Effect of exercise training on cardiac oxytocin and natriuretic peptide systems in ovariectomized rats

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1 Laboratory of Cardiovascular Biochemistry, Research Centre, Centre Hospitalier de l’Université de Montréal-Hôtel-Dieu, and Department of Medicine, Université de Montréal; and 2 Department of Kinesiology, Université de Montréal, Montréal, Québec, Canada

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Gutkowska J, Paquette A, Wang D, Lavoie J-M, Jankowski M. Effect of exercise training on cardiac oxytocin and natriuretic peptide systems in ovariectomized rats. Am J Physiol Regul Integr Comp Physiol 293: R267–R275, 2007. First published May 2, 2007; doi:10.1152/ajpregu.00071.2007.—Exercise training results in cardiovascular and metabolic adaptations that may be beneficial in menopausal women by reducing blood pressure, insulin resistance, and cholesterol level. The adaptation of the cardiac hormonal systems oxytocin (OT), natriuretic peptides (NPs), and nitric oxide synthase (NOS) in response to exercise training was investigated in intact and ovariectomized (OVX) rats. Ovariectomy significantly augmented body weight (BW), left ventricle (LV) mass, and intra-abdominal fat pad weight and decreased the expression of oxytocin receptor (OTR), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and guanylyl cyclase-A (GC-A), in the right atrium (RA) and LV, indicating estrogenic control of these genes. These effects of ovariectomy were counteracted by 8-wk-long exercise training which decreased fat pad weight (33.4 ± 2.3 to 23.4 ± 3.1 g, n = 8, P < 0.05), plasma free fatty acids (0.124 ± 0.033 to 0.057 ± 0.010 mM, n = 8, P < 0.01), and plasma triacylglycerol (0.978 ± 0.174 to 0.588 ± 0.115 mM, n = 8, P < 0.05). Chronic exercise tended to decrease BW and stimulated ANP (4- to 5-fold) and OTR gene expression in the LV and RA and BNP and inducible NOS (iNOS) mRNA in the LV. In sham-operated rats, exercise augmented ANP expression in the RA, downregulated GC-A mRNA in the LV and RA, but increased its expression threefold in the RA of OVX animals. Endothelial NOS and iNOS expression was enhanced in the left atrium of sham-operated rats. Altogether, these data indicate that in OVX animals, chronic exercise significantly enhances cardiac OT, NPs, and NOS, thus implicating all three hormonal systems in the beneficial effects of exercise training.

heart; atrial natriuretic peptide; ovariectomy

THE PASSAGE FROM GREAT PHYSICAL activity to sedentary living occurred in Western societies during the last century. These changes in lifestyle were accompanied by a growing prevalence of obesity, hypertension, diabetes, atherosclerosis, and their consequences, increasing the number of cardiovascular diseases, the leading cause of death in industrialized countries.

Numerous epidemiological studies have convincingly shown that physical training has a beneficial effect on cardiovascular disease outcome. Exercise training reduces heart rate and blood pressure (BP), augments myocardial oxygen uptake, and regulates circulating blood volume as well as various metabolic processes. Regular exercise appears to be particularly beneficial in menopausal women (3), whose lack of ovarian steroids has detrimental effects on bone mass and progression of cardiovascular diseases. The incidence of cardiovascular diseases rises sharply in women after menopause (55), where a fourfold higher prevalence of hypertension has been documented compared with premenopausal women (49). Exercising improves the metabolic and lipid profile and reduces inflammation and cell adhesion molecules in postmenopausal women, even in the absence of caloric restriction (57). Furthermore, postmenopausal women who engage in intermittent, moderate-intensity physical training experience a significant reduction in BP (50), with a decreased risk of pressure-related cardiovascular complications.

Several factors contribute to the beneficial effects of exercise in maintaining cardiovascular homeostasis. Exercise training increases vascular nitric oxide (NO) bioactivity as a result of enhanced endothelial nitric oxide synthase (eNOS) expression and reduces its oxidative stress-mediated destruction, thus improving vascular endothelial health (17, 26). In addition, we (14, 52) and others (1, 7, 37) showed that in humans and animals, exercise provokes the increased synthesis and release of atrial natriuretic peptide (ANP) into the circulation. ANP is a potent diuretic, natriuretic, and vasorelaxant hormone with anti-inflammatory, antihypertrophic, and antifibrotic properties (34, 36). The mechanisms of ANP stimulation by exercise are not yet known.

Recently chronic exercise training was demonstrated to increase oxytocin (OT) content and gene expression in the rat hypothalamus (4, 31). Therefore, we hypothesized that exercise training may have a direct influence on cardiac OT peptide and its receptor (OTR), which we uncovered recently (15, 20) in the rat and human heart as physiological regulators of ANP release. On the other hand, hypothalamic OT and cardiac ANP synthesis and release are controlled by estrogen receptor-mediated mechanisms (21). Plasma ANP levels are low in ovariectomized (OVX) rats but are restored by 17β-estradiol administration (56), and circulating ANP rises in response to exercise. Based on the interrelationship between estrogen and OT, it appears likely that a reduced cardiac OT system in OVX animals, stimulated after endurance training, could contribute to increased cardiac ANP gene expression and ANP release via local cardiac effects involving NO stimulation (5) and providing benefits to the heart. Accordingly, the following study was undertaken to test the hypothesis that chronic exercise stimulates the cardiac OT and ANP systems, leading to positive outcomes in normal and estrogen-deficient conditions. The effects of chronic exercise on the cardiac OT,
ANP, and NO systems were evaluated in normal and OVX female rats.

**MATERIALS AND METHODS**

**Animal care.** Female Sprague-Dawley rats (n = 32) weighing 180–200 g (8 wk old), obtained from Charles River (St. Constant, PQ, Canada), were housed individually under a 12:12-h light-dark cycle starting at 6 AM, with room temperature at 20–23°C. The animals were given free access to rat chow (12.5% fat, 63.2% carbohydrate, 24.3% protein, Agribrands Purina Canada, Woodstock, ON) and tap water. All groups were treated similarly in terms of daily manipulations. The experiments were conducted according to the Guidelines of the Canadian Council on Animal Care.

**Surgical procedure.** Two days after their arrival in our laboratory, the rats were randomly assigned to four groups (n = 8/group). Two groups underwent OVX, and two groups were sham-operated. OVX was performed according to the technique described by Robertson et al. (42). The animals were anesthetized with a mixture of ketamine-hydrochloride (100 mg/kg), xylazine (5 mg/kg), and acepromazine maleate (10 mg/kg). A peritoneal incision was performed, and the ovaries were removed and weighed (Mettler AE 100) and then frozen in liquid nitrogen and stored at −80°C. All rats were inspected visually for the presence or absence of ovaries, and uteri were excised and weighed to confirm the ovariectomy and sham surgery.

**Analytical procedures.** Plasma free fatty acids (FFA) were quantified by enzymatic colorimetric assay available from Roche Diagnostics (Mannheim, Germany). Plasma triacylglycerol (TAG) concentrations were measured by enzymatic colorimetric assay with kits available from Sigma (St. Louis, MO).

**Quantitative real-time-PCR.** Total RNA was extracted from frozen samples with TRIzol (Invitrogen Life Technologies, Burlington, ON), according to the manufacturer’s protocol. To remove genomic DNA, RNA samples were incubated with 2 U deoxyribonuclease I (DNase I; Invitrogen Life Technologies)/μg RNA for 30 min at 37°C. PCR was performed in an iCycler IQ Real Time PCR detection system (Bio-Rad Laboratories, Hercules, CA), using SYBR green chemistry. Samples were analyzed in duplicate or triplicate. For amplification, 2 μl of diluted cDNA were added to a 20-μl reaction mixture containing 1× iQ SYBR Green Supermix (Bio-Rad Laboratories) and 200 nM forward and reverse primers. The thermal cycling program was 95°C for 2 min, followed by 40 cycles at 95°C for 30 s, at 60°C for 30 s, and at 72°C for 30 s. The primers were purchased from Invitrogen Life Technologies. The oligonucleotide primers used for real-time quantitative PCR are listed in Table 1.

The relative expression of the RT-PCR products was determined according to the ΔΔCt method, which calculates relative expression with the equation: fold induction = 2^-ΔΔCt, where Ct = the threshold cycle, i.e., the cycle number at which the sample’s relative fluorescence rises above background fluorescence, and ΔΔCt = Ct gene of interest (unknown sample) – Ct GAPDH (unknown sample) – Ct gene of interest (calibrator sample) – Ct GAPDH (calibrator sample). One of the control samples, chosen as the calibrator sample, was employed in each PCR. Each sample was run in duplicate, and mean Ct was determined in the ΔΔCt equation. GAPDH was chosen for normalization because this gene showed consistent expression relative to other housekeeping genes among the treatment groups in our experiments. As a control, melting point analysis revealed that each of the primer pairs described (Table 1) amplified a single predominant product, and agarose electrophoresis demonstrated PCR products of single bands of predicted size.

**Semiquantitative PCR.** Semiquantitative PCR was performed to compare NOS mRNA expression in different groups. A volume of 5 μl first-strand cDNA was added to a PCR mixture and amplified for 30–35 cycles by incubation at 95°C for 30 s, at 65°C for 30 s, and at 72°C for 45 s, with a final incubation at 72°C for 5 min, all in a Robocycler gradient 40 thermocycler (Stratagene, La Jolla, CA). The specificity of the amplified products was verified by sequencing. Amplification of 18S RNA, used as an internal standard, followed the manufacturer’s protocol (Ambion, Austin, TX). The PCR products were fractionated onto 1.2% agarose gels containing ethidium bromide. The intensity of the bands was measured by densitometry with Molecular Imaging Software (Image-Quant Software, Molecular Dy-

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**Table 1. PCR primer sequences**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense Primer (5’-3’)</th>
<th>Antisense Primer (5’-3’)</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT</td>
<td>GACGCTGATCCTGAGACTGAA</td>
<td>CGCCCCTAAAGGATATCAAGAAA</td>
<td>NM_012996</td>
</tr>
<tr>
<td>OTR</td>
<td>CGTCAATGGCGCGCAAGAGAG</td>
<td>ATGCAAACGAATAGACCT</td>
<td>NM_018271</td>
</tr>
<tr>
<td>ANP</td>
<td>CAGATGAGGCTCCTTTCACA</td>
<td>GTTCAATCTACGCDGAAAGCT</td>
<td>NM_012613</td>
</tr>
<tr>
<td>BNP</td>
<td>GCATCCGAGCTGCGGCATCAATTTCTG</td>
<td>GACTGCGGAGATCCT</td>
<td>NM_031545</td>
</tr>
<tr>
<td>GC-A</td>
<td>ATTCCGCTGATGATCGCCTACGG</td>
<td>AGATCTGAGTAACTGCGGGTTCG</td>
<td>NM_021623</td>
</tr>
<tr>
<td>eNOS</td>
<td>TTAGCAGTGAGACAAACATCCC</td>
<td>CAGGCTGAGCTTTCAGTTC</td>
<td>NM_021838</td>
</tr>
<tr>
<td>iNOS</td>
<td>GATCAATTCTGGAGACCT</td>
<td>GCCTGTTTCTGCTCATAG</td>
<td>NM_021611</td>
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<tr>
<td>GAPDH</td>
<td>TTTCTAGATGACGACTCAAGG</td>
<td>TCACCCAGATGTTGATG</td>
<td>NM_017008</td>
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</table>

OT, oxytocin; OTR, oxytocin receptor; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; GC-A, guanylyl cyclase type A receptor; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
nmetics, Sunnyvale, CA). To validate this reverse transcription-PCR assay as a tool for the semiquantitative assessment of mRNA, dose-response curves were established for different amounts of total RNA extracted from the rat left ventricle (LV), and the samples were quantified in the curvilinear phase of PCR amplification. Expression levels were normalized against 18S RNA or GAPDH. No difference in 18S RNA or GAPDH levels was observed in any experimental group.

Western blot analysis. Tissue samples were prepared by homogenization in modified RIPA buffer [1× PBS, 1% Igepal CA-630 (Sigma), 0.5% sodium deoxycholate, 0.1% SDS, 10 mg/ml PMSF, 100 mM sodium orthovanadate, and 4% protease inhibitor] and then centrifuged at 10,000 g for 10 min at 4°C. The supernatants were collected, and protein concentrations were determined by modified Bradford assay. Twenty micrograms of total protein were applied to each well of 8% SDS-polyacrylamide gel and electrophoresed for 2 h at 130 V along with a set of molecular weight markers (RPN800, Amersham-Pharmacia Canada, Baie d’Urfé, QC). The resolved protein bands were then transferred onto PVDF membranes (Hybond-C; Amersham-Pharmacia) at 30 V for 120 min, at room temperature, using a transfer buffer (25 mMol/l Tris base, 192 mMol/l glycine, and 20% methanol). The blots were blocked overnight at 4°C with blocking buffer (10% nonfat milk in 10 mMol/l Tris, pH 7.5, 100 mMol/l NaCl, 0.1% Tween 20). The blocking buffer was then decanted, and the blots were incubated for 1 h at room temperature with primary antibodies obtained from Santa Cruz Biotechnology (Santa Cruz, CA): goat anti-OTR (1:1000, sc-654), and mouse anti-eNOS (1:1000, sc-8102), rabbit anti-eNOS (1:1000, sc-654), and mouse anti-β-actin antibody (Sigma). They were then washed in TBS washing buffer (10 mMol/l Tris, pH 7.5, 100 mMol/l NaCl, 0.1% Tween 20) and incubated with horseradish peroxidase-conjugated immunoglobulin G (anti-goat for OTR, 1:10,000; anti-rabbit for eNOS, 1:10,000; anti-mouse for iNOS, 1:10,000; Santa Cruz Biotechnology). The blots were visualized in a chemiluminescence detection system (RPN2132, Amersham-Pharmacia) and exposed to Kodak X-Omat film. Band density was measured by SCION software (http://www.scioncorp.com).

Statistical analysis. The significance of expression level differences between the treatment groups and the data in Table 2 were processed by two-way ANOVA with Fisher post hoc analysis. All values are expressed as means ± SE with significance defined as P < 0.05. Correlation was estimated with Pearson’s linear model.

**RESULTS**

Effect of O VX and training on physiological parameters.

The baseline characteristics of the physiological parameters in sham-operated and OVX animals and the effects of chronic 8-wk exercise are reported in Table 2. Uterus weight was largely decreased, and the uterus weight-to-BW ratio was diminished significantly (P < 0.01) in OVX animals, indicating the efficiency of ovariectomy. A significant interaction of training and ovariectomy influenced the increase in BW (ΔBW, P = 0.006). However, the increment of LV mass was influenced by ovariectomy (P = 0.001) only, not significantly by training (P = 0.10), and interaction of these factors was not observed (P = 0.8). In sham-operated rats, both sedentary and trained, LV mass was correlated with ΔBW (r = 0.93, P = 0.02; r = 0.95, P = 0.01, respectively). This correlation was absent in sedentary (r = 0.72; P = 0.16) and trained (r = 0.45; P = 0.44) OVX rats. In fact, the LV/BW ratio, commonly flagged as an index of cardiac hypertrophy, was affected by the interaction between training and ovariectomy (P = 0.01) which complicated interpretation of the results obtained. The LV/BW index was lower in OVX rats (1.84 ± 0.03) than in the controls (2.11 ± 0.06; P < 0.01). This index is useful when BW is unaltered (7). Since diet was not restricted in our experiments, ovariectomy significantly augmented BW as a consequence, at least in part, of augmented food intake. The BW increment was relatively greater (37%), from 287 ± 7 to 394 ± 9 g (n = 8, P < 0.01), than that of LV mass (18%), which increased from 617 ± 28 mg in sham-operated animals to 732 ± 27 mg (n = 8, P < 0.01) in OVX rats. This was largely responsible for the lower LV/BW index in OVX animals than in sham-operated controls. However, LV hypertrophy in OVX rats was evident not only from augmented LV mass but also from the LV/femur weight ratio, which was significantly higher (0.922 ± 0.032 mg/mg, n = 8, P < 0.01) in OVX animals than sham-operated controls (0.816 ± 0.042 mg/mg). Femur weight was not affected by ovariectomy (Table 2), which significantly increased intra-abdominal fat pad weight from 15.4 ± 1.8 to 33.4 ± 2.3 g (n = 8, P < 0.01; Table 2).

In OVX rats, exercise training had a beneficial effect on body composition, tending to reduce BW, and significantly

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**Table 2. Effect of ovariectomy and training on physiological parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>Sham + T</th>
<th>OVX</th>
<th>OVX + T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>287 ± 7</td>
<td>330 ± 14†</td>
<td>394 ± 9*</td>
<td>377 ± 9†</td>
</tr>
<tr>
<td>Energy intake, kJ/day</td>
<td>250 ± 6</td>
<td>288 ± 10†</td>
<td>321 ± 8*</td>
<td>310 ± 8*</td>
</tr>
<tr>
<td>Uterus, mg</td>
<td>608 ± 77</td>
<td>807 ± 66</td>
<td>105 ± 5*</td>
<td>102 ± 11*</td>
</tr>
<tr>
<td>Uterus/body weight, mg/g</td>
<td>2.14 ± 0.30</td>
<td>2.49 ± 0.26</td>
<td>0.27 ± 0.02*</td>
<td>0.27 ± 0.03†</td>
</tr>
<tr>
<td>Intra-abdominal fat pads, g</td>
<td>15.4 ± 1.8</td>
<td>14.3 ± 0.9</td>
<td>33.4 ± 2.3*</td>
<td>23.4 ± 3.1*</td>
</tr>
<tr>
<td>Intra-abdominal fat pads/body weight, g/g</td>
<td>0.053 ± 0.005</td>
<td>0.044 ± 0.003§</td>
<td>0.084 ± 0.005*</td>
<td>0.061 ± 0.007‡</td>
</tr>
<tr>
<td>Femur weight, mg</td>
<td>728 ± 18</td>
<td>838 ± 39½</td>
<td>772 ± 18</td>
<td>851 ± 13½</td>
</tr>
<tr>
<td>Right atrium, mg</td>
<td>22 ± 3</td>
<td>28 ± 2</td>
<td>32 ± 4</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>Right atrium/body weight, mg/g</td>
<td>0.077 ± 0.010</td>
<td>0.084 ± 0.008</td>
<td>0.080 ± 0.009</td>
<td>0.080 ± 0.012</td>
</tr>
<tr>
<td>Left ventricle, mg</td>
<td>617 ± 28</td>
<td>660 ± 35</td>
<td>732 ± 27*</td>
<td>791 ± 30*</td>
</tr>
<tr>
<td>Left ventricle/femur weight, mg/mg</td>
<td>0.816 ± 0.042</td>
<td>0.750 ± 0.015</td>
<td>0.922 ± 0.032*</td>
<td>0.910 ± 0.043*</td>
</tr>
<tr>
<td>Left ventricle/body weight, mg/g</td>
<td>2.11 ± 0.06</td>
<td>1.99 ± 0.04</td>
<td>1.84 ± 0.03*</td>
<td>2.09 ± 0.09†</td>
</tr>
<tr>
<td>Plasma FFA acids, mM</td>
<td>0.109 ± 0.027</td>
<td>0.035 ± 0.007§</td>
<td>0.124 ± 0.033</td>
<td>0.057 ± 0.010‡</td>
</tr>
<tr>
<td>Plasma TAG, mM</td>
<td>0.839 ± 0.120</td>
<td>0.746 ± 0.088§</td>
<td>0.978 ± 0.174</td>
<td>0.588 ± 0.115†</td>
</tr>
</tbody>
</table>

Significant difference between ovariectomized (OVX) and sham-operated (sham) rats: *P < 0.05; †P < 0.01 (OVX effect). Significantly different from sedentary rats: ‡P < 0.05; §P < 0.01 [training effect (T)]. FFA, plasma free fatty acids; TAG, plasma triacylglycerol.
decreased intra-abdominal fat pad weight, plasma FFA, and plasma TAG (Table 2). Exercise training significantly increased the LV weight/BW ratio in OVX rats to 2.09 ± 0.09 (n = 8, P < 0.01), suggesting the development of physiological hypertrophy. In sham-operated controls, chronic exercise augmented BW to 330 ± 14 g (n = 8, P < 0.05), and LV mass tended to grow from 617 ± 28 to 660 ± 35 mg, but the difference was not statistically significant. Also, exercise training significantly reduced the intra-abdominal fat pad-to-BW ratio, plasma FFA, and TAG (Table 2). Altogether, these data indicate that in both sham-operated and OVX rats, chronic exercise results in significant improvement of body composition, confirming the efficiency of the training program.

Effect of OVX and training on OT in the hypothalamus and heart. Figure 1A shows the changes of OT mRNA in the hypothalamus, the main site of OT synthesis. Ovariectomy decreased OT mRNA, confirming estrogenic control of the hypothalamic OT gene. Exercise training enhanced OT mRNA expression in the hypothalamus of sham-operated controls, confirming the results of Martins et al. (31) and reversed it to normal in OVX rats. On the other hand, ovariectomy did not alter hypothalamic OTR mRNA, and exercise training increased its expression, only in sham-operated animals (Fig. 1B). These results indicated that hypothalamic OT transcripts responded to the physiological changes induced by chronic exercise.

The LV and right atrium (RA) were chosen for evaluation in this study because increased LV ANP mRNA has been observed to be a compensatory mechanism in various forms of overload, and the RA is the main site of ANP synthesis and release (43).

Ovariectomy and training influenced gene expression in both the LV and RA (Figs. 2 and 3). As presented in Table 3, the interaction of ovariectomy and exercise training modulated the mRNA level of OTR and guanylyl cyclase A (GC-A) in the RA as well as ANP, brain type natriuretic peptide (BNP), OTR, and GC-A expression in the LV (P < 0.05). In the case of ANP (P = 0.08) in the RA (Fig. 3) and eNOS (P = 0.07) expression in the LV (Fig. 4), ANOVA of the interaction between ovariectomy and training did not show statistical significance (Table 3). Ovariectomy significantly downregulated ANP, GC-A, and OTR expression in the RA as well as ANP, BNP, and GC-A in the LV, indicating that all these genes are under estrogenic control. The effect of exercise training was especially evident in OTR mRNA in both the LV and RA, causing more than 10-fold increase in OVX rats but only 2- and 4.7-fold in the RA and LV, respectively, in sham-operated animals. The results obtained for the LV by real-time PCR correlated with those of Western blotting (r = 0.67, P = 0.005; Fig. 2B).

In OVX rats, chronic exercise counteracted the effect of estrogen deficiency and resulted in potent (4- to 5-fold) stimulation of ANP gene expression in the LV and RA (Figs. 2 and 3). It also enhanced LV BNP gene expression (Fig. 2D). In sham-operated rats, chronic exercise augmented ANP expression in the RA but not in the LV. Interestingly, GC-A was downregulated by exercise training in the LV and RA of sham-operated controls, but its expression was augmented threefold in the RA of OVX rats subjected to exercise training.

Chronic exercise heightened iNOS expression in control and OVX rats (Fig. 4). However, eNOS only increased in sham-operated controls and remained unchanged in OVX animals. These results were confirmed by Western blotting (Fig. 4C).

**DISCUSSION**

The present study reports alterations of OTR, natriuretic peptides (NPs), and both iNOS and eNOS in the heart of normal and estrogen-deficient rats subjected to chronic exercise training. Chronic exercise reduced plasma FFA, TAG, and intra-abdominal fat pad weight/BW in sham-operated and OVX animals. These beneficial metabolic effects of training were paralleled by the heightened expression of cardiac OTR, NP systems, iNOS, and eNOS.

The key findings were 1) ovariectomy had an adverse effect on BW and plasma lipid profile, induced LV hypertrophy, as determined by augmented LV weight, and increased intra-abdominal fat pad weight. This was accompanied by decreased OTR, ANP, BNP, and GC-A gene expression in both the LV and RA, suggesting that the lower expression of these genes may be related to the detrimental effect of ovariectomy. 2) Chronic (8 wk) exercise training counteracted the effects of OVX, normalizing or enhancing most of these genes in the heart, and supporting the contention that the OT and ANP systems may play a role in cardioprotection. 3) Chronic exercise also enhanced iNOS expression in normal and OVX rats, whereas the effect on eNOS was observed only in control animals. 4) ANP gene expression in the RA of sham-operated and OVX rats paralleled the changes in OTR expression, indicating a close relationship between the cardiac OT and
ANP systems and reinforcing our concept that stimulation of the cardiac OT system is a potent activator of ANP synthesis and release.

Based on these results, we propose a new hypothesis: that the cardioprotective effects of exercise training may be due, at least in part, to stimulation of the cardiac OT system, with subsequent upregulation of the NP and NO systems in the heart. These activations could result in increased cGMP, which exerts beneficial effects on the heart and is associated with a reduced risk of cardiovascular diseases.

An important finding of this study is the first ever demonstration that cardiac OTR respond to estrogen deficiency and to
chronic exercise training. Our interest in the cardiac OT system in relation to physical activity emerged from longitudinal investigations on the role of the brain in the regulation of cardio-renal homeostasis (32). These experiments led to the observation of OT and OTR synthesis in cardiomyocytes, and the identification of OT as a potent, naturally occurring cardiomyogenic factor, which promotes the differentiation of stem cells to cardiomyocytes (19, 39). Furthermore, we showed that activation of cardiac OTR is coupled with ANP release (15). Therefore, the beneficial effects of exercise may be due, at least in part, to activation of cardiac OTR and subsequent enhancement of ANP synthesis and release. However, a direct effect of exercise on the NP system cannot be excluded.

Moreover, recent data indicate that OT per se may exert cardioprotective actions. We demonstrated negative inotropic and chronotropic effects of OT in the isolated dog RA (35) and perfused rat heart (9). Although some contradictory results may be found in the literature, with both pressor and depressor actions of OT reported, most data point to its hypotensive properties. Increased oxytocinergic activity was recently linked to lowered BP and vascular resistance during stress in postmenopausal women on estrogen replacement therapy (28). Another important feature of OT is its role in cell proliferation. OTR mediates cell growth inhibition when coupled with Gi protein (41); therefore, an antihypertrophic impact of OT on the heart cannot be excluded. Additionally, the biological activities of OT may be mediated by natriuretic peptides (14) or NO (34). Both natriuretic peptides, ANP and BNP, are mainly synthesized in the mammalian heart (43). The physiological actions of NPs are conveyed by binding to particulate GC-coupled cell surface functional receptors GC-A and GC-B, whose activation promotes the intracellular generation of cGMP (40). The activation of these receptors leads to a reduction in preload by the elimination of water and sodium, and by shifting plasma volume from intravascular to extravascular spaces (36).

The role of ANP in BP regulation has been established in early research, by direct ANP infusion in humans and animals, and from further evidence derived from transgenic animal models. Mice overexpressing ANP or BNP have significantly lower BP (51), and mice with deleted GC-A have elevated BP and cardiac hypertrophy (23, 29, 38).

This study describes that exercise training-induced increases in atrial and ventricular ANP expression in overweight OVX rats in correlation with the reduction of abdominal fat pad weight, plasma FFA, and TAG levels. Those results are in agreement with the known role of NPs in the regulation of fat metabolism (45). Consistent with our observation of changes in adiposity and plasma lipid profile, ANP could be involved in the alteration of fat metabolism after exercise training. Indeed, ANP-stimulated lipolysis has been demonstrated in both isolated human fat cells (47) and in vivo by ANP infusion. The mechanism of ANP-induced lipolysis is cGMP dependent; therefore, some effects of exercise may be modulated by GC-A. The possible metabolic role of ANP-dependent lipolysis has been suggested by an increase in ANP-dependent lipolysis in obese women fed a low-calorie diet (46). The physiological relevance of the ANP-dependent pathway was recently disclosed by Moro et al. (33) who demonstrated that the lipid-mobilizing action seen under oral beta blockade was related to the effect of ANP. Therefore, exercise-induced higher ANP production may lead to higher lipolytic efficiency in adipose tissue, and this may be an important benefit of exercise training in obese patients. Consistent with these observations are our results showing exercise-induced increases in atrial and ventricular ANP expression in overweight OVX rats, which correlate with the reduction of abdominal fat pad weight, plasma FFA, and TAG levels.

Our results indicate that cardiac ANP is controlled by estrogen, as reported previously by us and others (22, 54). In agreement with this observation, van Eickels et al. (54) showed that estrogen treatment of mice reverses the cardiac hypertrophy induced by transverse aortic constriction, through an increase in ventricular ANP gene expression. Both ANP and BNP are downregulated in OVX animals, and this is accompanied by LV hypertrophy. However, the enhancement of OTR and ANP expression in LV and RA after training was accompanied by further increases in LV mass in the trained OVX group (as measured by the LV/BW index), and heightened OTR expression in the sham-trained group of rats was not accompanied by ANP and BNP changes in the LV. The further increase in LV mass of OVX-T animals may represent physiologic cardiac hypertrophy helping the myocardium to satisfy the higher demands of exercise while maintaining or enhancing

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Table 3. Results of two-way ANOVA analysis of gene expression in hypothalamus, right atrium, and left ventricle of rats subjected to ovariectomy and exercise training

<table>
<thead>
<tr>
<th>Organ</th>
<th>Gene</th>
<th>Interaction</th>
<th>Source of Variation</th>
<th>Ovariectomy</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>OT</td>
<td>3.2</td>
<td>0.11</td>
<td>34.1</td>
</tr>
<tr>
<td></td>
<td>OTR</td>
<td>2.9</td>
<td>0.11</td>
<td>34.1</td>
</tr>
<tr>
<td>Right atrium</td>
<td>OTR</td>
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<td>0.0003</td>
<td>120.3</td>
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<td></td>
<td>ANP</td>
<td>3.7</td>
<td>0.08</td>
<td>169.8</td>
</tr>
<tr>
<td></td>
<td>GC-A</td>
<td>38.2</td>
<td>&lt;0.0001</td>
<td>5.5</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>OTR</td>
<td>4.7</td>
<td>0.048</td>
<td>132.1</td>
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<tr>
<td></td>
<td>ANP</td>
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<td>54.0</td>
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<tr>
<td></td>
<td>BNP</td>
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<td>418.1</td>
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<tr>
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<tr>
<td></td>
<td>eNOSp</td>
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<td>9.8</td>
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P, probability level; F, test value.
normal function. Therefore, the lowered level of GC-A in the LV of rats subjected to exercise training may favor the beneficial hypertrophic cascade in both sham-operated and OVX animals as we already reported in a previous study of physiological cardiac hypertrophy in pregnant rats (22).

Furthermore, our data demonstrate that left ventricular BNP, considered as a marker of pathological hypertrophy, was normalized but not increased upon exercise training, countering the estrogen deficiency effect. At the same time, OTR and ANP, a cardioprotective hormone (36), were significantly augmented above normal levels. Therefore, the elevation of ANP mRNA by regular exercise may be beneficial in pathological ventricular hypertrophy observed in postmenopausal women, and exercise training, by the reconstitution of ANP levels and its effects on GC-A gene expression, may represent a novel approach for treatment.

The biological functions of NPs may be mediated not only by specific membrane-bound receptors but also via NO activation. Recent results presented by Costa et al. (5) suggest that the signaling cascade of ANP involves NOS activation in heart ventricles. NO binds to soluble GC, to increase cGMP and to maintain cardiovascular homeostasis (26). It is widely known that the three isoforms of NOS, eNOS, iNOS, and neuronal NOS, are expressed in various tissues, including the heart. Recent evidence indicates that some physiological responses to chronic exercise in rats may involve the NO pathway (13, 17). In dogs and rats, chronic exercise heightens eNOS expression in aortic endothelial cells (6, 48). Similar effects have been observed in humans, where chronic exercise elevated plasma NO, as measured by stable end products, the nitrates (30). Although the iNOS action in the heart remains controversial and some detrimental outcomes may occur in response to high NO concentrations produced by iNOS, suggesting that cardiac iNOS activation plays a role in the cardioprotective effects of 17β-estradiol (10). Furthermore, iNOS overexpression in the heart of eNOS gene-deleted mice restored, in part, the benefits attributed to eNOS (24).

However, we have not observed the increase in cardiac eNOS expression in exercised OVX rats, which has been shown to be induced in the coronary arteries and microvessels from hearts of exercised dogs (48). The increased eNOS most likely improves the bioavailability of endogenous NO and not only produces vasodilation but also inhibits platelet aggregation and has antioxidant, antiproliferative, and antiapoptotic properties (12). These effects suggest that increased NO production can contribute to control of cardiac hypertrophy during exercise. Lack of eNOS expression increase in LV in response to the exercise training in OVX rats could influence cardiac responses in these animals.

Several studies have shown that the plasma levels of ANP and BNP rise during dynamic exercise, not only in healthy subjects (18), but also in patients with some cardiac pathologies, such as atrial fibrillation (8), left ventricular dysfunction, and congestive heart failure (16, 25). The enhanced synthesis and release of these potent vasodilating and natriuretic agents may represent an important factor for fluid regulation, preventing cardiac hypertrophy and leading to increased lipid mobilization, functions that may significantly help obese patients.

Epidemiological studies indicate that physical training has beneficial effect on the outcome of cardiovascular diseases, via positive metabolic actions, direct vascular function improvement, and decreased BP. The results of our experiments reveal that physical exercise is associated with the activation of three cardiac hormonal systems, OT, NPs, and NOS. The activation of these hormonal systems has been demonstrated to be implicated in attenuating risk factors for cardiovascular diseases, such as hypertension, hypercholesterolemia, insulin resistance, and obesity. Our data support the contention that regularly performed exercise is a useful and inexpensive intervention for the prevention and treatment of these pathologies, particularly in postmenopausal women.

Fig. 4. Left ventricular inducible nitric oxide synthase (iNOS) mRNA (A) and endothelial NOS (eNOS) mRNA (B) expression in sham and OVX rats subjected to exercise training (T), as determined by RT-PCR. The results on eNOS mRNA were confirmed by Western blotting (C). Bars show means ± SE, n = 5, of 2–3 independent experiments. Significance of the data by the post hoc test: *P < 0.05, **P < 0.01, difference between sedentary and trained animals (T effect).
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