Exercíc Training Improves Baroreflex Sensitivity Associated With Oxidative Stress Reduction in Ovariectomized Rats


Abstract—The protection from coronary events that young women have is sharply reduced at menopause. Oxidative stress and baroreflex sensitivity impairment of the circulation have been demonstrated to increase cardiovascular risk. On the other hand, exercise training has been indicated as a nonpharmacological treatment for many diseases. The aim of the present study was to test the hypothesis that exercise training can improve baroreflex sensitivity associated with reduction in oxidative stress in ovariectomized rats, an experimental model of menopause. Exercise training was performed on a treadmill for 8 weeks. Arterial pressure and baroreflex sensitivity, which were evaluated by tachycardic and bradycardic responses to changes in arterial pressure, were monitored. Oxidative stress was evaluated by chemiluminescence and superoxide dismutase and catalase antioxidant enzyme activities. Exercise training reduced resting mean arterial pressure (112±2 vs 122±3 mm Hg in the sedentary group) and heart rate (325±4 vs 356±12 bpm in the sedentary group) and also improved baroreflex sensitivity (tachycardic response, 63% and bradycardic response, 58%). Myocardium (25%) and gastrocnemius muscle (48%) chemiluminescence were reduced, and myocardial superoxide dismutase (44%) and gastrocnemius catalase (97%) activities were enhanced in trained rats in comparison with sedentary rats. Myocardium chemiluminescence was positively correlated with systolic arterial pressure (r=0.6) and inversely correlated with baroreflex sensitivity (tachycardic response, r=−0.8 and bradycardic response, r=−0.7). These results indicate that exercise training in ovariectomized rats improves resting hemodynamic status and reflex control of the circulation, probably associated with oxidative stress reduction, suggesting a homeostatic role for exercise training in reducing cardiovascular risk in postmenopausal women. (Hypertension. 2005;46[part 2]:998-1003.)

Key Words: exercise ■ baroreflex ■ oxidative stress ■ rat ■ estrogen ■ menopause

Menopause has been associated with impairment of aerobic fitness, muscle strength, and bone mineral density, as well as an increase in body weight, type 2 diabetes, osteoporotic fractures, and cardiovascular disease (CVD). Many CVD states are associated with baroreflex impairment, the most important short-term regulator of arterial blood pressure. Moreover, the baroreflex has been recognized as a marker of autonomic control and as a predictor of CV mortality. Estrogen deprivation induces endothelial dysfunction and autonomic impairment and increases oxidative stress in fertile young women and postmenopausal women, thus increasing the CV risk. Oxidative stress has been implicated in the pathophysiology of a large number of diseases, and it plays a possible mechanistic role in baroreflex dysfunction, because antioxidant substances seem to improve baroreflex sensitivity (BRS) in different species. However, the role of oxidative stress on CV autonomic dysfunction during estrogen deprivation is not well understood.

Since the Women’s Health Initiative study has made medical practitioners review the risks and benefits to each patient and made women reconsider their use of hormone therapy, the significance of lifestyle and its impact on CV function for menopause management are of increasing relevance. In this sense, multiple studies have led to the rational suggestion that exercise favorably influences CV risk factors associated with pathological situations. A recent systematic review of randomized, controlled trials reported benefits of exercise on body weight, bone constitution, muscle strength and endurance, flexibility, oxygen consumption, blood pressure, and metabolic control after menopause. Jurca et al gave evidence of an increase in heart rate variability after 8 weeks of exercise training in postmenopausal women. However, the effects of exercise training on BRS, as well as the possible role of oxidative stress in the CV alterations induced by training in female rats subjected to estrogen deprivation, are unknown. Therefore, the purpose of the present study was...
to test the hypothesis that 8 weeks of exercise training would decrease arterial pressure (AP) and increase BRS in ovariec-
tomized (OVX) female rats. A secondary aim was to test the hypothesis that the hemodynamic changes induced by exercise training, if observed, would be associated with an improvement in oxidative stress profile.

Methods

Animals

Experiments were performed on 18 female, virgin Wistar rats (210±3 g) from the Animal Shelter of the University of Sao Paulo, Sao Paulo, Brazil, that were given standard laboratory chow and water ad libitum. The animals were housed in individual cages in a temperature-controlled room (22°C) with a 12-hour dark/ light cycle. All rats were treated similarly in terms of daily manipulation. All surgical procedures and protocols used were in accordance with the Guidelines for Ethical Care of Experimental Animals, which was approved by the International Animal Care and Use Committee. The rats were randomly assigned to 1 of 2 groups: sedentary OVX (SO, n=10) and trained ovariectomized (TO, n=8).

Ovariectomy

At 10 weeks of age, animals were anesthetized (80 mg/kg ketamine and 12 mg/kg xylazine), and a small abdominal incision was made. The ovaries were then located, and a silk thread was tightly tied around the oviduct, including the ovarian blood vessels. The oviduct was sectioned and the ovary removed. The skin and muscle wall were then sutured with silk thread. After surgery, the animals received an injection of antibiotics (40 000 U/kg penicillin G procaine IM).12

Exercise Training

Exercise training was performed on a motor treadmill at low-
moderate intensity (~50% to 70% maximal running speed) for 1 hour a day, 5 days a week, for 8 weeks, with a gradual increase in speed from 0.3 to 1.2 km/h. All animals were adapted to the procedure (10 min/d, 0.3 km/h) for 1 week before beginning the exercise training protocol. This adaptation period began 24 hours after OVX. Sedentary and trained female rats were subjected to a maximal treadmill test, as described in detail in a previous publica-
tion.13 The tests were performed (1) at the beginning of the experiment, (2) in the fourth week, and (3) in the eighth week of the training protocol. The purpose was to determine aerobic capacity and exercise training intensity.

CV Assessments

After the last training session, 2 catheters filled with 0.06 mL saline were implanted in anesthetized rats (80 mg/kg ketamine and 12 mg/kg xylazine) into the carotid artery and jugular vein (PE-10) for direct measurements of AP and drug administration, respectively. Rats receiving food and water ad libitum were studied 1 day after catheter placement; the rats were conscious and allowed to move freely during the experiments. The arterial cannula was connected to a strain-gauge transducer (P23Db, Gould-Statham), and blood pressure signals were recorded over a 20-minute period by a microcom-
puter equipped with an analog-to-digital converter board (CODAS, 2-KHz sampling frequency; Dataq Instruments, Inc). The recorded data were analyzed on a beat-to-beat basis to quantify changes in mean AP (MAP) and heart rate (HR). Increasing doses of phenyl-
ephrine (0.25 to 32, μg/kg) and sodium nitroprusside (0.05 to 1.6 μg/kg) were given as sequential bolus injections (0.1 mL) to produce pressure responses ranging from 5 to 40 mm Hg. A 3- to 5-minute interval between doses was necessary for blood pressure to return to baseline. Peak increases or decreases in MAP after phenylephrine or sodium nitroprusside injection and the corresponding peak reflex changes in HR were recorded for each dose of the drug. BRS was evaluated by a mean index relating changes in HR to the changes in MAP, allowing a separate analysis of gain for reflex bradycardia and reflex tachycardia. The mean index was expressed as beats per minute per millimeter of mercury, as described elsewhere.14–16

Oxidative Stress Profile

After animals were killed by decapitation, the heart (ventricles) and gastrocnemius muscle were immediately removed, rinsed in saline, and trimmed to remove fat tissue and visible connective tissue. These tissues were cut into small pieces, placed in ice-cold buffer, and homogenized in an ultra-Turrax blender with 1 g of tissue per 5 mL of 150 mmol/L KCl and 20 mmol/L phosphate buffer, pH 7.4. The homogenates were centrifuged at 600g for 10 minutes at −2°C. Chemiluminescence (CL) assay was carried out with an LKB Rack Beta liquid scintillation spectrometer 1215 (LKB Producer AB) in the out-of-coincidence mode at room temperature (25°C to 27°C). The supernatants were diluted in 140 mmol/L KCl and 20 mmol/L phosphate buffer, pH 7.4, and added to glass tubes, which were placed in scintillation vials; 3 mmol/L tert-butylhydroperoxide was added, and CL was determined up to the maximal level of emission.17 Catalase (CAT) activity was measured spectrophotometri-
cally by monitoring the decrease in H2O2 concentration over time. Aliquots of the samples were added to 50 mmol/L phosphate buffer in a quartz cuvette. After determining the baseline of the instrument, H2O2 was added to a final concentration of 10 mmol/L in 0.9 mL, and absorbance was measured at 240 nm.18 Superoxide dismutase (SOD) activity was determined in the homogenates by measuring the inhibition of the rate of autacatalytic adenrinochondrome formation at 480 nm in a reaction medium containing 1 mmol/L epinephrine and 50 mmol/L glycine-NaOH, pH 10.5.19 Glutathione peroxidase (GPx) activity was assessed by adding to the assay a mixture of 1 U/mL glutathione reductase and 2 mol/L glutathione in 1 mL phosphate buffer. Mixtures were preincubated at 37°C for 30 minutes. Subse-
sequently, NAPDH and tert-butylhydroperoxide were added, and the change in absorbance at 340 nm was recorded to calculate GPx activity, as previously described.20 Proteins were assayed by the method of Lowry et al.21

Statistical Analysis

Data are presented as mean±SEM. Comparisons between the 2 groups were performed with Student unpaired t tests. Pearson correlation was used to study the association between variables. The significance level was established at P<0.05.

Results

Body weight was not different between the groups at the beginning of the protocol (210±2 vs 211±3 g in the SO group). At the end of the training period, TO OVX animals (302±5 g) had a lower body weight than SO (328±5 g).

Maximal Exercise Protocol

Aerobic physical performance was evaluated by the response to the maximal treadmill test. At the beginning of the experiment and after 4 weeks of training, the aerobic physical performance was similar between groups (initial, 2.3±0.2 vs 2.4±0.1 km/h in the SO group; fourth week, 2.3±0.2 vs 2.2±0.1 km/h in SO). However, the animals subjected to exercise training showed an increase in the maximum speed of running when compared with the SO group after 8 weeks of exercise training (2.9±0.2 vs 2.1±0.1 km/h in the SO group).

Hemodynamic Assessments

As shown in the Table, exercise training induced reductions in systolic AP (124±3 vs 136±4 mm Hg in SO), diastolic AP (102±3 vs 107±3 mm Hg in SO), MAP (112±2 vs 122±3 mm Hg in SO), and resting HR (325±5 vs 356±10 bpm in SO) in OVX rats (Figure 1A and 1B). The tachycardic...
Oxidative Stress Profile in SO and TO OVX Groups

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Sedentary OVX</th>
<th>Trained OVX</th>
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<tbody>
<tr>
<td>CL, cps/mg protein</td>
<td>7348±373</td>
<td>5895±126*</td>
</tr>
<tr>
<td></td>
<td>2306±149</td>
<td>1562±218*</td>
</tr>
<tr>
<td>CAT, pmol/mg protein</td>
<td>32±2.4</td>
<td>40±2.8</td>
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<tr>
<td></td>
<td>7±0.6</td>
<td>14±2.1*</td>
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<td>SOD, U/mg protein</td>
<td>31.1±1.27</td>
<td>44.9±2.5*</td>
</tr>
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<td></td>
<td>38.1±2.06</td>
<td>31.7±2.53</td>
</tr>
<tr>
<td>GPx, µmol·min⁻¹·mg protein⁻¹</td>
<td>3.6±0.23</td>
<td>3.5±0.28</td>
</tr>
<tr>
<td></td>
<td>10.4±0.42</td>
<td>8.6±1.07</td>
</tr>
</tbody>
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cps indicates counts per second; other abbreviations are as defined in text. Values are mean±SEM.

*P<0.05 vs SO OVX group.

(4.4±0.5 vs 2.7±0.3 bpm/mm Hg in SO) and the bradycardic (∼2±0.1 vs ∼1.2±0.2 bpm/mm Hg in SO) responses evoked by baroreflex during AP falls and rises, respectively, were enhanced in TO OVX animals when compared with the SO group (Figure 1C).

Oxidative Stress Assessment

Membrane lipid peroxidation as assessed by CL showed a significant reduction in heart (∼25%) and gastrocnemius muscle (∼48%) homogenates from animals subjected to exercise training when compared with the SO group (Table 1). These changes were accompanied by significant alterations in antioxidant enzymes in these tissues. The SOD enzyme, which catalyzes the dismutation of superoxide into less toxic H₂O₂, presented an enhancement (∼44%) in TO OVX rat myocardium when compared with SO OVX rat myocardium. The CAT and GPx activities were similar in heart tissue between the studied groups. However, the activity of CAT, an enzyme that catalyzes the reduction of H₂O₂ to water and O₂, was greatly increased (∼97%) in the gastrocnemius muscle of TO OVX females in relation to SO rats. The SOD and GPx activities of gastrocnemius muscle were unchanged by exercise training (Table 1).

Correlation analysis involving all animals studied showed a significant, positive relation between CL and systolic AP (r=0.6, P<0.05; Figure 2A) and between SOD activity and bradycardic response induced by AP increases (r=0.65, P<0.05; Figure 2B). However, SOD activity was not correlated to tachycardic response (r=0.55, P=0.06) or to systolic AP (r=0.5, P=0.09). Moreover, CL was inversely correlated to the tachycardic (r=−0.8, P<0.05, Figure 2C) and bradycardic (r=−0.7, P<0.05; Figure 2D) responses evoked by the AP decrease and increase, respectively.

Discussion

There were 3 important insights from the present study. First, the TO OVX rats had a reduced AP and HR at rest when compared with their SO peers. Second, the BRS was higher in physically active rats than in sedentary ones. Finally, the major finding of this investigation is that exercise training induces a reduction not only in oxidative stress but also in AP associated with an increase in BRS in OVX animals. This suggests that oxidative stress may contribute mechanistically to the estrogen deprivation–associated CV impairment that has been observed in postmenopausal women. Because there are no rat models of menopause that are considered ideal,
most investigators have used relatively young (6 to 12 weeks) female rats OVX for short periods (3 to 5 weeks).\textsuperscript{22} Although this model may not be appropriate to determine long-term changes in CV regulation during menopause, the OVX procedure is an efficient way to simulate menopause status, because it suppresses ovarian hormonal levels. Furthermore, the persistent estrus (constant sexual receptivity) observed in aged female rats suggests an incomplete suppression of sex hormones with aging see.\textsuperscript{25} In the present study, female rats were OVX at 10 weeks of age, and the physiologic measurements were performed 9 weeks later.

The incidence of CVD in women increases sharply after menopause\textsuperscript{23} and probably involves changes in AP and its regulation associated with estrogen loss. The incidence of hypertension rises after menopause,\textsuperscript{24} thus corroborating this hypothesis. In the present study, we showed elevated AP values in OVX rats compared with AP values previously observed not only in intact female rats but also in male rats.\textsuperscript{9,12,15,25} In fact, Hernandez et al\textsuperscript{12} have previously reported that OVX in rats induced increase in AP values reaching values similar to the ones observed in our experiments. However, other study has reported no changes in AP after OVX.\textsuperscript{25} This discrepancy may be explained by the different observation time after OVX. Nickening et al\textsuperscript{25} killed the rats 5 weeks after OVX, instead of 8 or 9 week which were allowed after OVX in Hernandez et al\textsuperscript{12} protocol\textsuperscript{12} or in the present study, respectively. Importantly, we demonstrated that 8 weeks of exercise training induced significant reduction on AP values. In a study with normotensive postmenopausal women, 15 weeks of walking (65% maximum oxygen consumption) decreased diastolic AP by 3 mm Hg.\textsuperscript{26} However, other studies did not demonstrate improvement in AP after training in normotensive postmenopausal women.\textsuperscript{10} Moreover, previous studies\textsuperscript{27–30} have shown that low- to moderate-intensity exercise training is an efficient nonpharmacological treatment of hypertension.

Regarding the mechanisms involved in exercise-induced AP reduction, some investigators\textsuperscript{27} have reported that exercise training causes a reduction in cardiac output in hypertensive humans and animals, whereas others\textsuperscript{30} have observed a decrease in total peripheral resistance in humans. In the present work, the resting bradycardia observed in trained female OVX could induce cardiac output decrease and consequently contribute to AP reduction after training. Despite the fact that we have not studied the causes of resting bradycardia in trained OVX rats, we can speculate that it was related to changes in autonomic balance or in intrinsic heart rate as previously documented in male rats and mice.\textsuperscript{15,16}

Changes in AP may also be related to oxidative stress and endothelial dysfunction. Indeed, the decrease in myocardial and gastrocnemius muscle CL in trained OVX rats may reflect an improvement in the redox state toward oxidative process that may be associated with increases in nitric oxide (NO) bioavailability.\textsuperscript{31,32} A previous study has reported that a free radical scavenger (N-acetyl-l-cysteine) reverted endothelial dysfunction in the aortic rings of OVX rats.\textsuperscript{33} Hernandez et al\textsuperscript{12} showed that estrogen administration decreases blood pressure and increases vascular conductance in OVX rats. This effect may be related to increasing NO synthesis and/or preventing oxidative stress, thus improving endothelial function. The positive correlation obtained in the present work between systolic AP and CL also reinforces the role of oxidative stress in AP changes during female hormone deprivation.

Impairment in BRS is associated with both higher AP and severity of CVD.\textsuperscript{2} Thus, the identification of mechanism underlying a depressed BRS has important clinical implications, as well as the study of therapies to improve this CV reflex. Hunt et al\textsuperscript{34} demonstrated that long-term estrogen replacement therapy in postmenopausal women has effects on CV regulation, as evidenced by an increase in vascular sympathetic baroreflex gain, that may be not reflected in resting blood pressure or in cardiovagal baroreflex gain. However, hormonal therapy is actually questionable and not applicable to all postmenopausal women. Davy et al\textsuperscript{35} reported that physically active postmenopausal women present higher BRS and levels of HR variability compared with age-matched less active women, providing insight into a possible cardioprotective mechanism in physically active postmenopausal women. In the present study, we demonstrated that exercise training applied to an experimental model of ovarian hormone deprivation induces improvement in BRS.

An increased BRS after exercise training has been found in borderline-hypertensive humans\textsuperscript{28} and in male normotensive and
male SHR. In the present study, we observed that exercise training in OVX rats improved BRS for bradycardiac and tachycardiac responses. Despite the fact that we did not study changes in parasympathetic function in OVX rats in this investigation, the resting bradycardia observed could be due to an increase in vagal tonus to the heart, indicating an improvement in vagal reserve used during HR responses evoked by baroreceptors. Increased shear stress during exercise may also enhance the release of endothelial factors. All of these mechanisms may increase the sensitivity of the arterial baroreceptor afferents, thus increasing the BRS. In fact, Brum et al observed an increased BRS in the baroreflex function curves after exercise training of male normotensive rats and of male SHR, suggesting that similar alterations can occur in OVX rats. However, we cannot exclude the possibility that exercise effects are associated with other alterations in the central and efferent components of the baroreflex pathway.

Moreover, the increased levels of oxidative stress could impair baroreflex function through a direct suppressive influence on baroreceptors, because direct administration of SOD and CAT on the carotid sinus improved BRS in rabbits with experimentally induced atherosclerosis. In addition, because NO appears to be an important modulator of baroreflex function in humans and its bioavailability is reduced with estrogen deprivation, it is possible that oxidative stress could suppress baroreflex function indirectly by reducing NO bioavailability. Monahan et al recently reported that acute intravenous ascorbic acid administration increase BRS in older men without clinical disease, suggesting that oxidative stress contributes mechanistically to the age-associated reduction in BRS. In fact, the negative correlations obtained between CL and both bradycardic and tachycardic responses to AP changes in the present investigation suggest a role for oxidative stress in the improvement of BRS observed after training in OVX rats.

Other previous studies confirm the role of oxidative stress and antioxidant treatment in improving BRS in young healthy adults, heart failure patients, and male rats. Antioxidant therapy, by increasing the bioavailability of NO in sinus node, may influence baroreflex function at several sites in the baroreflex arc, increasing barosensory arterial wall distensibility, preventing the inhibition of baroreceptor firing caused by free radicals, and improving the effectiveness of the cardiac response. In this sense, the exercise-induced enhancement in antioxidant enzymes, SOD in heart, and CAT in gastrocnemius could provide similar improvement in the baroreflex arc, as previously demonstrated by antioxidant therapy.

A previous study from our group demonstrated reduced SOD and unchanged CAT and GPx activities after OVX in the myocardium of rats. Exercise training applied in the present study induced enhancement of myocardial SOD activity in OVX rats and no alteration in CAT or GPx activities. Because SOD detoxifies the superoxide anion, which inactivates NO, the increased myocardial SOD activity in trained rats may be associated with an NO-dependent facilitation of vagally induced resting or reflex bradycardia after exercise training. The positive correlation between myocardial SOD and the bradycardic response to AP rises \( (P<0.05) \), as well as the trend of a correlation with the tachycardic response to AP falls \( (P=0.06) \), reinforces this rationale.

Oxidative stress was also evaluated in gastrocnemius muscle, in both red and white type fibers, allowing a general view of oxidative stress balance in skeletal muscle. The increase in gastrocnemius CAT activity after training may reflect increased levels of fatty acyl coenzyme A oxidase that initiates \( \beta \)-oxidation of fatty acids in peroxisomes. Finally, it is important to emphasize that reactive oxygen species modulate contractile function because excessive oxidative stress accumulation inhibits force. In this aspect, the reduction in CL and the improvement of antioxidant enzymes after training in the heart and in the gastrocnemius muscles of OVX rats could represent an improvement in performance of these muscles, also suggesting a positive role of exercise training in preventing muscle performance impairment in postmenopausal women.

One question is whether the exercise protocol used in our study was effective in producing physical training in the female rats. In the present study, trained rats showed a marked increase in estimated aerobic physiologic capacity as evaluated by their response to the maximal exercise test. A significant association \( (r=0.83) \) between oxygen consumption and running velocity was reported in untrained rats. Furthermore, the finding of resting bradycardia is a good indication of the efficacy of the exercise training protocol to produce overall fitness. Additional information provided by our study is the demonstration of reduced body weight in TO OVX rats. In a recent review, Asikanen et al showed that 9 studies in postmenopausal women have reported improvement in body weight after training.

In conclusion, exercise training in OVX rats improves resting hemodynamic status and reflex control of circulation, probably associated with oxidative stress reduction, suggesting a homeostatic role for exercise training in reducing CV risk after menopause.

Perspectives

Menopause is recognized as a period of increased risk for coronary heart disease. Vulnerability to this condition is often attributed to the naturally occurring estrogen deficiency characteristic of this part of the life cycle. Indeed, premenopausal reductions in endogenous estrogen occasioned by functional ovarian abnormalities or failure are hypothesized to be similarly pathogenic and to accelerate development of CVD prematurely, thereby increasing the health burden of older women. In fact, as life expectancy increases, women are spending more time in the postmenopausal phase of life and are naturally exposed to the risk of more prevalent diseases at this time, like hypertension, diabetes, myocardial infarction, and heart failure. After data from randomized, controlled trials became available, postmenopausal hormonal therapy was believed to be ineffective for coronary disease prevention; consequently, other pharmacological and nonpharmacological interventions must be invoked to reduce coronary risk. Exercise training as a tool to improve oxidative stress and the baroreflex control of the circulation associated with estrogen deprivation seems to be an alternative in the management of the increased risk for developing chronic diseases in this condition. The contribution of physical activity to changing the time course of estrogen deprivation associated with experimental models of hypertension, diabetes, or myocardial...
infarction as well as the pathophysiologic principles of these changes should be further evaluated.

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