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Exercise training associated with estrogen therapy induced cardiovascular benefits after ovarian hormones deprivation

Karin Flues[a], Janaina Paulini[a], Sebastião Brito[b], Iris Callado Sanches[b], Fernanda Consolim-Colombo[a], Maria-Cláudia Irigoyen[a], Kátia De Angelis[b,*]

[a] Hypertension Unit, Heart Institute (InCor), University of Sao Paulo Medical School, Sao Paulo, Brazil
[b] Human Movement Laboratory, Sao Judas Tadeu University, Rua. Taquari, 546 Sao Paulo, Sao Paulo 03166-000, Brazil

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ABSTRACT

Menopause is recognized as a period of increased risk for coronary heart disease. Although the benefits of exercise training in lowering cardiovascular risk factors are well established, the risks and benefits of hormone therapy have been questioned. The purpose of the present study was to investigate the effects of estrogen therapy (HT) associated or not with exercise training (ET) in autonomic cardiovascular control in ovariectomized (OVX) rats. Female rats were divided into: control, OVX, OVX + HT, OVX + ET and OVX + HT + ET. HT was performed using a 0.25 mg 8-weeks sustained release pellet. Trained groups were submitted to an 8-week exercise training protocol on treadmill. Baroreflex sensitivity (BRS) was evaluated by heart rate responses to arterial pressure (AP) changes, and vagal and sympathetic tonus by pharmacological blockade. Ovariectomy induced an AP increase (123 ± 2 mmHg vs. 108 ± 2 mmHg), BRS impairment (~69%), sympathetic activation (~100%) and vagal tonus reduction (~77%) compared to controls. HT or ET normalized the changes in parasympathetic tonus. However, only the association HT + ET was able to promote normalization of AP, BRS and sympathetic tonus, as compared to controls. These results indicate that ET induces cardiovascular and autonomic benefits in OVX rats under HT, suggesting a positive role of this association in the management of cardiovascular risk factor in postmenopausal women.

1. Introduction

Heart disease is a major cause of illness and death in women, and its occurrence is increased after menopause [1]. Although the causes of higher cardiovascular morbidity and mortality in postmenopausal women are not well understood, they may involve changes in arterial pressure (AP) and its regulation after estrogen deprivation [2–4]. Moreover, low arterial baroreflex sensitivity (BRS), a marker of autonomic control, is associated with both higher AP and severity of cardiovascular disease [5,6].

Despite the description of a number of biologically plausible mechanisms for coronary protection from estrogen, results of well-designed and conducted primary and secondary prevention randomized clinical trials of hormone therapy (HT) documented cardiovascular risk rather than protection [7,8]. Since the Women's Health Initiative Study [9] has made medical practitioners review the risks and benefits to each patient and made women reconsider the use of HT, according to U.S. Preventive Services Task Force [5], the significance of lifestyle and its impact on cardiovascular function for menopause management are of increasing relevance.

According to the consistent benefits of regular physical exercise to general population [10], a systematic review of randomized controlled trials reported benefits of exercise on metabolic and cardiovascular parameters in postmenopausal women [11]. Recently, we have demonstrated that female ovariectomized (OVX) rats had bradycardia, reduced AP and BRS improvement after 8 weeks of exercise training [12].

Nowadays, the trend is to redirect focus to proved coronary risk reduction interventions for menopausal women, such as smoking cessation, a heart-healthy diet, physical activity, weight management and pharmacologic control of hypertension and hypercholesterolemia [1,8]. However, the effects of estrogen therapy associated with exercise training are not well known. Therefore, the purpose of the present study was to investigate the effects of HT associated or not with 8 weeks of exercise training in autonomic cardiovascular control in OVX rats.

* Corresponding author. Tel.: +55 11 27991909x1635; fax: +55 11 27991909x1634.
E-mail address: prof.kangelis@usjt.br (K. De Angelis).

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2. Methods

2.1. Animals

Experiments were performed on 35 female virgin Wistar rats (192 ± 4 g) from the Animal Shelter of University of Sao Paulo, Sao Paulo, Brazil, receiving standard laboratory chow and water *ad libitum*. The animals were housed in individual cages in a temperature-controlled room (22 °C) with a 12-h dark–light cycle. All rats were treated similarly in terms of daily manipulation. All surgical procedures and protocols used were approved by the Experimental Animal Use Committee of the Sao Judas University and were conducted in accordance with National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals. The rats were randomly assigned to one of four groups: sedentary control (SC, $n = 7$), sedentary OVX (SO, $n = 10$), sedentary OVX + estrogen therapy (SOE, $n = 9$), trained OVX (TO, $n = 8$) and trained OVX + estrogen therapy (TOE, $n = 9$).

2.2. Ovariectomy

At 10 weeks of age, animals were anesthetized (Ketamine 80 mg/kg + Xylazin 12 mg/kg), and a small abdominal incision was made. The ovaries were then located. The oviduct was sectioned, and the ovary was removed [12,13].

2.3. 17β-Estradiol replacement

Seven days after ovariectomy and under the same anesthesia, the SOE and TOE groups were subcutaneously implanted with a pellet releasing 1.5 mg/day 17β-estradiol (Innovative Research of America, Toledo, OH over an 8-week period). As reported recently, the concentrations of 17β-estradiol decreases in OVX rats and increases after 17β-estradiol pellets implantation in these animals [13]. In this study, the estrogen concentration, measured by immunoassay, was non-detectable in SO and TO groups, and the estrogen concentration was similar between SC, SOE and SOT groups.

2.4. Exercise training

All animals were adapted (beginning 24 h after ovariectomy) to the treadmill (10 min/day; 0.3 km/h) for 1 week prior to beginning the exercise training protocol. A maximal treadmill test [12,14,15] was performed in all groups: (1) at the beginning of the experiment; (2) in the fourth and (3) eighth weeks of the training protocol. The purpose was to determine aerobic capacity and exercise training intensity. Exercise training was performed on a motor treadmill at low-moderate intensity (~50–70% maximal running speed) for one hour a day, 5 days a week for 8 weeks, with a gradual increase in speed from 0.3 to 1.2 km/h [12,14].

2.5. Cardiovascular assessments

After the last training session, 2 catheters filled with 0.06 mL saline were implanted into the carotid artery and jugular vein (PE-10) of the anesthetized rats anesthetized (Ketamine 80 mg/kg + Xylazin 12 mg/kg) for direct measurements of AP and drug administration, respectively. Rats received food and water *ad libitum* and were studied 1 day after catheter placement; the rats were conscious in their cages and allowed to move freely during the experiments. The arterial catheter was connected to a transducer (Blood Pressure XDCR, Kent© Scientific, Litchfield, CT), and AP signals were recorded over a 30-min period by a microcomputer equipped with an analog-to-digital converter board (CODAS, 2-kHz sampling frequency; Dataq Instruments, Inc., Akron, OH) [12,14,16].

Increasing doses of phenylephrine (0.25–32 μg/kg) and sodium nitroprusside (0.05–1.6 μg/kg) were given as sequential bolus injections (0.1 mL) to produce AP responses ranging from 5 to 40 mmHg. A 3–5 min interval between doses was necessary for AP to return to baseline. BRS was evaluated by a mean index, relating changes in heart rate (HR) to the changes in mean AP (MAP) after phenylephrine or sodium nitroprusside injection, allowing a separate analysis of reflex bradycardia and reflex tachycardia, respectively [12,14,16].

After BRS evaluation, resting HR was recorded and methylatropine (3 μg/kg, *iv.*) was injected. Because the HR response to these drugs reaches its peak within 3–5 min, this time interval was allowed to elapse before HR measurement. Atenolol (8 μg/kg, *iv.*) was injected 10 min after methylatropine, and again the HR response was evaluated after simultaneous blockade to obtain the intrinsic HR. On the subsequent day, the sequence of injections was inverted (first atenolol and then methylatropine). Sympathetic tonus was determined as the difference between maximum HR after methylatropine injection and the intrinsic HR. Vagal tonus was obtained by the difference between the lowest HR after atenolol injection and intrinsic HR [14,16].

2.6. Statistical analysis

Data are reported as means ± SEM, and ANOVA (one- or two-way) was used to compare groups, followed by the Student–Newman–Keuls test. The significance level was established as $p < 0.05$.

3. Results

Body weight was not different among the groups at the beginning of the protocol. At the end of the protocol, SC group presented lower body weight than SO and TO groups. At the same time, SOE, TO and TOE groups presented reduced body weight in relation to SO group (Table 1).

The aerobic physical performance was similar among groups at the beginning of the experiment and after 4 weeks of training. However, the animals submitted to exercise training (TO and TOE) presented an increase in the maximum speed of running when compared to SC, SO and SOE groups after 8 weeks of exercise training (Table 1).

As described in Table 2, SO and SOE rats presented higher systolic (SAP), diastolic (DAP) and MAP compared to SC rats. Exercise training, associated or not to HT, induced a reduction in SAP, DAP and MAP compared to SO and SOE groups. HR was similar among the groups.

Estrogen deprivation caused a decrease in tachycardic response evoked by baroreceptor activation during AP falls (SO: 2.6 ± 0.2 bpm/mmHg vs. SC: 4.4 ± 0.3 bpm/mmHg). HT did not change tachycardic response (SO: 2.6 ± 0.2 bpm/mmHg vs. SOE: 2.4 ± 0.2 bpm/mmHg), but both trained groups (TO: 4.4 ± 0.46 and TOE: 3.6 ± 0.17 bpm/mmHg) had a significant benefit in this response compared to sedentary OVX groups. The bradycardic response to AP rises was similar among SC, SO, SOE, TO and TOE groups (−1.47 ± 0.1, −1.2 ± 0.1, −1.16 ± 0.15, −1.88 ± 0.1 and −1.62 ± 0.1 bpm/mmHg, respectively) (Fig. 1).

Ovariectomy reduced vagal tonus and increased sympathetic tonus, and both HT and exercise training were able to enhance vagal tonus. However, the HT associated to exercise training was more efficient in normalizing vagal and sympathetic tonus. The intrinsic HR, obtained after simultaneous methylatropine and atenolol injections was similar between studied groups (Table 2).