Effect of Cardiorespiratory Fitness on Vascular Regulation and Oxidative Stress in Postmenopausal Women

Vincent Pialoux, Allison D. Brown, Richard Leigh, Christine M. Friedenreich, Marc J. Poulin

Abstract—Increasing evidence exists suggesting an important role for oxidative stress in the pathogenesis of hypertensive models in animals.1,5 This hypothesis is supported by mechanistic evidence whereby superoxide reacts with NO synthesized by endothelial NO synthase to form the oxidant peroxynitrite, thereby inducing NO inactivation and an uncoupling of endothelial NO synthase.2 In this context, oxidative stress, defined as the imbalance in favor of ROS generation over antioxidant defense, is usually higher in the hypertensive population, as shown by accumulation of ROS end products from oxidized lipids and DNA in patients with essential hypertension.3 Moreover, studies on spontaneously hypertensive rats suggest that oxidative stress involves enhanced NADPH oxidase activity, leading to ROS overgeneration and dysfunctional endothelial NO synthase.1,4 Conversely, inhibition of ROS generation or treatment with radical scavengers prevents development of arterial hypertension in most hypertensive models in animals.1,5

Although the beneficial effect of hormone therapy on cardiovascular risk after menopause is still controversial,6,7 the increased incidence of cardiovascular disease in postmenopausal women compared with premenopausal women8 may suggest that estrogen may have a protective effect on the cardiovascular system. Oxidative stress is reported to increase after menopause,9 suggesting that the decrease in sex hormones occurring at the time of menopause could predispose women to higher levels of ROS. Estradiol is thought to have antioxidant properties, because it increases the expression of antioxidant enzymes and decreases NADPH oxidase enzyme activity and superoxide production. Some studies have suggested that the increase in oxidative stress in postmenopausal women could underlie, at least in part, the mechanism whereby postmenopausal women are at increased risk of cardiovascular disease.8 Indeed, it has...
been shown recently that reduced carotid artery compliance of sedentary postmenopausal women was partially restored by infusion of ascorbic acid.\textsuperscript{10} Interestingly, it also appears that this compliance is not different between exercise-trained postmenopausal and premenopausal women.\textsuperscript{11} Taken together, these data suggest that regular exercise might suppress the reduction of arterial compliance via a decrease in oxidative stress. In this context, it has been demonstrated clearly that, in a premenopausal population, regular physical training\textsuperscript{12,13} upregulates antioxidant enzymatic systems, which may have implications for attenuating the usual increase in postmenopausal-related oxidative stress. This paradigm is supported by 2 recent studies\textsuperscript{14,15} that reported a decrease in plasma thiobarbituric acid reactive substances after 8 and 24 weeks of exercise training in postmenopausal women. However, a recent study reported no significant difference in lipid peroxidation between premenopausal and postmenopausal women randomly matched for body mass index (BMI) and waist circumference.\textsuperscript{16} The correlation reported between oxidative stress and body fat\textsuperscript{17} might suggest that the increase in fat mass occurring at menopause\textsuperscript{18} may partially explain the elevated levels of oxidative stress markers after menopause.

Finally, we demonstrated recently that cardiorespiratory fitness and cerebral blood flow significantly impact cognitive outcomes in older women.\textsuperscript{19} In addition, recent findings demonstrating that exercise restored endothelium-dependent dilation via improvement of antioxidant enzyme efficiency and NO bioavailability in old mice.\textsuperscript{20} Here, we tested the hypothesis that increases in antioxidant enzyme efficiency and decreased oxidative stress, leading to NO bioavailability, might be a potential mechanism to account for this observed association between fitness and cardiovascular parameters. In the current study, we investigated the impact of fitness status on enzymatic antioxidant efficiency, oxidative stress, and NO production in the same study population of postmenopausal women who had participated in our original study.\textsuperscript{19} Secondary objectives were to determine the strength of the associations among oxidative stress, enzymatic antioxidant and NO production, and arterial blood pressure and cerebrovascular regulation.

**Methods**

**Protocol**

The study design and participant sample have been described previously.\textsuperscript{19} In brief, a cross-sectional study was conducted of 42 white postmenopausal women aged 50 to 90 years identified in the community through advertisements at the University of Calgary, Calgary Health Region, and in the general community. The study protocol was approved by the cohort health research ethics board at the University of Calgary. Additional details are included in an online Data Supplement (please see http://hyper.ahajournals.org).

The subject's menopausal status was determined by a self-report of absence of menses for ≥12 months and by the assessment of ovarian hormones levels. Additional details are included in the online Data Supplement.

Subjects refrained from eating or drinking any caffeine-containing beverages for ≥4 hours before each of the testing sessions. The first visit was a screening session and involved collecting participant demographic data and anthropometric measurements, completion of a lifestyle questionnaire adapted from the Past Year Total Physical Activity Questionnaire\textsuperscript{21,22} (from which a level of physical activity in the past 12 months was estimated as the metabolic equivalent hours per week of activity), spirometry testing, and a carotid artery ultrasound screening. The second visit involved a graded exercise test on a modified recumbent cycle ergometer to determine maximal oxygen consumption (\(\dot{V_O}_2\)max), as described by Brown et al.\textsuperscript{19} During the third session, blood testing was performed. Resting blood pressure and transcranial Doppler ultrasound recordings from the right middle cerebral artery were made while subjects sat quietly. Systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure (MABP) were determined as the mean of 3 values using an automated blood pressure monitoring device (Dinamap; Johnson and Johnson Medical, Inc). MABP recorded was also continuously recorded at the finger by photoplethysmography (Portapress, TPD Biomedical Instrumentation).

Measurements of middle cerebral artery peak velocity, as an index of cerebral blood flow, were made at rest using transcranial Doppler ultrasound.\textsuperscript{23,24} Additional details are included in an online Data Supplement.

**Biochemical Analyses**

Blood was collected from the antecubital vein in two 7-ml EDTA tubes for biochemical analysis at the beginning of the third session. The plasma was obtained by centrifugation of the samples at 1000g for 10 minutes at 4°C. Plasma was separated into aliquots and frozen at −80°C until assays could be performed.

Plasma levels of oxidative stress (ie, 8-hydroxy-2′-deoxyguanosine [8-OHdG], malondialdehyde [MDA], and 8-isoprostaglandin F2α [8-iso-PGF2α]), antioxidant enzymatic activities (ie, plasma glutathione peroxidase [GPX] and catalase activities), end product of NO (nitrites and nitrates), and ovarian hormone levels were measured at rest. Additional details are included in an online Data Supplement.

**Statistical Analysis**

To examine the effects of fitness on our primary outcome variables, we divided the study population into 2 groups, on the basis of aged-predicted \(\dot{V}_O_2\)max values.\textsuperscript{25,26} Participants assigned to the fit group had a \(\dot{V}_O_2\)max >100% of age-predicted values, whereas participants assigned to the sedentary group had a \(\dot{V}_O_2\)max <100% of age-predicted values.

Independent sample \(t\) tests were used to compare the fit and sedentary groups. Pearson correlation coefficients between vascular measures and biochemical markers (oxidative stress and enzymatic antioxidant markers and end product of NO) were estimated. Regression analyses were also controlled for BMI and fat mass using partial correlations. Because of the exploratory nature of the study, we did not perform multiple linear regressions. Statistical analyses were performed with SPSS (version 15.0, SPSS, Inc). Differences were considered significant at \(P<0.05\).

**Results**

Of the 42 women who were tested, 2 subjects were excluded from the statistical analyses; 1 woman did not complete the \(\dot{V}_O_2\)max test, and a blood sample was not collected on another woman. Thus, of the 42 subjects tested, complete data sets were available on 40 women (19 fit and 21 sedentary). However, CVC data were calculated on only 39 subjects because of the failure to locate a suitable transcranial Doppler signal in 1 woman.

Anthropometric, fitness, and physical activity data of our cohort are presented in Table 1. The study population included older women who were, on average, moderately active and fit, normal weight for height, and with a mean level of education equivalent to a university undergraduate diploma. A difference between the fit and sedentary women was found, particularly with respect to education, BMI, and physical fitness.
Vascular Outcomes
MABP was higher in the sedentary group compared with the fit group (77.3 ± 12.6 versus 71.6 ± 13.3 mm Hg; *P* < 0.05). The increase in MABP was attributable mainly to a higher systolic blood pressure (113.0 ± 20.7 versus 103.3 ± 14.2 mm Hg; *P* = 0.045) in the sedentary group, although diastolic blood pressure was also somewhat higher in this group (57.0 ± 11.6 versus 52.0 ± 11.3 mm Hg; *P* = 0.08) as compared with the active group. CVC was higher in the fit group compared with the sedentary group (1.15 ± 0.21 versus 1.01 ± 0.17 cm.s⁻¹.mm Hg⁻¹; *P* = 0.04).

Ovarian Hormones
Group mean values of serum estradiol and progesterone are included in Table 1. Estradiol and progesterone were not significantly correlated with any antioxidant variables.

Biochemical Outcomes
End product of NO (NOx) was not statistically significantly different in the fit group (40.2 ± 21.5 mmol.L⁻¹) compared with the sedentary group (33.7 ± 17.3 mmol.L⁻¹; Table 2). Oxidative stress was evaluated by analyzing blood oxidation markers (Table 2). The sedentary group exhibited values that were 270% and 93% higher than the fit group for 8-iso-PGF2α (*P* = 0.001) and 8-OHdG (*P* = 0.02), respectively. The antioxidant status response was evaluated by analyzing blood antioxidant enzymatic activities (Table 2). GPX and catalase activities were 26% (*P* < 0.0001) and 36% (*P* = 0.05) higher, respectively, in the fit group (Table 2).

Physical Activity and Physical Fitness
Physical activity was positively correlated with absolute VO₂max (*r* = 0.43; *P* = 0.006) and VO₂max expressed as a percentage of age-predicted values (*r* = 0.48; *P* = 0.003). Correlation analyses between oxidative stress markers and fitness parameters (with and without controlling for BMI and fat mass) are presented in Table 3. The correlations between VO₂max (normalized for body weight) and oxidative stress markers are presented in an online Data Supplement (Table S1 and Figure S1, available at http://hyper.ahajournals.org).

Starting with markers of oxidative stress, 8-OHdG, 8-iso-PGF2α, and MDA were negatively correlated with VO₂max expressed as a percentage of age-predicted values and with total physical activity over the past year (Figure 1 and Table 3). The correlation with 8-OHdG and 8-iso-PGF2α remained statistically significant, even after controlling for BMI and fat mass.

Turning to markers of antioxidant enzyme activity, GPX was positively correlated with VO₂max expressed as a percentage of age-predicted values (Table 3). The correlations between VO₂max (normalized for body weight) and antioxidant enzyme activities are presented in an online Data Supplement (Table S1 and Figure S2).

Looking at the relationships between oxidative stress and antioxidant enzyme activity, GPX was negatively correlated with 8-OHdG (*r* = −0.39; *P* = 0.011) and with 8-iso-PGF2α

### Table 1. Descriptive Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Overall (n = 40)</th>
<th>Fit (n = 19)</th>
<th>Sedentary (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65.1 ± 8.4</td>
<td>67.8 ± 8.1</td>
<td>62.1 ± 7.8</td>
</tr>
<tr>
<td>Height, cm</td>
<td>161.3 ± 6.0</td>
<td>160.2 ± 4.9</td>
<td>161.9 ± 6.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>66.7 ± 10.0</td>
<td>61.9 ± 7.2</td>
<td>71.1 ± 10.5*</td>
</tr>
<tr>
<td>BMI, kg/m⁻²</td>
<td>25.6 ± 3.7</td>
<td>24.2 ± 2.7</td>
<td>28.2 ± 3.9*</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>36.5 ± 5.3</td>
<td>34.7 ± 5.3</td>
<td>38.4 ± 4.9*</td>
</tr>
<tr>
<td>V0₂ max relative, mL · kg⁻¹ · min⁻¹</td>
<td>25.6 ± 5.6</td>
<td>28.9 ± 4.8</td>
<td>22.3 ± 4.3*</td>
</tr>
<tr>
<td>V0₂ max absolute, L · min⁻¹</td>
<td>1.67 ± 0.3</td>
<td>1.79 ± 0.3</td>
<td>1.55 ± 0.2*</td>
</tr>
<tr>
<td>V0₂ max percentage predicted, %</td>
<td>101.2 ± 22.6</td>
<td>119.6 ± 12.8</td>
<td>82.9 ± 13.5*</td>
</tr>
<tr>
<td>Physical activity, MET·h · wk⁻¹ · y⁻¹</td>
<td>81.2 ± 47.6</td>
<td>98.3 ± 48.3</td>
<td>48.5 ± 20.5*</td>
</tr>
<tr>
<td>Serum estradiol, pmol · L⁻¹</td>
<td>25.7 ± 37.4</td>
<td>13.8 ± 35.1</td>
<td>34.8 ± 37.3</td>
</tr>
<tr>
<td>Serum progesterone, nmol · L⁻¹</td>
<td>1.13 ± 0.54</td>
<td>1.19 ± 0.47</td>
<td>1.44 ± 0.57</td>
</tr>
</tbody>
</table>

Values represent mean ± SD. Participants assigned to the fit group had a VO₂max that was >100% of age-predicted values, whereas participants assigned to the sedentary group had a VO₂max defined as <100% of age-predicted values. VO₂max indicates maximal oxygen consumption; MET, metabolic equivalent.

*Data were significantly different from fit group by *P* < 0.05.

### Table 2. Mean Values of Plasma MDA, 8-OHdG, 8-iso-PGF2α, Nitrotyrosine, Catalase, and GPX Activities and NOx

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fit (n = 19)</th>
<th>Sedentary (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OHdG, µg · L⁻¹</td>
<td>51.3 ± 54.0</td>
<td>98.9 ± 70.5†</td>
</tr>
<tr>
<td>MDA, µmol · L⁻¹</td>
<td>4.19 ± 0.87</td>
<td>4.62 ± 0.70</td>
</tr>
<tr>
<td>8-iso-PGF2α, ng · L⁻¹</td>
<td>3.62 ± 3.60</td>
<td>9.78 ± 5.07†</td>
</tr>
<tr>
<td>Nitrotyrosine, nmol · L⁻¹</td>
<td>157 ± 19</td>
<td>163 ± 18</td>
</tr>
<tr>
<td>GPX, µmol · L⁻¹ · min⁻¹</td>
<td>14.1 ± 2.7</td>
<td>11.1 ± 1.5*</td>
</tr>
<tr>
<td>Catalase, µmol · L⁻¹ · min⁻¹</td>
<td>11.70 ± 0.83</td>
<td>8.6 ± 4.1*</td>
</tr>
<tr>
<td>NOx, µmol · L⁻¹</td>
<td>40.2 ± 21.5</td>
<td>33.7 ± 17.3</td>
</tr>
</tbody>
</table>

Values represent mean ± SD. Participants assigned to the fit group had a VO₂max that was >100% of age-predicted values, whereas participants assigned to the sedentary group had a VO₂max defined as <100% of age-predicted values.

*Data are significantly different from the fit group at *P* < 0.05.

†Data are significantly different from the fit group at *P* < 0.01.
of oxidative stress. The finding in this study of a positive}

It has been suggested previously that the decrease in levels of}

menopause is associated with an increase in oxidative stress

correlated with higher MABP values and lower values for

cerebrovascular resistance. We found greater oxidative stress

Correlations among oxidative stress, NO levels, and vascular

Correlations among oxidative stress, NO levels, and vascular

Associations Among Oxidative Stress, NO Level, and

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Effect of Fitness Level on Oxidative Stress

Effect of Fitness Level on Oxidative Stress

Figure 1. Relationship between predicted VO₂max and plasma

Figure 1. Relationship between predicted VO₂max and plasma

\[
\begin{array}{c|c|c|c}
\text{Variables} & \text{Pearson } r & F & P \\
\hline
8-OhDg, \mu g \cdot L^{-1} & \text{vs predicted } VO_2max, \% & -0.35 & 0.033 & 0.35 & 0.04 \\
\text{vs physical activity, MET-h \cdot wk^{-1} \cdot y^{-1}} & -0.38 & 0.015 & 0.33 & 0.05 \\
\text{MDA, } \mu \text{mol} \cdot L^{-1} & \text{vs predicted } VO_2max, \% & -0.33 & 0.03 & -0.31 & 0.06 \\
\text{vs physical activity, MET-h \cdot wk^{-1} \cdot y^{-1}} & -0.30 & 0.05 & -0.30 & 0.07 \\
8-iso-PGF2α, ng \cdot L^{-1} & \text{vs predicted } VO_2max, \% & -0.42 & 0.007 & -0.37 & 0.03 \\
\text{vs physical activity, MET-h \cdot wk^{-1} \cdot y^{-1}} & -0.52 & 0.001 & -0.35 & 0.04 \\
\text{GPX, } \mu \text{mol} \cdot L^{-1} \cdot min^{-1} & \text{vs predicted } VO_2max, \% & 0.55 & 0.001 & 0.58 & 0.001 \\
\end{array}
\]

8-OhDg indicates maximal oxygen consumption; MET, metabolic equivalent. Correlation analyses were conducted on data from all of the volunteers (n=40).

(\(r = -0.29; P = 0.05\)), whereas catalase was negatively correlated with 8-iso-PGF2α (\(r = -0.37; P = 0.01\)).

**Table 3. Correlations Among Fitness Parameters and Plasma Oxidative Stress and Antioxidant Enzyme Activity Controlled for BMI and Fat Mass**

Effect of Oxidative Stress on Blood Pressure and Cerebrovascular Function

Although oxidative stress has been reported to contribute to the increase in arterial blood pressure and the subsequent progression of hypertension, \(1, \) we are the first to report a positive association between oxidative stress and blood pressure in a normotensive population of postmeno-
pausal women. This finding corroborates previous data in humans and animals in the context of hypertension. The negative correlations between both 8-OHdG and nitrotyrosine and CVC suggest that the regulation of cerebrovascular tone may be determined, at least in part, by ROS and peroxynitrite. These findings have broad clinical implications in relation to mechanisms that regulate cerebral blood flow and imply that oxidative stress–induced changes may contribute to the progression of cerebrovascular disease, such as stroke.

Clinical trials of hypertension treatment with antioxidants have been inconclusive and failed to demonstrate significant beneficial effects of nonenzymatic antioxidants, such as vitamins C and E, or β-carotene in the management of MABP.31 The results of the present study suggest that the improvement of enzymatic antioxidant defense by means of greater physical activity may reduce MABP. On the basis of our reported correlations between MAPB and both nitrotyrosine and 8-OHdG, a 25% reduction of oxidative stress decreased MABP by 10 mm Hg. Although this result is not necessarily generalizable to the clinical management of hypertensive patients, a decrease of blood pressure by 10 mm Hg (eg, 110 to 100 mm Hg) using medication reduces the risk for a stroke event from 0.85 to 0.65.

**Table 4. Correlations Among Cardiovascular Parameters and Plasma Oxidative Stress and End Products of NO Controlled for BMI and Fat Mass**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pearson Correlation Coefficient</th>
<th>P</th>
<th>Controlled for BMI and Fat Mass</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrotyrosine, nmol · L⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs MABP, mm Hg</td>
<td>0.41</td>
<td>0.01</td>
<td>0.52</td>
<td>0.003</td>
</tr>
<tr>
<td>vs CVC, cm · s⁻¹ · mm Hg⁻¹*</td>
<td>0.32</td>
<td>0.04</td>
<td>0.33</td>
<td>0.05</td>
</tr>
<tr>
<td>vs NOx, μmol · L⁻¹</td>
<td>−0.62</td>
<td>&lt;0.001</td>
<td>−0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8-OHdG, μg · L⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs MABP, mm Hg</td>
<td>0.43</td>
<td>0.006</td>
<td>0.33</td>
<td>0.05</td>
</tr>
<tr>
<td>vs CVC, cm · s⁻¹ · mm Hg⁻¹*</td>
<td>−0.37</td>
<td>0.04</td>
<td>−0.33</td>
<td>0.05</td>
</tr>
<tr>
<td>vs NOx, μmol · L⁻¹</td>
<td>−0.25</td>
<td>NS</td>
<td>0.22</td>
<td>NS</td>
</tr>
<tr>
<td>8-iso-PGF2α, ng · L⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs MABP, mm Hg</td>
<td>0.10</td>
<td>NS</td>
<td>0.06</td>
<td>NS</td>
</tr>
<tr>
<td>vs CVC, cm · s⁻¹ · mm Hg⁻¹*</td>
<td>−0.27</td>
<td>NS</td>
<td>−0.30</td>
<td>NS</td>
</tr>
<tr>
<td>vs NOx, μmol · L⁻¹</td>
<td>0.18</td>
<td>NS</td>
<td>−0.12</td>
<td>NS</td>
</tr>
<tr>
<td>MDA, nmol · L⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs MABP, mm Hg</td>
<td>0.10</td>
<td>NS</td>
<td>0.29</td>
<td>NS</td>
</tr>
<tr>
<td>vs CVC, cm · s⁻¹ · mm Hg⁻¹*</td>
<td>−0.17</td>
<td>NS</td>
<td>−0.18</td>
<td>NS</td>
</tr>
<tr>
<td>vs NOx, μmol · L⁻¹</td>
<td>−0.05</td>
<td>NS</td>
<td>0.009</td>
<td>NS</td>
</tr>
<tr>
<td>NOx, μmol · L⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs MABP, mm Hg</td>
<td>−0.54</td>
<td>&lt;0.001</td>
<td>−0.49</td>
<td>0.005</td>
</tr>
<tr>
<td>vs CVC, cm · s⁻¹ · mm Hg⁻¹*</td>
<td>0.41</td>
<td>0.02</td>
<td>0.37</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Correlation analyses were conducted on data from all of the volunteers (n=40). NS indicates not significant.

*Analyses were conducted on n=39.

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**Figure 2.** Relationship between MABP and NOx concentration in serum in postmenopausal women (r=−0.54; P<0.001). ○ (n=21), Sedentary group (<100% age-predicted VO₂max); ● (n=19), fit group (>100% age-predicted VO₂max).

**Figure 3.** Relationship between MABP and nitrotyrosine concentration in plasma in postmenopausal women (r=0.41; P=0.01). ○ (n=21), Sedentary group (<100% age-predicted VO₂max); ● (n=19), fit group (>100% age-predicted VO₂max).
Effect of NO Production on Blood Pressure and Cerebrovascular Function

Exercise training in healthy individuals is known to elevate NO bioavailability through a variety of mechanisms, including increased endothelial NO synthase enzyme expression and activity. Moreover, in elderly women, Maeda et al. reported that the plasma concentration of NOx was significantly increased by exercise training. In this present study, despite a trend (+19%; P=0.10), NOx was not significantly different in the fit group compared with the sedentary group, most likely because of the relatively small number of subjects and wide interindividual variability.

Nevertheless, NOx was negatively correlated with MABP, systolic blood pressure, and diastolic blood pressure, thus highlighting the role of NO in blood pressure regulation in a postmenopausal population with a normal range of blood pressure values. Our results also suggest that the negative association found between plasma NO metabolites and the incidence of hypertension in the general population might also occur in hypertensive postmenopausal women. Finally, NOx was positively correlated with cerebral vascular conductance, thereby supporting the hypothesis that the lower vasodilator ability of cerebral vessels is likely attributable to reduced bioavailability of NO.

Interestingly, NOx was negatively correlated with nitrotyrosine, supporting emerging evidence for a role for improved NO bioavailability after exercise training as a result of reduced oxidative stress. However, other mechanisms activated by ROS are probably involved in endothelial dysfunction independent of the impairment of the NO pathway. By mediating the increase in intracellular calcium concentration, ROS plays a central role in the molecular cascade between angiotensin II and the final vasoconstriction of vascular smooth muscle. ROS is also known to induce atherosclerosis and subsequent endothelial dysfunction via an increase in adhesion molecules, proinflammatory cytokines, and permeability of the endothelium.

Perspectives

This research reveals that the modulation of oxidative stress by regular physical exercise mediates blood pressure and cerebral vascular conductance after menopause. This study also shows how lifestyle may complement current medical therapies in the prevention and management of cardiovascular disease. Nevertheless, this cross-sectional study needs to be replicated in a larger randomized, controlled trial to further verify our results. Moreover, direct causality among exercise, oxidative stress, and blood pressure cannot be established from these clinical studies of association. Therefore, future intervention studies should determine whether enzymatic antioxidant improvement resulting from physical training can target and reverse the oxidative stress that occurs at the time of the menopause and that likely contributes to elevated blood pressure.

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Disclosures

None.

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EFFECT OF CARDIORESPIRATORY FITNESS ON VASCULAR REGULATION AND OXIDATIVE STRESS IN POSTMENOPAUSAL WOMEN

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EXPENDED MATERIALS AND METHODS

Protocol

Eligibility criteria included: non-smokers within the past 12 months, ability to perform moderate exercise independently, body mass index (BMI) ≤30 kg/m², normal spirometry, less than 20% stenosis of either carotid arteries, and no evidence of significant co-morbid disease or pharmacologic therapies, as determined by the study physician, that would interfere with their ability to exercise or with study outcomes. The study requirements were fully explained to each study participants, and written informed consent was obtained.

Estradiol was measured by electrochemiluminescent immunoassay using an Elecsys 2010 from Roche Diagnostics. The measuring range was 18.4 – 15781 pmol/l, with an accuracy of 6.0%. Progesterone was measured by chemiluminescent immunoassay using an Advia Centaur by Siemens/Bayer Diagnostics. The measuring range was 0.48 – 190.8 nmol/l, with an accuracy of 6.0%.

Measurements of middle cerebral artery peak velocity, as an index of cerebral blood flow, were made at rest using transcranial Doppler ultrasound (1,2). The right middle cerebral artery was identified through the temporal window just above the zygomatic arch using search techniques previously described (3;4). As there were no changes in $P$ throughout the experimental sessions $V$ $P$ was used as the primary index of cerebral blood flow. The resting cerebral vascular tone was calculated as cerebrovascular conductance (CVC), which is the ratio $V_P / MABP$ (5;6). All cardiovascular parameters were acquired at 10 ms intervals and the values for each determined beat-by-beat by specifically designed computer software (Chamber V2.43, University Laboratory of Physiology, Oxford, UK).

Biochemical analyses

Concentrations of plasma 8-OHdG was determined using an ELISA kit from Cell BioLabs (Cell Biolabs, Inc. San Diego, CA). The limits of detection for this assay are 1-200µg.l⁻¹.

Concentrations of plasma MDA were determined as thiobarbituric reactive substances by a modified method of Ohkawa et al (7), as previously described (8). Although MDA assay is often associated with relevant methodological limitations (9), it was the most common lipid peroxidation marker and it still widely used as marker of oxidative stress in the area of blood pressure regulation.

Concentrations of plasma 8-iso-PGF2α were measured in the plasma using an ELISA kit (Cells bioLabs, Inc. San Diego, CA). The limits of detection for this assay are 0.05-200 ng.l⁻¹.

Concentrations of plasma Nitrotyrosine, as end product of protein nitration by ONOO-, were measured using an ELISA kit from Cell BioLabs (Cell Biolabs, Inc. San Diego, CA). The limits of detection for this assay are 1-8000 nmol.l⁻¹.
GPX in the plasma was determined by the modified method of Paglia and Valentine (10) using hydroperoxide (H₂O₂) as a substrate. GPX was determined by the rate of oxidation of NADPH to NADP⁺ after addition of glutathione reductase (GR), reduced glutathione (GSH), NADPH.

Catalase activity in the plasma was determined by the method of Johansson and Borg (11) using hydroperoxide (H₂O₂) as substrate, and formaldehyde as standard. Catalase activity was determined by the formation rate of formaldehyde induced by the reaction of methanol and H₂O₂ using catalase as enzyme.

The end-products of endothelium nitric oxide, nitrites and nitrates, were measured in the serum using a commercially available kit (Cayman Chemical Company, Ann Arbor, MI, USA) based on methods previously described (12). The sum of nitrite and nitrate in the plasma (NOₓ) is considered an index of nitric oxide production (13).
REFERENCES


Table S1: Correlations between maximal oxygen consumption (normalized to body weight) and plasma oxidative stress and antioxidant enzyme activity controlled or not for body mass index and fat mass.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pearson Correlation Coefficient</th>
<th>P</th>
<th>Controlled for BMI and Fat mass</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OHdG (μg.l⁻¹) vs. VO₂max (ml.kg⁻¹.min⁻¹)</td>
<td>-0.52</td>
<td>0.001</td>
<td>-0.30</td>
<td>0.07</td>
</tr>
<tr>
<td>8-iso-PGF2α (ng.l⁻¹) vs. VO₂max (ml.kg⁻¹.min⁻¹)</td>
<td>-0.41</td>
<td>0.008</td>
<td>-0.17</td>
<td>ns</td>
</tr>
<tr>
<td>Nitrotyrosine (nmol.l⁻¹) vs. VO₂max (ml.kg⁻¹.min⁻¹)</td>
<td>-0.31</td>
<td>0.04</td>
<td>0.18</td>
<td>ns</td>
</tr>
<tr>
<td>GPX (μmol.l⁻¹.min⁻¹) vs. VO₂max (ml.kg⁻¹.min⁻¹)</td>
<td>0.40</td>
<td>0.001</td>
<td>0.32</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Abbreviations: VO₂max, maximal oxygen consumption; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; 8-iso-PGF2α, 8-iso-Prostaglandin F2α, MDA: malondialdehydes; GPX: glutathione peroxidase activity. Correlation analyses conducted on data from all volunteers (n=40).
Table S2: Correlations between cardiovascular parameters and plasma oxidative stress and end-products of NO controlled or not for body mass index and fat mass.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pearson Correlation Coefficient</th>
<th>P</th>
<th>Controlled for BMI and Fat mass</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nitrotyrosine (nmol.l⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs. SBP (mmHg)</td>
<td>0.24</td>
<td>0.13</td>
<td>0.23</td>
<td>0.16</td>
</tr>
<tr>
<td>vs. DBP (mmHg)</td>
<td>0.44</td>
<td>0.005</td>
<td>0.58</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>8-OHdG (µg.l⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs. SBP (mmHg)</td>
<td>0.48</td>
<td>0.002</td>
<td>0.43</td>
<td>0.01</td>
</tr>
<tr>
<td>vs. DBP (mmHg)</td>
<td>0.33</td>
<td>0.04</td>
<td>0.32</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>8-iso-PGF2α (ng.l⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs. SBP (mmHg)</td>
<td>0.16</td>
<td>ns</td>
<td>0.15</td>
<td>ns</td>
</tr>
<tr>
<td>vs. DBP (mmHg)</td>
<td>0.07</td>
<td>ns</td>
<td>0.005</td>
<td>ns</td>
</tr>
<tr>
<td><strong>MDA (nmol.l⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs. SBP (mmHg)</td>
<td>0.36</td>
<td>0.02</td>
<td>0.46</td>
<td>0.01</td>
</tr>
<tr>
<td>vs. DBP (mmHg)</td>
<td>0.06</td>
<td>ns</td>
<td>0.04</td>
<td>ns</td>
</tr>
<tr>
<td><strong>NOx (µmol.l⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs. DBP (mmHg)</td>
<td>-0.50</td>
<td>0.003</td>
<td>-0.45</td>
<td>0.01</td>
</tr>
<tr>
<td>vs. SBP (mmHg)</td>
<td>-0.42</td>
<td>0.01</td>
<td>-0.39</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Abbreviations: 8-OHdG: 8-hydroxy-2'-deoxyguanosine, 8-iso-PGF2α: 8-iso-Prostaglandin F2α, SBP: systolic blood pressure, DBP: diastolic blood pressure, MDA: malondialdehydes, NOx: end-products of NO metabolism. Correlation analyses conducted on data from all volunteers (n=40).
**Figure S1:** Relationship between maximal oxygen consumption (VO$_2$max) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentration in plasma in postmenopausal women. Open circles (n=21) represent sedentary group (≤ 100% age predicted VO$_2$max) and solid circles (n=19) represent fit group (>100% age predicted VO$_2$max).
Figure S2: Relationship between maximal oxygen consumption (VO$_2$max) and plasmatic glutathione peroxidase activity (GPX) in postmenopausal women. Open circles (n=21) represent sedentary group ($\leq$ 100% age predicted VO$_2$max) and solid circles (n=19) represent fit group (>100% age predicted VO$_2$max).