Aerobic physical training increases contractile response and reduces cardiac fibrosis in rats subjected to early ovarian hormone deprivation

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Felix AC, Dutra SG, Tezini GC, Simões MV, de Souza HC. Aerobic physical training increases contractile response and reduces cardiac fibrosis in rats subjected to early ovarian hormone deprivation. J Appl Physiol 118: 1276–1285, 2015. First published March 15, 2015; doi:10.1152/japplphysiol.00483.2014.—We investigated the effects of early ovarian hormone deprivation on the heart and the role of physical training in this condition using different approaches: cardiac autonomic tone, contractility, morphology and function, and cardiac fibrosis. Female Wistar rats (n = 48) were assigned into two groups: ovariectomized (Ovx; 10-wk-old) and control rats (Sham; 10-wk-old). Each group was further divided into two subgroups, sedentary and trained (aerobic training by swimming for 10 wk). The sedentary groups showed similar cardiac autonomic tone values; however, only the Sham group had an increase in vagal participation for the determination of the basal heart rate after physical training. The contractile responses to cardiac β-agonists of the sedentary groups were similar, including an increased response to a β1-agonist (dobutamine) observed after physical training. The Ovx sedentary group presented changes in cardiac morphology, which resulted in decreases in the ejection fraction, fractional shortening, and cardiac index compared with the Sham sedentary group. Physical training did little to alter these findings. Moreover, histology analysis showed a significant increase in cardiac fibrosis in the sedentary Ovx group, which was not observed in the trained Ovx group. We conclude that early ovarian hormone deprivation in rats impairs autonomic control, cardiac morphology, and cardiac function and increases cardiac fibrosis; however, it does not affect the contractility induced by dobutamine and salbutamol. Furthermore, this model of physical training prevented an increase in fibrosis and promoted an increase in the cardiac contractile response but had little effect on cardiac autonomic control or morphological and functional parameters.

ovarian hormones; autonomic control; cardiac function; cardiac remodeling; physical training

POSTMENOPAUSAL WOMEN HAVE an increased risk of developing cardiovascular disease compared with men of the same age (3, 75, 77), which has been associated with the loss of the cardioprotective effects of ovarian hormones, particularly estrogen (4, 75). The average age at which menopause occurs in women in developed countries is ~51 yr, but in some cases, it can occur before 40 yr of age, which is defined as early menopause (17, 79a) and is due to many causes, including surgical, genetic, autoimmune, iatrogenic, and idiopathic (13, 39) causes. Early menopause has been associated with a further increased risk of cardiovascular disease and mortality compared with physiological menopause (45, 74).

The cardioprotective effects of ovarian hormones involve different components that are mainly related to cardiovascular control, tissue remodeling, and cardiac function. In this context, studies have shown that estrogen deficiency causes changes in cardiac autonomic influence, reducing vagal participation and increasing the sympathetic drive to the heart (35, 49, 62–63). Furthermore, changes have been observed in the structure and cardiac tissue as a result of pathological hypertrophy and increased fibrosis (1, 9, 22, 55, 76). All of these changes seem to contribute to the development of cardiac dysfunction, which is often observed in experimental and clinical studies (2, 12, 23, 31, 43, 53).

The usual treatment for the prevention of cardiovascular risk as well as other complications arising from menopause involves supplementation with exogenous female sex hormones (estrogen and/or progesterone). However, due to differences in the results obtained with this treatment (30, 36, 40, 60, 72), the use of hormone therapy has been widely investigated by the scientific community, and new forms of therapy have been sought. It is known that physical exercise confers benefits to the cardiovascular system that antagonize the systemic damage promoted by ovarian hormone deficiency.

In fact, physical training has unquestionable beneficial effects on human health and quality of life. Its effects, which have been evaluated in experimental models, involve a range of cardiovascular adaptations, including reductions in blood pressure and bradycardia at rest and a change in autonomic balance to the heart characterized by increased vagal influence (11, 19, 38, 65, 70–71). Furthermore, it has been shown that physical training is beneficial to cardiac remodeling and function (15, 25, 27, 29). Clinical studies have revealed an improvement in left ventricular function in physiological postmenopausal women due to physical training characterized by increases in the ejection fraction (EF) and shortening fraction (FS) (20, 50), while studies of isolated hearts have shown that physical training promotes cardiac remodeling by inducing the development of physiological eccentric hypertrophy (26) and a reduction in cardiac fibrosis (47).

Despite the few reports indicating the beneficial cardiovascular effects of exercise training in menopausal women, no studies have investigated these parameters (autonomic control, histology, function, and cardiac morphology) together, particularly in the assessment of early menopause, as well as the effects of aerobic physical training. Accordingly, we tested the hypothesis that early ovariectomy promotes pathological hypertrophy and increased fibrosis in the heart and consequent loss of cardiac function associated with an imbalance in autonomic tone control. Additionally, physical training could...
counteract the cardiovascular impairment promoted by ovarian hormone deprivation.

Therefore, the aim of this study was to investigate the effects of early ovariectomy on cardiovascular autonomic control and cardiac functional and histomorphologic parameters in young rats as well as the therapeutic role of aerobic physical training.

METHODS

Animals

Research trials were conducted on young female Wistar rats aged 10 wk that weighed ~250 g upon arrival. The rats were supplied by the Animal Facility of the Ribeirão Preto Medical School, University of São Paulo, Brazil, and they were housed in a room with a strictly controlled temperature (21 ± 1°C) and a 12-h light-dark cycle with unrestricted access to tap water and standard rat chow (Nuvilab CR-1; Nuvital). All of the experimental protocols performed in the current study were approved by the Committee on Animal Research and Ethics of the Ribeirão Preto Medical School, University of São Paulo (Protocol No. 178/2011).

Experimental Groups

The animals (n = 48) were assigned to four groups as follows: sedentary Sham rats (n = 12), trained Sham rats (n = 12), sedentary ovariectomized (Ovx) rats (n = 12), and trained Ovx rats (n = 12). Animals in the trained groups were subjected to swimming training over a period of 10 wk before the experimental protocol was performed.

Ovariectomy

At 10 wk of age, the rats were anesthetized with tribromoethanol (250 mg/kg ip; Sigma-Aldrich, St. Louis, MO), 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 was removed. The contralateral ovary was removed in a similar manner. The skin and muscle wall were then sutured with silk thread. All animals received prophylactic antibiotic therapy (penicillin G procaine; 4,000 IU/kg im) following the surgical procedure. Sham rats underwent the same procedure except for the sectioning of the oviducts and the removal of the ovaries. The rats were housed individually, and a 2-wk postsurgical recovery period was allowed. Next, the rats were housed in groups of three per cage (60 × 50 × 22 cm; Insight). Daily vaginal smears were collected from all rats previously described (46). This procedure allowed for the phase of the estrus cycle to be determined by daily analysis of the types of cells that sloughed off of the vaginal epithelium. With this approach, four different stages can be observed as follows: proestrus (nucleated epithelial cells), estrus (cornified cells), metestrus (some cornified cells in addition to nucleated cells and a large number of leukocytes), and diestrus (leukocyte infiltration). Thus collected vaginal fluid was placed on glass slides and examined by light microscopy (×40). In the Sham group, estrous cycle regularity was confirmed by the presence of vaginal epithelial cells characteristic of each of the four stages described above. In the Ovx groups, the absence of the estrous cycle was confirmed by a permanent diestrus phase.

Physical Training

The rats in the training groups underwent a protocol of aerobic physical training that consisted of swimming sessions in a glass aquarium (100-cm long × 80-cm wide × 80-cm high), which allowed for the simultaneous training of six animals. The tank was filled with 50 cm of warm water (30 ± 2°C), which was changed after every group training session.

The training program was conducted in two different stages over a total of 10 wk. The first stage consisted of a 2-wk adaptation period, during which the session length was gradually increased from 5 to 50 min per day, five times per week (in increments of 5 min per day). The second stage consisted of 8 wk of 1-h physical training sessions, five times per week. To evaluate physical training intensity, blood was collected from the tail vein of the animals at the 3rd, 5th, and 8th wk immediately before and after 30 min of exercise, and the lactate concentration was measured (Accutrend Plus; Roche Diagnostics, Mannheim, Germany). The expected lactate level ranged from 5.5 to 6 mmol/l, as previously determined (28). If the animals did not achieve the expected lactate concentration, the level of training exertion was increased by fastening an impermeable, lead-containing Velcro strap to the chest to increase body weight by 2 to 6% (28).

Experimental Protocol

Echocardiography. At 22 wk of age, half of the rats (n = 6) were subjected to echocardiography using an ultrasound Vevo 2100 High-Resolution Imaging System (VisualSonics, Toronto, ON, Canada) with a high-resolution transducer at 21 MHz. For this procedure, the anterior regions of the thorax had been previously trichotomized (Veet; Reckitt Benckiser, São Paulo, SP, Brazil), and all animals were anesthetized with 1.5% isoflurane supplemented with 1% O2 and placed on a heated (37°C) platform. Electrocardiogram and temperature were monitored.

High-resolution B-mode and M-mode images were acquired. Wall thicknesses and left ventricle dimensions were obtained from a short-axis view at the level of the papillary muscles. Diastolic measurements were performed at the point of greatest cavity dimension, and systolic measurements were conducted at the point of minimal cavity dimension. All measurements were performed according to the standards of the American Society of Echocardiography (61) and were carried out by a single individual who was blind to the characteristics of each group.

The following parameters were obtained from the images: interventricular septum thickness (IVST), posterior wall thickness (PWT), end-diastolic diameter of the left ventricle (LVEDD), and end-systolic diameter of the left ventricle (LVESD). The shortening fraction was calculated as follows: \( FS(%) = \frac{LVESV-LVEDV}{LVEDV} \times 100 \), and the ejection fraction (EF) was calculated according to Teichholz as follows: \( \text{EF} = \frac{\text{LVEDV} - \text{LVESV}}{\text{LVEDV}} \times 100 \) (69). The left ventricle mass (LV mass/final body weight) was obtained with the following formula: 1.047 × \( \left( \frac{\text{LVEDD} + \text{PWD} + \text{IVST}}{3} - \text{LVEDD} \right)^3 \) (44), and the relative wall thickness (RWT) was calculated as follows: \( \left( \frac{2 \times \text{PWD} \times \text{LVEDD}}{\text{LVEDD}} \right) \) (58, 79). For the quantifications of left ventricular volumes, the following formula was used: \( \text{LVEDV} = \text{LVESV} \times (7 + 2.4 \times \text{LVEDD}^3) \) and \( \text{LVEDV} = \text{LVESV} \times (7 + 2.4 \times \text{LVEDD}^3) \) (69).

Recording of arterial pressure and heart rate. At 22 wk of age, the other half of the rats (n = 6) were anesthetized with tribromoethanol (250 mg/kg ip; Sigma-Aldrich) and polyethylene catheters made in our laboratory (PE-50 soldered to PE-10; Intramedic; Clay Adams, Parsippany, NJ) were implanted into the left femoral artery for the subsequent recording of hemodynamic parameters, systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), and heart rate (HR) and into the left femoral vein for drug administration. Catheters were tunneled subcutaneously and exteriorized at the nape. To prevent blood from clotting, the catheters were filled with a heparinized saline solution (500 IU/ml). The rats were then allowed to recover for 24 h before the cardiac sympathovagal assessment protocol, which was carried out without anesthesia.

Cardiac sympathovagal balance. Twenty-four hours after the surgical procedure, the femoral artery catheter was attached to a pressure transducer (MLT844; ADInstruments, Bella Vista, Australia), which converts blood pressure fluctuations into electrical signals. Next, signals were amplified through a bridge amplifier.
Cardiac contractility. For isolation and perfusion of rat hearts, following echocardiography, rats were anesthetized and administered heparin (5,000 IU/kg iv). After a 15-min period, the animals were killed by cervical vessel transection. Following exsanguination, the hearts were quickly excised and perfused at a constant flow of 10 ml/min with Krebs buffer (in mM: 118.4 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄·7H₂O, 25.0 NaHCO₃, 1.2 KH₂PO₄, 11.2 glucose, and 2.0 pyruvic acid) through a cannula inserted into the aorta. The nutrient solution was continuously gassed with 95% O₂-5% CO₂ (pH 7.4) at a pressure of 80 cm of H₂O and maintained at 37°C. The coronary perfusion pressure was measured using a pressure transducer (HP-1280 C; Hewlett-Packard). To verify the viabilities of the preparations, ventricular contractility was monitored throughout the experiment. A metal hook coupled to a force transducer (Statham) was placed in the heart apex, and an initial tension of 6 g was applied to the organ. The coronary perfusion pressure and force of contraction were recorded using a polygraph (R 611; Beckman). After a 30-min period of stabilization, a dose-response curve was obtained for dobutamine and salbutamol. Increasing doses of the drugs (1–100 nmol) were applied. The maximum responses to dobutamine and salbutamol were recorded for 30 min, atropine was injected into half of the animals of the same group, and HR was recorded for another 15 min to determine IHR. All sequences were reversed again after 24 h. Data from the atropine-propranolol and propranolol-atropine sequences were pooled to determine basal HR (before any previous sequence) and IHR. In half of the animals, the atropine-propranolol sequence was reversed to propranolol-atropine, following the same recording procedure (15 min) for each drug as that used in the previous sequence to determine IHR. All sequences were reversed again after 24 h. Data from the atropine-propranolol and propranolol-atropine sequences were pooled to determine basal HR (before any drugs) and IHR.

Histological analysis. The hearts from the cardiac sympathovagal balance protocol were removed and weighed for normalization according to body weights [relative heart weight (mg/g) = heart weight/ final body weight] and immediately fixed in 10% formaldehyde. Subsequently, the hearts were sectioned transversely at the level of the middle third of the ventricle at a 3-mm thickness. After collection, the two fragments were processed for routine paraffin embedding. Paraffin blocks were cut, obeying the transverse direction in the middle third of the ventricle (5-μm thickness), and stained with Picro-Sirius Red for the quantification of the areas of cardiac fibrosis.

For the quantification of fibrosis, we used a common light microscope (AxioLab; Carl Zeiss, Oberkochen, Germany) coupled to a polarizing filter that detected the birefringence of collagen fibers. The images of the left ventricle were captured with a high-resolution camera (AxioCam-MCR; Carl Zeiss) that was adapted for light microscopy (×40; AxioLab; Carl Zeiss) and were transmitted to a computer using AxioVision Rel 4.8 software (Carl Zeiss). Twenty random fields for each heart were recorded, and the quantification of fibrosis corresponding to each field was calculated according to the percentage of occupied area using ImageJ software (National Institutes of Health, Bethesda, MD).

RESULTS

Hemodynamic Values

Table 1 shows the baseline AP and HR values for the different groups studied. The results showed that ovariectomy and physical training did not alter the SAP, DAP, or MAP. Moreover, the groups undergoing physical training had bradycardia compared with their respective sedentary counterparts (physical training factor, F(1,20) = 29.9; P < 0.001).

Table 1. Values for the arterial pressure and heart rate, before and after autonomic pharmacological blockade with atropine and propranolol observed in the control and ovariecotimized rats, both sedentary and trained

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 12)</th>
<th>Ovx (n = 12)</th>
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<tbody>
<tr>
<td></td>
<td>Sedentary</td>
<td>Trained</td>
<td>Sedentary</td>
<td>Trained</td>
<td>F_1,20</td>
<td>P</td>
<td>F_1,20</td>
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<td>Baseline values</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SAP, mmHg</td>
<td>113 ± 3</td>
<td>114 ± 3</td>
<td>112 ± 2</td>
<td>118 ± 2</td>
<td>0.4</td>
<td>NS</td>
<td>2.1</td>
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<tr>
<td>DAP, mmHg</td>
<td>81 ± 5</td>
<td>77 ± 3</td>
<td>82 ± 2</td>
<td>81 ± 2</td>
<td>0.8</td>
<td>NS</td>
<td>0.8</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>94 ± 4</td>
<td>91 ± 2</td>
<td>94 ± 1</td>
<td>96 ± 1</td>
<td>1.5</td>
<td>NS</td>
<td>0.1</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>360 ± 2</td>
<td>330 ± 5*</td>
<td>362 ± 5</td>
<td>332 ± 8†</td>
<td>0.1</td>
<td>NS</td>
<td>29.9</td>
</tr>
<tr>
<td>Tonic autonomic control</td>
<td></td>
<td></td>
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<tr>
<td>∆HR atropine, beats/min</td>
<td>76 ± 9</td>
<td>103 ± 10*</td>
<td>78 ± 10</td>
<td>81 ± 14</td>
<td>1.1</td>
<td>NS</td>
<td>12.7</td>
</tr>
<tr>
<td>%HR atropine, beats/min</td>
<td>67 ± 4</td>
<td>88 ± 2*</td>
<td>68 ± 3</td>
<td>73 ± 6</td>
<td>2.8</td>
<td>NS</td>
<td>10.0</td>
</tr>
<tr>
<td>∆HR propranolol, beats/min</td>
<td>37 ± 6</td>
<td>15 ± 4</td>
<td>36 ± 3</td>
<td>26 ± 3</td>
<td>0.7</td>
<td>NS</td>
<td>1.9</td>
</tr>
<tr>
<td>%HR propranolol, beats/min</td>
<td>33 ± 4</td>
<td>12 ± 2*</td>
<td>32 ± 3</td>
<td>27 ± 6</td>
<td>2.8</td>
<td>NS</td>
<td>10.0</td>
</tr>
<tr>
<td>IHR, beats/min</td>
<td>362 ± 2</td>
<td>326 ± 5*</td>
<td>366 ± 3</td>
<td>324 ± 4†</td>
<td>0.1</td>
<td>NS</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Values are expressed as the means ± SE. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; HR, heart rate; IHR, intrinsic heart rate; Sham, control group; Ovx, ovariecotimized group; NS, not significant. *P < 0.05, compared with the sedentary Sham group; †P < 0.05, compared with the sedentary Ovx group.
Cardiac morphology

Parameters

General characteristics and cardiac morphology parameters observed in the control and ovariectomized rats, both sedentary and trained.

Table 2. General characteristics and cardiac morphology parameters observed in the control and ovariectomized rats, both sedentary and trained.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham (n = 12)</th>
<th>Ovx (n = 12)</th>
<th>Ovariectomy Factor</th>
<th>Physical Training Factor</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sedentary</td>
<td>Trained</td>
<td>F(DF) P</td>
<td>F(DF) P</td>
<td>F(DF) P</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>374 ± 7</td>
<td>374 ± 5</td>
<td>443 ± 12*</td>
<td>430 ± 12†</td>
<td>F(1,20): 43.9; &lt;0.001</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>0.93 ± 0.02</td>
<td>1.13 ± 0.02*</td>
<td>1.04 ± 0.03*</td>
<td>1.19 ± 0.02†</td>
<td>F(1,20): 8.1; 0.01</td>
</tr>
<tr>
<td>Relative heart weight,</td>
<td>2.49 ± 0.09</td>
<td>3.03 ± 0.08*</td>
<td>2.37 ± 0.13</td>
<td>2.78 ± 0.07†</td>
<td>F(1,20): 3.5; NS</td>
</tr>
<tr>
<td>mg/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cardiac morphology</td>
<td></td>
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</tr>
<tr>
<td>PWT, mm</td>
<td>1.75 ± 0.06</td>
<td>1.72 ± 0.09</td>
<td>1.81 ± 0.06</td>
<td>1.51 ± 0.07†</td>
<td>F(1,20): 8.5; 0.01</td>
</tr>
<tr>
<td>IVST, mm</td>
<td>1.69 ± 0.04</td>
<td>1.43 ± 0.06*</td>
<td>1.50 ± 0.03*</td>
<td>1.34 ± 0.04†</td>
<td>F(1,20): 7.3; 0.01</td>
</tr>
<tr>
<td>RWT, mm</td>
<td>0.45 ± 0.02</td>
<td>0.43 ± 0.02</td>
<td>0.47 ± 0.02</td>
<td>0.37 ± 0.01†</td>
<td>F(1,20): 1.2; 0.01</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>7.71 ± 0.10</td>
<td>7.86 ± 0.23</td>
<td>7.76 ± 0.25</td>
<td>8.09 ± 0.17</td>
<td>F(1,20): 0.4; NS</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>4.49 ± 0.19</td>
<td>5.27 ± 0.21*</td>
<td>5.05 ± 0.20*</td>
<td>5.59 ± 0.20†</td>
<td>F(1,20): 5.2; 0.03</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>979 ± 31</td>
<td>900 ± 77</td>
<td>943 ± 69</td>
<td>831 ± 64</td>
<td>F(1,20): 0.6; NS</td>
</tr>
</tbody>
</table>

All values are presented as the means ± SE. PWT, posterior wall thickness; IVST, interventricular septum thickness; RWT, relative wall thickness; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LV, left ventricular. *P < 0.05, compared with the sedentary Sham group; †P < 0.05, compared with the sedentary Ovx group; ‡P < 0.05, compared with the trained Sham group.

The sedentary Ovx group showed higher body weight [ovariectomy factor, F(1,20): 43.9; P < 0.001] and absolute heart weight [ovariectomy factor, F(1,20): 8.1; P = 0.01] compared with the sedentary Sham group. However, the values for relative heart weight were similar between the groups.

After physical training, both the Sham and the Ovx groups showed the highest absolute [physical training factor, F(1,20): 34.5; P < 0.001] and relative heart weights [physical training factor, F(1,20): 21.7; P < 0.001] compared with their respective sedentary counterparts. In addition, when the trained groups were compared, the trained Ovx group showed only a higher body weight [ovariectomy factor, F(1,20): 43.9; P < 0.001].

Morphological Parameters

Table 2 also shows the absolute values for the morphological parameters. The sedentary Sham and Ovx groups showed similar values for PWT, RWT, LV mass, and LVEDD values. However, the sedentary Ovx group showed lower values for IVST [ovariectomy factor, F(1,20): 7.3; P = 0.01] and augmentation of LVESD [ovariectomy factor, F(1,20): 5.2; P = 0.03].

Moreover, following training, the Sham group showed a reduction in IVST [physical training factor, F(1,20): 17.2; P < 0.001] and an increase in LVEDD [physical training factor, F(1,20): 11.8; P = 0.003] compared with their respective control group. On the other hand, the trained Ovx group presented with reduced PWT [physical training factor, F(1,20): 4.5; P = 0.04], IVST [physical training factor, F(1,20): 17.2; P < 0.001], and RWT values [physical training factor, F(1,20): 6.2; P = 0.02] and an augmentation of the LVEDS value [physical training factor, F(1,20); 11.8; P = 0.003] compared with the sedentary Ovx group.

Table 3 shows the values for the morphological parameters normalized by body weight. The sedentary Ovx group showed smaller values for IVST [ovariectomy factor, F(1,20): 39.6; P < 0.001], LVEDD [ovariectomy factor, F(1,20): 26.0; P < 0.001], and LV mass/final body weight [ovariectomy factor, F(1,20): 9.0; P = 0.007] compared with the sedentary Sham group.

Furthermore, following training, the Sham group showed a reduction in IVST [physical training factor, F(1,20): 10.9; P = 0.003] and an increase in LVEDD [physical training factor, F(1,20): 11.8; P = 0.003] compared with their respective sedentary group.
Table 3. Cardiac morphology parameters normalized for body weight observed in the control and ovariectomized rats, both sedentary and trained

<table>
<thead>
<tr>
<th>Cardiac Morphology</th>
<th>Sham (n = 12)</th>
<th>Ovx (n = 12)</th>
<th>Ovariectomy Factor</th>
<th>Physical Training Factor</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sedentary</td>
<td>Trained</td>
<td>P(SED)</td>
<td>P(TRA)</td>
<td>P(SPD)</td>
</tr>
<tr>
<td>PWT, mm/kg</td>
<td>4.60 ± 0.12</td>
<td>4.61 ± 0.29</td>
<td>4.12 ± 0.21</td>
<td>3.56 ± 0.25†</td>
<td>F(1,20): 1.24</td>
</tr>
<tr>
<td>LVST, mm/kg</td>
<td>4.54 ± 0.08</td>
<td>3.84 ± 0.21*</td>
<td>3.41 ± 0.10*</td>
<td>3.14 ± 0.13‡</td>
<td>F(1,20): 39.6</td>
</tr>
<tr>
<td>RWT, mm/kg</td>
<td>1.21 ± 0.04</td>
<td>1.17 ± 0.08</td>
<td>1.07 ± 0.07</td>
<td>0.88 ± 0.06</td>
<td>F(1,20): 11.0</td>
</tr>
<tr>
<td>LVEDD, mm/kg</td>
<td>20.65 ± 0.51</td>
<td>21.02 ± 0.47</td>
<td>17.63 ± 0.51*</td>
<td>18.87 ± 0.55</td>
<td>F(1,20): 26.0</td>
</tr>
<tr>
<td>LVESD, mm/kg</td>
<td>12.06 ± 0.68</td>
<td>14.10 ± 0.56*</td>
<td>11.41 ± 0.43</td>
<td>13.04 ± 0.40‡</td>
<td>F(1,20): 2.5</td>
</tr>
<tr>
<td>LV mass, mg/g</td>
<td>2.61 ± 0.04</td>
<td>2.40 ± 0.21</td>
<td>2.13 ± 0.15*</td>
<td>1.94 ± 0.16‡</td>
<td>F(1,20): 9.0</td>
</tr>
</tbody>
</table>

All values are presented as the means ± SE. *P < 0.05, compared with the sedentary Sham group; †P < 0.05, compared with the sedentary Ovx group; ‡P < 0.05, compared with the trained Sham group.

On the other hand, the trained Ovx group only presented with an increase in LVESD [physical training factor, F(1,20): 11.8; P = 0.003] compared with the sedentary Ovx group.

**Functional Parameters**

Table 4 shows that the sedentary Ovx group exhibited reductions in the index [ovariectomy factor, F(1,20): 9.6; P = 0.006], EF [ovariectomy factor, F(1,20): 6.0; P = 0.02], and FS [ovariectomy factor, F(1,20): 7.9; P = 0.01] in association with an increase in LVEDV [ovariectomy factor, F(1,20): 4.6; P = 0.06] compared with the sedentary Sham group.

Physical training of the Sham group promoted reductions in EF [physical training factor, F(1,20): 15.2; P < 0.001] and FS [physical training factor, F(1,20): 16.7; P < 0.001]; this also resulted in an increase in LVESV [physical training factor, F(1,20): 11.5; P = 0.003]. Physical training of the Ovx group promoted an increase only in LVESV [physical training factor, F(1,20): 11.5; P = 0.003].

However, cardiac output, stroke volume, and LVEDV were similar among all groups.

**Cardiac Contractility–Dobutamine and Salbutamol**

Table 5 shows the values for cardiac contractility at baseline and after the administration of multiple doses of dobutamine (β1-adrenergic agonist, 0.5–50 nmol) and salbutamol (β2-adrenergic agonist, 0.5–50 nmol), while Fig. 2 shows the maximum contractile responses (50 nmol). The administration of salbutamol and dobutamine similarly induced dose-dependent contraction in all groups (Fig. 2, A and C).

The sedentary Sham and Ovx groups showed similar responses with regard to basal contractility after the administration of both drugs. On the other hand, the physical training of the Sham and Ovx groups (Fig. 2, B and D) resulted in similar increases in the basal contractile response [physical training factor, F(1,20): 13.7; P = 0.001] and in the maximum contractile response after the administration of dobutamine [physical training factor, F(1,20): 6.1; P = 0.02].

**Percentage of Cardiac Fibrosis–Histological Analysis**

Figure 3 shows the results of histological analysis for the determination of cardiac fibrosis (show as percentages). The sedentary Ovx group showed a significant increase in cardiac fibrosis compared with the sedentary Sham group [ovariectomy factor, F(1,20): 23.8; P < 0.001; Fig. 3, A and C]. On the other hand, this increase was not observed in the trained Ovx group [physical training factor, F(1,20): 24.6; P = 0.001], which showed similar values as the Sham groups (Fig. 3, B, D, and E).

**DISCUSSION**

We evaluated important parameters that guide cardiovascular homeostasis, including cardiac autonomic control, histomorphology, and cardiac function, in young ovariectomized rats. We also investigated the therapeutic effects of physical training for the prevention and treatment of possible heart damage arising from the loss of ovarian function.

Our findings showed that ovariectomy caused significant damage to cardiac morphology and histology in young rats that interfered with the function of the left ventricle, but no changes occurred in autonomic balance or cardiac contractility. On the other hand, low-intensity aerobic exercise prevented the increase in cardiac fibrosis in these rats, modified some morphological and functional parameters, and increased cardiac contractility; however, it did not affect cardiac autonomic balance, as observed in the Sham group.
Aerobic physical training is known to improve cardiovascular autonomic control. Our study showed that young sham rats subjected to low-intensity aerobic exercise showed an increase in vagal participation and/or a reduction in sympathetic participation in cardiac autonomic balance, which was associated with a reduction in basal HR and IHR. These adaptations were expected, because previous studies have shown similar results (32, 52), including those performed in our laboratory (65, 70–71).

Indeed, reductions in basal HR and IHR have been extensively characterized and investigated by several studies (52). However, the adjustments in cardiac autonomic balance are of interest, mainly because the mechanisms involved are still uncertain. One of the most quoted hypotheses is that aerobic physical training promotes adaptations in central neural regions, such as the hypothalamus, the nucleus of the solitary tract, and the rostral ventrolateral medulla, promoting a reduction in the sympathetic autonomic drive to the heart (21, 33, 41, 48, 64, 81–82).

However, physical training did not seem to promote any changes in the autonomic components involved in determining HR in the young rats that underwent early ovariectomy in contrast with the sham group, suggesting that ovarian...
hormones may influence cardiac autonomic control. This suggestion is based on previous studies that have show that estrogen deficiency can interfere with autonomic cardiovascular control and affect areas of the central nervous system. This possible central nervous system action is based on the identification of estrogen receptors in central nuclei that are involved in cardiac autonomic control (56, 66). Although the underlying mechanisms have not yet been defined, it is known that the blockade or activation of these receptors at specific sites seems to produce different cardiovascular responses (62, 63). Although our results did not reveal the underlying mechanisms, we have demonstrated strong evidence of the importance of ovarian hormones in cardiac autonomic control, and these findings should be further investigated in the future.

**Body Weight, Heart Weight, and Relative Heart Weight**

Our results showed that the loss of ovarian function promoted an increase in final body weight, confirming previous results of clinical and experimental studies (16, 68). With respect to absolute heart weight, we observed that both ovariectomy and exercise training were responsible for higher values compared with the control groups. However, when the values were normalized, only physical training was responsible for the increase in heart weight. This relative increase in heart weight is associated with adaptations induced by aerobic exercise due to increased preload (6).

**Cardiac Morphological and Functional Parameters**

The adaptations promoted by aerobic exercise training on cardiac remodeling and function in rats as well as the involvement of ovarian hormones in these processes are also controversial. Our results showed that the young sham rats subjected to low-intensity aerobic physical training had reduced EF but no changes in cardiac output or cardiac index. However, morphological assessment showed reductions in IVST and FS, resulting in an increased in LVESD and the consequent enhancement of LVESV, as shown by both the absolute and normalized values. Despite these findings, we emphasize that cardiac output and cardiac index were not affected, possibly because of the upward trend of LVEDV.

Regarding the effects of ovariectomy on cardiac morphologic and functional parameters, our study showed significant changes characterized by increased fibrosis, decreased IVST and increased LVESD (for the absolute values) and decreases in the normalized values for IVST, LVEDD, and LV mass. As a consequence, these alterations led to a reduction in FS and an increase in LVESV, resulting in reductions EF and cardiac index.

Indeed, ovariectomy appears to promote changes in parameters similar to those induced by physical training, although these alterations most likely occurred by different mechanisms, because in contrast with the animals that underwent physical training, the ovariectomized rats showed additional reductions in LV mass/final body weight and cardiac index. The cause of these findings is unknown. However, considering these results together with the increased fibrosis that was detected suggests that a loss of cardiomyocytes occurred due to ovariectomy, leading to the antiapoptotic activity of estrogens (24, 34, 54, 67) in addition to their beneficial effects on cardiac function (18, 78).

Some studies have also shown that estrogens may be related to cardiac fibrosis because they appear to inhibit transforming growth factor-β1 (TGF-β1) and the enzyme c-Jun NH2-terminal (JNK) kinase, which are considered to play main roles in the differentiation of fibroblasts into myofibroblasts. These cells are responsible for producing collagen in the heart (51, 55). However, despite increased fibrosis, the assessment of contractility by the administration of dobutamine and salbutamol showed similar results between the Ovx and Sham groups (both trained and sedentary), indicating that at least in young rats, increased cardiac apoptosis and fibrosis resulting from a decrease in estrogen did not affect contractility as opposed to what we have recently observed in aged rats (70).

In addition, aerobic physical training prevented the development of cardiac fibrosis in ovariectomized rats, demonstrat-
ing the cardioprotective effects of exercise in this experimental model due to the disproportionate increase in collagen, which has been reported to promote alterations in the electrical, mechanical, and metabolic properties of the heart (7). This cardioprotection was the main finding of the current study, primarily because the observations were performed on young rats and because there was no therapeutic strategy for counteracting the development of fibrosis in the heart (8). These results corroborate with previous studies that have been conducted using old male and female rats that have demonstrated that physical training promotes lower collagen levels in the heart (14, 42, 73, 80), including the hearts of ovariectomized, spontaneously hypertensive rats (47). The mechanisms responsible for the reduction in cardiac fibrosis induced by physical training were not fully identified. However, in an experimental model of heart failure, physical training has been observed to promote decreased levels of angiotensin II, which is a major model of heart failure, physical training has been observed to promote decreased levels of angiotensin II, which is a major factor related to increased fibrosis in the heart (57). Additionally, a recent study has also shown that aerobic exercise inhibits TGF-β1 and tissue inhibitor of metalloproteinases (TIMP-1), which are directly involved in the increased synthesis and accumulation of collagen (42).

With respect to morphological and functional parameters, we observed that physical training in the Ovx group promoted reductions in PWT, IVST, and RWT (for the absolute values only) and increases in the absolute and normalized values of LVESD accompanied by an increase in LVEFV. Physical training was observed to promote the attenuation of the decrease in the cardiac index, although this result was not significant. These absolute values differ from those reported by a previous study that also evaluated morphofunctional parameters in trained ovariectomized young rats, showing increases in LV mass and RWT but no change in FS (10). However, considering the normalized values, the majority of cardiac morphological parameters assessed in our study corroborate with the findings of a previous study that did not observe morphological changes in female rats subjected to aerobic exercise training (37).

We again emphasize the difficulty in comparing our results with those of the above-mentioned study due to several differences, including the differing ages and species of the animals assessed as well varying methodologies employed and accuracy of the equipment. Additionally, in the previously mentioned study, there was no calculation of functional parameters, such as output or cardiac index, once again highlighting the difficulties comparing two the studies.

In summary, it seems that ovariectomy causes important effects on histomorphology and cardiac function, as evidenced by the reductions in IVST and LV mass/final body weight as well as the reductions in the functional parameters. In the cardiac tissue, we observed an increase in cardiac fibrosis, which may have been due the significant reduction in LV mass/final body weight. Additionally, ovarian hormone deprivation appeared to attenuate adaptations in cardiac tonic autonomic balance resulting from aerobic exercise, suggesting that the local tissue changes may have influenced this response.

Finally, the most relevant finding of this study was that physical training can help to prevent the development of fibrosis in young ovariectomized rats, representing an important preventive strategy to reduce one of the main factors associated with dysfunction and heart failure, since fibrosis itself is considered to be a predictive factor for increased cardiovascular morbidity and mortality.

Conclusion

Our findings indicate that the early loss of ovarian function promotes significant changes in remodeling and cardiac function that are mainly characterized by a reduction in LV mass/final body weight and an increase in cardiac fibrosis as well a reduction in the cardiac index. However, despite these changes, ovariectomized rats exhibit the maintenance of cardiac autonomic balance and the cardiac contractile response.

Our results showed that the model of physical training that was used did not change the morphological parameters observed following ovariectomy. However, physical training promoted the attenuation of the decrease in the cardiac index and was responsible for preventing the development of cardiac fibrosis induced by ovariectomy in the young rats.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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