Effects of exercise on markers of oxidative stress: an Ancillary analysis of the Alberta Physical Activity and Breast Cancer Prevention Trial

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ABSTRACT

Background: Oxidative stress may contribute to cancer aetiology through several mechanisms involving damage to DNA, proteins and lipids leading to genetic mutations and genomic instability. The objective of this study was to determine the effects of aerobic exercise on markers of oxidative damage and antioxidant enzymes in postmenopausal women.

Methods: The Alberta Physical Activity and Breast Cancer Prevention Trial (ALPHA) was a two-centre, two-armed randomised trial of 320 inactive, healthy, postmenopausal women aged 50–74 years. Participants were randomly assigned to a year-long exercise intervention (225 min/week) or a control group while being asked to maintain a normal diet. Fasting blood samples were obtained and plasma concentrations of two oxidative damage markers (8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-isoprostaglandin F2α (8-Iso-PGF2α)) and two antioxidant enzymes (superoxide dismutase and catalase) were measured at baseline, 6 months and 12 months. Intention-to-treat (ITT) and per-protocol analyses were performed using linear mixed models adjusted for baseline biomarker concentrations. A further exercise adherence analysis, based on mean minutes of exercise per week, was also performed.

Results: In the ITT and per-protocol analyses, the exercise intervention did not have any statistically significant effect on either oxidative damage biomarkers or antioxidant enzyme activity.

Conclusions: A year-long aerobic exercise intervention did not have a significant impact on oxidative stress in healthy, postmenopausal women.

Trial registration number: NCT00522262.

Introduction

Breast cancer is the most common cancer among women worldwide.1–2 Since 2004, breast cancer incidence rates have stabilised in North America.3 However, the absolute number of cases being diagnosed is still increasing because of population growth and ageing.1 Consistent epidemiological evidence exists that physical activity reduces postmenopausal breast cancer risk by 20–25%.4 Several inter-related biological mechanisms are hypothesised to explain this association.5 Until now, five randomised controlled exercise trials have investigated the direct effect of exercise on hypothesised biomarkers of postmenopausal breast cancer.5–9 These studies have found that exercise decreases endogenous oestrogens, adiposity, leptin and markers of insulin resistance and inflammation.10–12

Systemic oxidative stress has been implicated in many diseases and disorders, including the pathogenesis and progression of breast cancer.13–15 Oxidative stress refers to the excessive production of reactive oxygen species (ROS), particularly from oxygen radicals, during a period of increased exposure to environmental stress. ROS are a normal by-product of metabolism and are necessary components of both cell signalling and homoeostasis;16 however, their presence

Summary box

- This is the first large-scale randomised controlled trial of 320 postmenopausal women to examine the effect of a year-long aerobic exercise intervention on markers of oxidative damage and antioxidant enzyme activity.
- Overall, we did not observe any effect of physical activity on 8-hydroxy-2′-deoxyguanosine (8-OHdG), 8-isoprostaglandin F2α (8-Iso-PGF2α), superoxide dismutase or catalase in this study.
- Oxidative stress may not be a predominant mechanism by which physical activity decreases breast cancer risk.


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in excess amounts can have a negative physiological effect. ROS can induce damage to lipids, proteins and DNA, in turn leading to genetic mutations and genomic instability, thus contributing to carcinogenesis. In addition, ROS have been shown to be involved in the signalling pathways of neoangiogenesis, a mechanism that aids tumour growth and metastasis development.

The balance of oxidative stress factors is mainly determined by endogenous enzymatic mechanisms, although exogenous lifestyle factors such as dietary intake, physical activity and medication use also play a major role. A single bout of intense exercise causes a transient increase in ROS. However, as part of a favourable biological adaptive response referred to as the exercise-induced oxidative stress paradox, exercise training enhances antioxidant and oxidative damage repair enzyme capacity and subsequently reduces overall oxidative damage. Enzymatic antioxidants may decrease breast cancer development by neutralising the over-generated ROS. Hence, antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) are established markers for assessing the beneficial effect of physical training on antioxidant status. It is also hypothesised that oxidative damage markers can be affected by physical exercise training through the enhanced activity of DNA damage repair mechanisms. These include: 8-hydroxy-2'-deoxyguanosine (8-OHdG), an accurate marker of DNA oxidation that is associated with breast cancer risk, and 8-isoprostaglandin F2α (8-iso-PGF2α), a lipid peroxidation product that is a reliable marker of in vivo oxidative stress and is also related to breast cancer risk.

Until now, very few studies conducted in humans have examined the effect of long-term exercise on markers of oxidative stress. Of those studies, most did not focus on cancer-free, previously inactive, postmenopausal women who are of greatest relevance to postmenopausal breast cancer risk. One randomised controlled trial did examine the effects of a year-long exercise intervention on oxidative stress in postmenopausal women, but investigated only one biomarker via urine, F2-isoprostane. To the best of our knowledge, no other randomised controlled trials conducted in humans have studied the effects of long-term exercise on accurate and reliable markers of DNA oxidation, lipid peroxidation and antioxidant enzyme levels. The Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial was a randomised controlled trial that examined how a 1 year aerobic exercise intervention influenced hypothesised biomarkers that can modulate risk of postmenopausal breast cancer compared to a sedentary lifestyle. Briefly, women were recruited from May 2003 to June 2005 through targeted mailings to participants in the Alberta Breast Screening Programme, brochures distributed to family physicians and not currently undertaking or planning to undertake a weight loss programme, through media campaigns in Calgary and Edmonton, Alberta. Specific eligibility criteria included: inactive women (<90 min/week recreational activity over the past year or, if between 90 and 120 min/week of physical activity had a VO2max <34.5 mL/kg/min); aged 50–74 years; postmenopausal for at least 24 months; no previous cancer diagnosis besides non-melanoma skin cancer; no major comorbidities; acceptable heart and lung function as assessed by baseline fitness test; physician clearance for unrestricted physical activity; normal fasting lipids, glucose thyroid stimulating hormone and alanine aminotransferase; body mass index (BMI) 22–40 kg/m²; non-smoker; <14 drinks of alcohol/week; without diabetes; breast tissue density greater than a zero density level; no medications or exogenous hormones that might influence oestrogen metabolism; not currently or planning to undertake a weight loss programme and no planned, extended absences in the 18 months subsequent to enrolment.

**METHODS**

**Study population**

Methods for the ALPHA Trial have been previously reported. Briefly, women were recruited from May 2003 to June 2005 through targeted mailings to participants in the Alberta Breast Screening Programme, brochures distributed to family physicians and not currently undertaking or planning to undertake a weight loss programme, through media campaigns in Calgary and Edmonton, Alberta. Specific eligibility criteria included: inactive women (<90 min/week recreational activity over the past year or, if between 90 and 120 min/week of physical activity had a VO2max <34.5 mL/kg/min); aged 50–74 years; postmenopausal for at least 24 months; no previous cancer diagnosis besides non-melanoma skin cancer; no major comorbidities; acceptable heart and lung function as assessed by baseline fitness test; physician clearance for unrestricted physical activity; normal fasting lipids, glucose thyroid stimulating hormone and alanine aminotransferase; body mass index (BMI) 22–40 kg/m²; non-smoker; <14 drinks of alcohol/week; without diabetes; breast tissue density greater than a zero density level; no medications or exogenous hormones that might influence oestrogen metabolism; not currently or planning to undertake a weight loss programme and no planned, extended absences in the 18 months subsequent to enrolment.

**Intervention**

Women were randomised to either a year-long, moderate-to-vigorous intensity aerobic exercise intervention of 225 min/week (n=160) or to a control group that was asked to maintain their current activity levels (n=160). The final exercise prescription was 45 min long sessions, five times per week at 70–80% heart rate reserve for 1 year. Of these sessions, at least three were facility-based with on-site exercise trainers while the remaining sessions were home based. The initial exercise prescription of three weekly sessions of 15–20 min duration at an intensity of 50–60% heart rate reserve was gradually increased over a period of 3 months to the final prescription. To monitor heart rate, participants wore Polar A3 heart rate monitors throughout the exercise period. Participants in both groups were also asked not to alter their diet for the duration of the study. Ethics approval was obtained from ethics review boards of the University of Calgary, the University of Alberta and the Alberta Cancer Board and each participant provided written informed consent.

**Blood collection and biomarker assays**

Fasting blood was collected at baseline (60 mL), 6 and 12 months (40 mL) after a minimum 10 hours fast. Participants were instructed not to exercise or consume alcohol within 24 hours of the test. All blood samples were collected, processed and stored within 4 hours of collection at −86°C at the Holy Cross Centre in Calgary until the time of the assays. The laboratory assays
described below were carried out at the University of Calgary with the oversight of VP (Lyon). All three samples for each participant were included in a single batch with equal numbers of samples from exercisers and controls and two pooled quality control samples. Samples were randomly allocated within each plate. Laboratory personnel were blinded to the samples. All samples were assayed in duplicate. For any sample, if the within-individual CV between repeats was above 20%, we used the value that was closest to the mean concentration for the plate. Values above four SDs from the mean of each plate were defined as outliers and removed. Values below the limit of detection (ie, below the optical density of the negative control) were removed from analyses for each assay. In order to adjust for potential plate effects, concentration values were adjusted for the overall within-plate mean concentrations.

Plasma 8-OHdG and 8-iso-PGF2α were measured using ELISA kits (Cell Biolabs, San Diego, California, USA). Detection limits, intra-assay and interassay coefficients of variation (CV) were 0.1 ng/mL, 5% and 5%, respectively, for 8-OHdG and 0.1 ng/mL and 6% and 6%, respectively, for 8-iso-PGF2α. The quantity of 8-OHdG and 8-iso-PGF2α was determined by comparing its absorbance at 450 nm in a sample with those of a standard curve using an M2e SpectraMax microplate reader.

Plasma CAT and SOD activity were measured spectrophotometrically using standard quantification methods. Catalase activity was quantified by the method of Johansson and Borg using hydrogen peroxide as a substrate and formaldehyde as a standard. SOD activity was quantified using the Beauchamp and Fridovich method, slightly modified by Oberley and Spitz. The intra-assay and interassay CVs were 5% and 12%, respectively, for catalase and 6% and 7%, respectively, for SOD.

Statistical methods
All biomarker levels were subjected to log-transformation to obtain an approximately symmetrical distribution after observing data to be askew. The primary ITT analysis included all values that were available at each study interval. The per-protocol analysis included all control group participants and only those in the exercise group who completed ≥90% of the exercise prescription (≥204 min/week) for weeks 13–52 of the exercise intervention (excluding the 12-week ramp-up period). A further analysis was performed based on exercise adherence, using mean minutes of activity per week for all 52 weeks of the study. Exercise adherence was classified into the following categories based on previously defined public health guidelines: <150, 150–225 and >225 min/week.

General linear mixed models were used for all biomarkers to observe the intervention effects using the measures at 6 and 12 months as repeated measurers. These models included the main effects of intervention and time, as well as their interaction term, and were adjusted for baseline values of each biomarker. Treatment effect ratios (TERs) were estimated as a geometric mean ratio of the exercisers compared to the controls from the linear mixed models. A TER > 1.0 indicates higher oxidative stress biomarker or antioxidant enzyme activity levels among exercisers compared to controls.

The secondary analysis examined if the effect of exercise on the biomarkers of interest was mediated by exercise adherence, changes in body composition (assessed by BMI, body fat percentage and intra-abdominal fat area), overall physical fitness throughout the study, (assessed by VO2max) and diet or vitamin intake. Effect modification (moderation) was evaluated based on statistical significance of the interaction term (Pinteraction) between the intervention group assignment and each proposed moderator baseline at the ITT analysis models. Hypothesised moderators included baseline levels of: fitness (VO2max), age, BMI, body fat percentage, intra-abdominal fat area and oxidative stress biomarkers or antioxidant enzyme activity. All statistical analyses completed for this study were performed with the use of SAS software (V.9.2; SAS Institute, Cary, North Carolina, USA).

RESULTS
Of the 320 women initially randomised, 311 (97%) completed the trial. Baseline characteristics of the study population have previously been described. At baseline, there were limited differences between the exercise and control groups with respect to baseline concentrations of the biomarkers of interest (table 1).

In the ITT analysis of the data (table 2), we did not observe any statistically significant differences in levels for the oxidative stress biomarkers or antioxidant enzyme activities between the two groups. Similarly, in the per-protocol analysis (table 3), no biomarkers showed a statistically significant difference between groups following the intervention.

In the exercise adherence analysis (table 4), no differences were observed in these biomarker levels between any levels of exercise adherence compared to the control group. Finally, we did not observe any statistically significant mediation or effect modification by fitness (VO2max), age, BMI, body fat percentage, intra-abdominal fat area and oxidative stress biomarkers or antioxidant enzyme activity.

DISCUSSION
Our year-long moderate-to-vigorous intensity exercise intervention among previously inactive, postmenopausal, cancer-free women did not cause any statistically significant effects compared to the controls for biomarkers of oxidative stress or antioxidant enzyme activity.

Oxidative stress is one of the more recently hypothesised mechanisms whereby physical activity can influence the risk of breast cancer and other chronic diseases. While there have been a limited number of studies in this area of research, the findings of this trial differ from
non-statistically significant, duration and scope. While this study showed a trial was comparable to the ALPHA Trial in terms of studies showed consistency across biomarkers, PAusal women and none considered all of the above markers simultaneously. Of these studies, most have shown a beneficial impact of physical activity in reducing level of oxidative stress or increasing antioxidant enzyme activity and decreased oxidative stress biomarkers in what some studies have shown no effect of exercise on oxidative stress paradox. Exercise has generally been found to increase antioxidant enzyme capacity while decreasing lipid peroxidation and DNA damage markers in healthy populations of men and women. Very few randomised controlled studies have examined these markers of oxidative stress in inactive, postmenopausal women and none considered all of the above markers.56 Only one previous research. Exercise has generally been found to increase antioxidant enzyme capacity while decreasing lipid peroxidation and DNA damage markers in healthy populations of men and women.39 However, not all studies showed consistency across biomarkers.40 42 45 46 52

Table 2

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Intention-to-treat analysis of circulating oxidative stress biomarker levels for exercisers and controls in the ALPHA Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline Geometric mean (95% CI)*</td>
</tr>
<tr>
<td>SOD (μmol/L/min)</td>
<td>Exercisers 12.4 (12.1 to 12.8) 155</td>
</tr>
<tr>
<td></td>
<td>Controls 12.2 (11.9 to 12.6) 159</td>
</tr>
<tr>
<td>CAT (μmol/L/min)</td>
<td>Exercisers 20.6 (18.8 to 22.6) 156</td>
</tr>
<tr>
<td></td>
<td>Controls 19.1 (17.5 to 20.9) 159</td>
</tr>
<tr>
<td>8-iso-PGF2α (ng/L)</td>
<td>Exercisers 228 (213 to 245) 149</td>
</tr>
<tr>
<td></td>
<td>Controls 230 (216 to 245) 154</td>
</tr>
<tr>
<td>8-OHdG (ng/L)</td>
<td>Exercisers 7.1 (6.8 to 7.3) 156</td>
</tr>
<tr>
<td></td>
<td>Controls 7.1 (6.8 to 7.4) 156</td>
</tr>
</tbody>
</table>

*Blood samples from n=159 participants in the control group and n=156 participants in the exercise group were obtained. A further n=1, 12 and 3 participants were removed from the SOD, 8-iso-PGF2α and 8-OHdG analyses, respectively, as they were outliers or below the limit of detection.
†The TER was calculated based on a linear mixed model for each biomarker, adjusted for time and baseline value of the biomarker. The TER represents the adjusted ratio of geometric means for the exercise group over the control group. A TER of <1.0 indicates lower oxidative stress biomarker or antioxidant activity levels in the exercise group relative to the control group at 6 and 12 months; a TER >1.0 indicates higher oxidative stress biomarker or antioxidant activity levels in the exercise group; and a ratio of 1.0 indicates no difference between groups.

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http://bmjopensem.bmj.com/
Table 3  Per-protocol* analysis of circulating oxidative stress biomarker levels for exercisers and controls in the ALPHA Trial

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Six months</th>
<th>Twelve months</th>
<th>Per cent change from baseline to 12 months</th>
<th>TER of exercise/control (95% CI)‡</th>
<th>Between-group p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric mean</td>
<td>Geometric mean</td>
<td>Geometric mean</td>
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<tr>
<td></td>
<td>(95% CI)†</td>
<td>(95% CI)†</td>
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<tr>
<td>SOD (μmol/L/min)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>12.3 (11.8 to 12.7)</td>
<td>12.3 (11.7 to 12.9)</td>
<td>12.2 (11.8 to 12.6)</td>
<td>–0.3</td>
<td>0.98 (0.94 to 1.02)</td>
<td>0.30</td>
</tr>
<tr>
<td>Controls</td>
<td>12.2 (11.9 to 12.6)</td>
<td>12.4 (12.0 to 12.7)</td>
<td>12.6 (12.2 to 13.0)</td>
<td>2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAT (μmol/L/min)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Exercisers</td>
<td>21.5 (19.2 to 24.0)</td>
<td>21.3 (18.8 to 24.2)</td>
<td>21.6 (19.1 to 24.4)</td>
<td>1.4</td>
<td>1.00 (0.92 to 1.09)</td>
<td>0.99</td>
</tr>
<tr>
<td>Controls</td>
<td>19.1 (17.5 to 20.9)</td>
<td>19.5 (17.8 to 21.3)</td>
<td>19.5 (17.8 to 21.5)</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-iso-PGF2α (ng/L)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Exercisers</td>
<td>220 (199 to 244)</td>
<td>224 (207 to 242)</td>
<td>212 (188 to 240)</td>
<td>–3.7</td>
<td>0.98 (0.90 to 1.08)</td>
<td>0.89</td>
</tr>
<tr>
<td>Controls</td>
<td>230 (216 to 245)</td>
<td>218 (202 to 237)</td>
<td>223 (209 to 239)</td>
<td>–3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-OHdG (ng/L)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>7.2 (6.9 to 7.5)</td>
<td>7.2 (6.9 to 7.5)</td>
<td>7.3 (7.1 to 7.6)</td>
<td>1.6</td>
<td>0.99 (0.95 to 1.02)</td>
<td>0.44</td>
</tr>
<tr>
<td>Controls</td>
<td>7.1 (6.8 to 7.4)</td>
<td>7.2 (6.9 to 7.5)</td>
<td>7.2 (7.0 to 7.5)</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Per-protocol analysis included all participants in the control group and only those participants who fulfilled ≥90% of the exercise prescription over weeks 13–52 of the study.
†Blood samples from n=159 participants in the control group and n=156 participants in the exercise group were obtained. A further n=1, 12 and 3 participants were removed from the SOD, 8-iso-PGF2α and 8-OHdG analyses, respectively, as they were outliers or below the limit of detection.
‡The TER was calculated based on a linear mixed model for each biomarker, adjusted for time and baseline values of the biomarker. The TER represents the adjusted ratio of geometric means for the exercise group over the control group. A TER of <1.0 indicates lower oxidative stress biomarker or antioxidant activity levels in the exercise group relative to the control group at 6 and 12 months; a TER >1.0 indicates higher oxidative stress biomarker or antioxidant activity levels in the exercise group; and a ratio of 1.0 indicates no difference between groups.
8-iso-PGF2α, 8-isoprostaglandin F2α; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; CAT, catalase; SOD, superoxide dismutase; TER, treatment effect ratio.
| Table 4 | Adherence level* analyses of oxidative stress biomarker levels at baseline and 12 months in exercisers and controls in the ALPHA Trial |
|-----------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | Baseline | Twelve months | Ratio 12 months/baseline | Per cent change§ | p Value¶ |
| | Geometric mean (95% CI)† | Geometric mean (95% CI)‡ | n | (95% CI)‡ | |
| | SOD (μmol/L/min) | | | | |
| Controls | 12.2 (11.9 to 12.6) | 12.6 (12.2 to 13.0) | 155 | 1.02 (1.00 to 1.05) | 2.5 |
| <150 min/week | 12.7 (12.1 to 13.3) | 12.1 (11.4 to 12.9) | 40 | 0.98 (0.92 to 1.03) | −2.5 |
| 150–225 min/week | 12.5 (11.9 to 13.0) | 12.2 (11.8 to 12.7) | 66 | 0.99 (0.95 to 1.03) | −1.1 |
| >225 min/week | 12.0 (11.3 to 12.6) | 12.3 (11.7 to 12.8) | 47 | 1.01 (0.96 to 1.06) | 0.6 |
| CAT (μmol/L/min) | | | | | |
| Controls | 18.9 (17.4 to 20.7) | 19.5 (17.8 to 21.4) | 156 | 1.02 (0.96 to 1.09) | 2.1 |
| <150 min/week | 21.2 (17.4 to 25.9) | 23.3 (19.2 to 28.5) | 40 | 1.12 (0.99 to 1.28) | 12.4 |
| 150–225 min/week | 19.4 (16.8 to 22.5) | 19.4 (16.7 to 22.6) | 67 | 0.99 (0.90 to 1.10) | −0.8 |
| >225 min/week | 22.0 (19.1 to 25.3) | 22.5 (19.1 to 26.5) | 47 | 1.05 (0.93 to 1.18) | 4.5 |
| 8-OHdG (ng/L) | | | | | |
| Controls | 7.1 (6.8 to 7.3) | 7.2 (7.0 to 7.5) | 153 | 1.03 (1.00 to 1.05) | 2.8 |
| <150 min/week | 6.7 (6.2 to 7.1) | 7.0 (6.4 to 7.5) | 40 | 1.03 (0.98 to 1.08) | −2.5 |
| 150–225 min/week | 7.2 (6.9 to 7.5) | 7.4 (7.0 to 7.7) | 67 | 1.03 (0.99 to 1.07) | −1.1 |
| >225 min/week | 7.2 (6.8 to 7.7) | 7.3 (7.0 to 7.7) | 47 | 1.02 (0.97 to 1.07) | 0.6 |
| 8-iso-PGF2α (ng/L) | | | | | |
| Controls | 234 (220 to 250) | 223 (209 to 239) | 143 | 0.97 (0.89 to 1.05) | −3.2 |
| <150 min/week | 240 (216 to 267) | 197 (156 to 249) | 38 | 0.85 (0.71 to 1.00) | −15.2 |
| 150–225 min/week | 226 (201 to 254) | 203 (175 to 234) | 63 | 0.90 (0.80 to 1.02) | −9.7 |
| >225 min/week | 223 (190 to 261) | 230 (197 to 269) | 42 | 0.99 (0.85 to 1.15) | −1.5 |

*Adherence was calculated as the mean minutes of exercise per week for the exercise group over the 52 weeks of the study.
†Blood samples from n=159 participants in the control group and n=156 participants in the exercise group were obtained. A further n=1, 12 and 3 participants were removed from the SOD, 8-iso-PGF2α and 8-OHdG analyses, respectively, as they were outliers or below the limit of detection.
‡Ratio of geometric means at 12 months to geometric means at baseline, adjusted for the baseline oxidative stress biomarker or antioxidant activity level and age.
§Percentage change in adherence group mean of each oxidative stress biomarker or antioxidant activity level at 12 months...activity level, where quintiles are numbered 1–5 and these values are treated as a continuous variable.
¶p Value for trend represents a test of linear association between the adherence quintile and change in oxidative stress biomarker or antioxidant activity level between the control group and the specified adherence group, adjusted for the baseline value of the oxidative stress biomarker or antioxidant activity level and age. A unified model, where the adherence group was treated as a categorical variable, was used to calculate the p values, which correspond to β coefficients for the other quintile groups, using the control group as the referent group.
**p Value for trend was calculated as the mean minutes of exercise per week for the exercise group over the 52 weeks of the study.

8-iso-PGF2α, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; CAT, catalase; SOD, superoxide dismutase.
increase; however, over time, this increase produces a compensation mechanism, by which adaptation occurs to higher levels of oxidative stress by upregulating the amount and efficiency of antioxidant enzymes. Increased ROS species have been attributed to a multitude of different cancers and other chronic diseases.\textsuperscript{13–15} Additionally, oxidative stress increases with increasing age.\textsuperscript{60} and there is evidence that postmenopausal women have higher levels of lipid peroxidation compared to premenopausal women.\textsuperscript{51,52} Thus, their reduction due to physical activity could serve as a plausible mechanism of cancer prevention, although this effect was not observed in our trial.

The study population of the ALPHA Trial consisted of healthy, postmenopausal women. Healthier individuals may have lower levels of oxidative stress; hence, an exercise intervention may not have had a significant impact in reducing ROS or increasing antioxidant enzyme activity. This hypothesis is supported by the most comparable study of a year-long exercise intervention in postmenopausal women that showed only a modest improvement compared to those in erythrocytes,\textsuperscript{61} 62 This is consistent with the hypothesis that antioxidant enzyme capacities and DNA damage biomarkers in plasma may not be responsive to aerobic exercise training as those in erythrocytes,\textsuperscript{59} 61 62 63 Furthermore, plasma levels of antioxidant activity may not correlate with tissue levels. Similarly, in terms of oxidative stress biomarkers, urinary measures of F2-isoprostanes have been shown to have a differential effect depending on whether enzyme activities were measured in plasma or in erythrocytes.\textsuperscript{46} Studies using plasma measures of activity have shown inconsistent results,\textsuperscript{45} 46 49, 56 with one study demonstrating a differential effect depending on whether enzyme activities were measured in plasma or in erythrocytes.\textsuperscript{46} Furthermore, plasma levels of antioxidant activity may not correlate with tissue levels. Similarly, in terms of oxidative stress biomarkers, urinary measures of F2-isoprostanes have been shown to display daily variability.\textsuperscript{63} In addition, concentrations of F2-isoprostanes in urine are 20–50 times lower than in plasma and depend on creatinine concentration, both suggesting that urine may be more sensitive to smaller changes.\textsuperscript{64} It is possible that antioxidant enzyme capacities and DNA damage biomarkers in plasma may not be as responsive to aerobic exercise training as those in erythrocytes, whole blood or urine.

Since the ALPHA Trial was a year-long intervention of 225 min/week of aerobic exercise, this time frame may be too long to observe an effect of the exercise intervention. Most trials that have shown increases in antioxidant enzyme capacity with exercise were much shorter in duration (8–24 weeks)\textsuperscript{45} 47 49 51 56 59 and we hypothesise that without an increase in aerobic exercise volume or intensity, the exercise effect on oxidative stress biomarkers and antioxidant enzyme activity could have an adaptive effect over time. It is possible that an increase in antioxidant activity as a result of the intervention would have occurred within the first few weeks of the aerobic exercise training. Since oxidative stress is a balance between ROS and the antioxidant system,\textsuperscript{65} we hypothesise that this early increase in antioxidant enzyme activity could be matched by a decrease in ROS generation in response to the exercise intervention such that, over time, the initial increase in antioxidant enzyme activity would begin to diminish and return to basal levels because of the decreased stimulus. Indeed, studies of prolonged aerobic exercise training do not always show an improvement in total antioxidant capacity.\textsuperscript{66–68} The lack of effect on antioxidant enzyme capacity due to decreased ROS is most likely complemented by the decreased adiposity and C reactive protein (CRP) levels seen in the exercise group of the ALPHA Trial.\textsuperscript{11} 69 Adipose tissue has been shown to be a potent source of ROS,\textsuperscript{70} 71 while increased CRP has been correlated with increased ROS.\textsuperscript{72} Overall, this study showed no effect of a year-long exercise intervention on these four markers of oxidative stress in healthy, postmenopausal women. While we did not find any statistically significant effects of aerobic exercise training on oxidative stress biomarkers in this study, based on the current literature and biological models, oxidative stress remains a plausible mechanism by which physical activity could influence breast cancer risk and further research is warranted.

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