al.* 2009), it was only recently proposed as a biomarker of breast cancer risk (Cleary *et al.* 2009, Jarde *et al.* 2009). Thus, our study provides a new insight, using a potentially stronger indicator of breast cancer risk than leptin or adiponectin on its own, into how exercise impacts the causal pathway between exercise and postmenopausal breast cancer.

Table 3 Concentrations of proposed biomarkers at baseline and 12 months in controls and exercisers by three adherence levels

	Baseline	12 months	Ratio 12 months/ baseline (95% CI) ^a	Percent change ^b	P value ^c	P for trend ^d
	Geometric mean (95% CI)	Geometric mean (95% CI)				
Insulin (μIU/ml)						
Controls ^e	5.6 (5.1 to 6.2)	5.9 (5.3 to 6.6)	1.05 (0.98 to 1.13)	5.4	Ref	<0.001
<150 min/week ^e	7.4 (5.9 to 9.3)	7.1 (5.8 to 8.7)	0.96 (0.81 to 1.13)	−4.4	0.679	
150–225 min/week ^e	5.6 (4.8 to 6.6)	4.9 (4.2 to 5.7)	0.87 (0.79 to 0.96)	−13.1	0.002	
>225 min/week ^e	5.3 (4.4 to 6.4)	4.7 (4.0 to 5.6)	0.89 (0.79 to 0.99)	−11.2	0.010	
HOMA-IR						
Controls	1.4 (1.2 to 1.5)	1.4 (1.3 to 1.6)	1.05 (0.97 to 1.13)	4.8	Ref	<0.001
<150 min/week	1.9 (1.4 to 2.4)	1.8 (1.4 to 2.3)	0.97 (0.81 to 1.16)	−3.4	0.860	
150–225 min/week	1.3 (1.1 to 1.6)	1.2 (1.0 to 1.4)	0.87 (0.78 to 0.97)	−12.8	0.007	
>225 min/week	1.3 (1.0 to 1.6)	1.1 (0.9 to 1.3)	0.84 (0.75 to 0.95)	−16.0	0.002	
Leptin (ng/ml)						
Controls	19.3 (17.5 to 21.2)	19.1 (17.4 to 21.1)	0.99 (0.94 to 1.05)	−0.7	Ref	<0.001
<150 min/week	18.0 (15.4 to 20.9)	17.3 (14.5 to 20.7)	0.96 (0.87 to 1.07)	−3.6	0.513	
150–225 min/week	19.9 (17.5 to 22.7)	16.1 (13.8 to 18.7)	0.81 (0.74 to 0.88)	−19.2	<0.001	
>225 min/week	16.8 (14.4 to 19.6)	11.8 (9.8 to 14.3)	0.70 (0.61 to 0.81)	−29.6	<0.001	
Adiponectin/leptin						
Controls	0.64 (0.56 to 0.72)	0.64 (0.56 to 0.72)	1.00 (0.94 to 1.07)	0.2	Ref	<0.001
<150 min/week	0.66 (0.51 to 0.86)	0.70 (0.52 to 0.94)	1.06 (0.94 to 1.20)	6.2	0.378	
150–225 min/week	0.63 (0.54 to 0.75)	0.77 (0.64 to 0.94)	1.22 (1.11 to 1.34)	21.9	0.001	
>225 min/week	0.68 (0.55 to 0.86)	1.02 (0.78 to 1.33)	1.48 (1.29 to 1.71)	48.4	<0.001	

CI, confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance.

^aRatio of geometric means at 12 months to geometric means at baseline.

^bPercent change in proposed biomarker at 12 months from baseline for that level or group.

^cP values for change in proposed biomarker at 12 months from baseline between controls and that level of exercise adherence group adjusted for the baseline value.

^dTest for trend in change in proposed biomarker at 12 months from baseline cross controls and three adherence groups adjusted for the baseline value.

^eN=156, 40, 67, and 47 for controls and three exercise adherence levels ≤150, 150–225, >225 min/week, respectively.

As with the McTiernan trial (Frank *et al.* 2005), our study demonstrated decreased insulin concentrations and HOMA-IR in exercisers that differed significantly from changes in controls across 12 months of exercise; no significant change in glucose was found in either trial. Exercise also lowered circulating insulin significantly in the Dose–Response to Exercise in Postmenopausal Women (DREW) trial, a 6-month exercise RCT in 349 overweight/obese postmenopausal women with elevated blood pressure; glucose levels also decreased significantly (Arsenault *et al.* 2009). In general, exercise with moderate weight loss has been found to improve insulin sensitivity (Albright *et al.* 2000, Ryan 2000, Wareham *et al.* 2005) and prevent type 2 diabetes (Gill & Cooper 2008, Physical Activity Guidelines Advisory Committee 2008).

The impact of our intervention on breast cancer prevention has not been tested. However, varying insulin levels, comparable with the 12-month changes that we observed in exercisers (6.1 vs 5.3 μIU/ml), have significantly altered breast cancer risk in non-diabetic, postmenopausal women not using hormone therapy (Gunter *et al.* 2009). In a large

etiologic study (Gunter *et al.* 2009), women with insulin levels 3.9 to <5.6 μIU/ml experienced a 41% lower risk of breast cancer than women with 5.6 to <8.8 μIU/ml insulin (midpoints of the ranges differed by 51%). Extrapolating those findings to our study, assuming a linear relation between insulin and breast cancer risk, we postulate that the 10% decrease in insulin experienced by our exercise group would lower breast cancer risk by ~8%.

It remains unclear what exercise dose is optimal for improving insulin sensitivity. In secondary analyses, we observed significant trends between exercise adherence and insulin and HOMA-IR changes respectively. Another RCT, the Studies of Targeted Risk Reduction Interventions through Defined Exercise (STRRIDE), found improvements in insulin sensitivity in 154 overweight/obese adults that were greater with 170 min/week of exercise than with 115 min/week, supporting our finding of dose–response trends for insulin and HOMA-IR when exercise exceeded 150 min/week (Houmard *et al.* 2004). In another comparable trial (Frank *et al.* 2005), there was no benefit from exercising <130 min/week, but decreases

Table 4 Treatment effect ratios for changes in proposed biomarkers over 6 and 12 months from baseline, before and after adjustment for adiposity change

	Treatment effect ratio of exercise/control (95% CI) ^a	P value
Insulin (μIU/ml)		
No adjustment	0.87 (0.81 to 0.93)	<0.001
Adjustment for weight change	0.90 (0.84 to 0.96)	0.002
Adjustment for % body fat change	0.92 (0.86 to 0.99)	0.021
Adjustment for total body fat change	0.92 (0.86 to 0.99)	0.021
Adjustment for intra-abdominal fat area change	0.91 (0.85 to 0.98)	0.011
HOMA-IR		
No adjustment	0.86 (0.80 to 0.93)	<0.001
Adjustment for weight change	0.91 (0.84 to 0.98)	0.009
Adjustment for % body fat change	0.93 (0.87 to 1.01)	0.073
Adjustment for total body fat change	0.93 (0.87 to 1.00)	0.065
Adjustment for intra-abdominal fat area change	0.92 (0.85 to 0.99)	0.027
Leptin (ng/ml)		
No adjustment	0.82 (0.78 to 0.87)	<0.001
Adjustment for weight change	0.91 (0.87 to 0.95)	<0.001
Adjustment for % body fat change	0.95 (0.90 to 0.99)	0.023
Adjustment for total body fat change	0.94 (0.90 to 0.99)	0.018
Adjustment for intra-abdominal fat area change	0.89 (0.85 to 0.95)	<0.001
Adiponectin/leptin		
No adjustment	1.21 (1.13 to 1.28)	<0.001
Adjustment for weight change	1.08 (1.03 to 1.14)	0.004
Adjustment for % body fat change	1.04 (0.98 to 1.09)	0.207
Adjustment for total body fat change	1.03 (0.98 to 1.09)	0.207
Adjustment for intra-abdominal fat area change	1.10 (1.03 to 1.17)	0.003

CI, confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance.

^aThe geometric mean ratios were estimated from least square means for the difference in treatment effect between exercisers and controls averaged across the entire study period adjusted for the baseline values, and then back log-transformed.

in insulin and HOMA-IR were observed for exercise durations of 131 to 190 min/week; smaller decreases occurred when duration exceeded 190 min/week.

Our findings for an effect of aerobic exercise on insulin and HOMA-IR scores, which was attenuated but not wholly explained by changes in adiposity, are biologically plausible. Exercise may lower serum insulin levels by promoting fat loss and preserving lean body mass, and independently of fat loss by increasing the number and activity of glucose transporters in muscle and adipose tissue (Ryan 2000). Another trial of older women (Frank *et al.* 2005) found decreased insulin and HOMA-IR scores only in women whose total body fat was decreased by the intervention, which suggests these changes were driven by fat loss. We similarly showed total and percent body fat loss to be mediators of exercise-induced changes in HOMA-IR (Table 4). The same trial (Frank *et al.* 2005) showed no difference across changes in body weight, percent body fat or intra-abdominal adiposity, concordant with our findings for insulin.

Early epidemiological evidence (Orenstein & Friedenreich 2004) does not support an inverse

association between chronic exercise and circulating levels of IGF1 and IGFBP3, particularly in older women. This evidence is consistent with our findings and other comparable RCTs (McTiernan *et al.* 2005, Seo *et al.* 2010) with one exception: that RCT (Orsatti *et al.* 2008) showed significantly increased IGF1 with exercise. A recent review also suggested that long-term exercise may actually increase circulating IGF1 (Nindl & Pierce 2010). Therefore, the evidence is weak that chronic exercise lowers IGF1 in postmenopausal women.

Given that leptin and adiponectin derive almost exclusively from adipose tissue, their levels should change with exercise-induced body fat loss and decreased central adiposity (Ritland *et al.* 2008), which was experienced in this trial (Friedenreich *et al.* 2010b). We observed reductions in leptin for exercisers, which increased in a dose-dependent manner with exercise adherence. Only a few studies have addressed the effects of exercise on leptin in postmenopausal women (Kohrt *et al.* 1996, Ryan *et al.* 2000, Hayase *et al.* 2002, Frank *et al.* 2005, Giannopoulou *et al.* 2005) and only two of them were RCTs (Frank *et al.* 2005, Giannopoulou *et al.* 2005).

One trial (Frank *et al.* 2005) showed decreased leptin concentrations with exercise; however, in our most adherent subgroup, we observed more than twice the decrease in leptin (12% with >190 min/week (Frank *et al.* 2005) versus 30% with >225 min/week). In another RCT (Giannopoulou *et al.* 2005), leptin decreased in exercisers, but the change was of borderline statistical significance ($P=0.06$); this difference might be attributable to the higher volume of exercise undertaken in our trial or the minimal fat loss in the earlier study. Adiponectin levels have an inverse association with adiposity. Exercise may lower the levels of the obesity-related inflammatory markers, tumor necrosis factor- α , and interleukin 6, thereby increasing adiponectin gene expression and secretion (Bruun *et al.* 2003). However, we and others found no changes in adiponectin levels with exercise (Giannopoulou *et al.* 2005, Arsenault *et al.* 2009, Coker *et al.* 2009, Ligibel *et al.* 2009). Although RCT evidence relating chronic exercise to circulating adiponectin has been inconclusive (Simpson & Singh 2008), it is hypothesized that levels increase with sufficient weight loss (Kraemer & Castracane 2007, Christiansen *et al.* 2010), perhaps more than the 2.3 kg weight loss in our trial (Friedenreich *et al.* 2010b) and the others (Giannopoulou *et al.* 2005, Arsenault *et al.* 2009). We observed significantly greater increases in the A/L ratio in exercisers than in controls, driven mainly by decreased leptin. This finding indicates improved insulin sensitivity, as did the changes we observed for insulin and HOMA-IR; all three indices have been strongly associated with the insulin sensitivity index from hyperinsulinaemic–euglycaemic clamp studies (Finucane *et al.* 2009). Our secondary analysis suggests that total and percent body fat loss influenced the effect of exercise on the A/L ratio.

Our study's strengths include its large size, long duration, low drop-out rate, excellent adherence, reliable assays, and quantitative assessments of total and central adiposity. We studied the isolated effects of exercise by not intervening on diet and this RCT was the first to investigate sustained exercise-induced changes in the A/L ratio in older women. Limitations include some lack of compliance among the control group: 10% of controls increased recreational activity by 20 MET-hours/week. We used insulin and HOMA-IR as IR indicators, which are practical alternatives to the gold standard euglycaemic hyperinsulinemic clamp method (DeFronzo *et al.* 1979, Bergman *et al.* 1985). Furthermore, given the number of secondary analyses of these data, type I error is a possibility. In terms of generalizability, our findings describe non-diabetic, primarily Caucasian, normal weight to overweight and

obese postmenopausal women who were inactive at baseline and, on average, had elevated leptin levels relative to normal weight women.

Conclusion

This RCT demonstrated that significant improvements in IR indicators, leptin, and A/L profiles can be achieved with sustained, moderate-to-vigorous aerobic exercise in previously inactive, postmenopausal women. Secondary analyses suggested that a decrease in total or percent body fat may be required to alter HOMA-IR and the A/L ratio. Similar to others, we observed no effect of long-term exercise on IGF1 or IGFBP3 levels. The benefits of improved insulin sensitivity include not only cancer risk reduction but also lower risk of metabolic syndrome, cardiovascular disease, and type 2 diabetes. Our finding of improved hormone levels with exercise ≥ 150 min/week is consistent with public health guidelines for prevention of all-cause mortality, cardiovascular disease, and type 2 diabetes (Physical Activity Guidelines Advisory Committee 2008) and lower than cancer prevention recommendations (World Cancer Research Fund and the American Institute for Cancer Research 2007), but because the ALPHA Trial was not designed to answer the question of exercise dose, further RCT evidence is needed to confirm our findings.

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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