

Effects of exercises on the right ventricle of female mice submitted to ovarian hormone deprivation

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Abstract

Introduction: Several studies indicate that the estrogen deficiency increase the incidence of the cardiovascular diseases in women in post-menopausal period and that the exercise program may prevent or relieve problems such as cardiovascular disease, obesity, muscle weakness, osteoporosis and depression. The effects of the estrogen deprivation in the myocardium still remain unclear, mainly in the right ventricle. The aim was to investigate the effects of exercises on the right myocardium in the female mice subjected to deprivation of ovarian hormones. **Material and methods:** A total of 15 female mice at 9 months of age, divided into 3 groups (n = 5) were studied: non-ovariectomized sedentary (S); ovariectomized sedentary (OS), ovariectomized trained (OT) animals. Ovariectomy was performed at 9 months of age and physical training began 7 days after surgery. The animals underwent a physical training protocol for 4 weeks on a treadmill with progressive speed and load (one hour a day/5 days a week at 50 to 65% of the maximum speed of running). After the experimental proceeding, the heart were removed and processed accordingly to conventional protocol for optical microscopy, and the slides stained by the methods of Hematoxylin and Eosin. Stereology was used to analyze the components of the myocardium. **Results:** Ours results indicate that the exercise training promoted cardiomyocyte hypertrophy, increase in connective tissue with decreased cardiomyocytes. **Conclusion:** Our data suggest that moderate exercise promoted right ventricular remodeling with cardiomyocyte hypertrophy and increase in connective tissue, to fit the modifications promoted by exercise in the left ventricle.

Keywords: aerobic physical, exercise, VD, estrogen, stereology.

1 Introduction

Cardiovascular diseases (CVD) have been shown to be an important cause of morbidity and mortality in individuals of both sexes. In women, the risk of death from CVD increases significantly at the onset of menopause (KANNEL, HJORTLAND, McNAMARA et al., 1976; TEEDE and McGRATH, 1999), especially in sedentary women, in whom the deleterious effects of lack of physical activity are already installed; especially because the hormonal changes that characterize this period potentiate these effects (BARBOSA, SANTARÉM, JACOB FILHO et al., 2002; EVIO, PEKKARINENT, SINTONEN et al., 2007; MARQUES, NASCIMENTO, MANDARIM-DE-LACERDA et al., 2006). Studies show that after sixty years of age, the prevalence of left ventricular hypertrophy in women increases 69% per decade of life, compared with only 15% in men (HAYWARD, KELLY and COLLINS, 2000).

It is known that a sedentary lifestyle results, in most individuals, in structural and particularly functional cardiovascular alterations that have been well characterized, such as loss of cardiomyocytes (with subsequent

hypertrophy of the remaining cells) and decreased arterial compliance (ÁGUILA, MANDARIM-DE-LACERDA and APFEL, 1998; MAIFRINO, ARAÚJO, FACCINI et al., 2009; FLORES, FIGUEROA, SANCHES et al., 2010).

It has been observed in recent years in postmenopausal women, an increase in the interest for physical activity as a means of maintaining health. Exercise stimulates processes, which, over time, alter the morphological body composition and biochemical function, promoting myocardial adaptation according to the type, intensity and duration of the exercise. (MAIFRINO, ARAÚJO, FACCINI et al., 2009; EMTER, McCUNE, SPARAGNA et al., 2005; WISLOFF, STOYLEN, LOENNECHEN et al., 2007; GARGAGLIONE, 2008; SCHULTZ, SWALLOW, WATERS et al., 2007). Most morphological studies involving the myocardium analyze the association between the left ventricle and the bloodstream, and there are few and limited studies correlating with the right ventricle. The aim of this study was to investigate the effects of exercise on the right ventricular myocardium in female mice submitted to ovarian hormone deprivation.

2 Material and methods

2.1 Animals and groups

The study was approved by the Ethics Committee in Research of Universidade São Judas Tadeu (COEP-USJT), according to the following protocol: 058/2007. A total of 15 female mice (C57BL/6J) were studied. The groups were 9 months of age and had initial weight ranging from 20 to 30 g. The mice were kept in cages in a room with controlled room temperature between 22-24 °C and light/dark cycle of 12/12 hours. All mice were fed standard chow and water “ad libitum”. The animals were randomly divided into three groups (n = 5): non-ovariectomized sedentary (S), ovariectomized sedentary (OS), ovariectomized trained (OT).

2.2 Ovariectomy

The ovariectomy was performed at 9 months of age. The animals were anesthetized with ketamine and xylazine solution (120 : 20 mg/kg IM) and a small incision was made in the lower third of the abdominal region. The ovaries were located and removed, and fallopian tubes were tied (MARSH, WALKER, CURTISS et al., 1999; IRIGOYEN, PAULINI, FLORES et al., 2005). The confirmation of the ovariectomy efficacy was determined by analysis of vaginal secretions for four consecutive days, the last day being when euthanasia of the animals was performed.

2.3 Physical training

Physical training started seven days after the ovariectomy surgery; the trained groups underwent a training protocol for 4 weeks on a treadmill with progressive velocity and load (one hour a day/5 days a week at 50 to 65% of maximum speed of running), according De Angelis, Santos, Irigoyn et al. (2004). The animals were adapted to the treadmill for 10 minutes during three days before the start of training.

2.4 Tissue preparation

After 4 weeks of training, which lasted the same time for the sedentary group, the animals were sacrificed by decapitation. A thoracotomy was performed for exposure and removal the heart. The atria and the ventricles were separated and the right ventricle was sectioned. Then the samples were washed (PBS 0.1 M, pH 7.4) and fixed in 10% buffered formalin for 72 hours. After that they were dehydrated, cleared, embedded in paraffin, sectioned in cross-sectional sections of 5 micrometers thick and collected on glass slides for analysis under light microscopy. The sections were stained with Hematoxylin and Eosin to verify the myocytes cross-sectional area, the volume density of myocytes and connective tissue. Images were captured with a 40× objective, and transferred to the image analysis program (Axio Vision Software, Zeiss). For stereological analysis, the photomicrographs of the VD were analyzed by the test system with 200 points, and values were expressed as a percentage.

2.5 Statistical analysis

Results are shown as mean and standard error of mean. One-way Analysis of variance (ANOVA), and post-hoc Tukey tests were duly applied in data analysis. The level of significance for all tests was set at $p < 0.05$.

Table 1. Parameters analyzed in the RV in the groups: sedentary (S), ovariectomized sedentary (OS) and ovariectomized trained (OT).

Parameters/ groups	S	OS	OT
A[my] (μm^2)	155.7 \pm 2.5	162.8 \pm 2.5	181.4 \pm 3.3**
Vv[my]%	88.8 \pm 0.4	87.8 \pm 0.4	87.0 \pm 0.6
Vv[int]%	11.1 \pm 0.4	12.1 \pm 0.4	13.0 \pm 0.6*

Values represent Mean \pm MSE. * $p < 0.05$ vs. S. ** $p < 0.05$ vs. OS.

3 Results

The results disclosed in Table 1 show that the right ventricle of animals from the OS group compared with the S group showed no significant difference in any of the parameters studied. When comparing the group OT with OS, we found that exercise promoted a significant increase in myocyte cross-sectional area ($p < 0.05$) and when compared with S, we observed an increase in the volume densities of the interstitium ($p < 0.05$).

4 Discussion

The results suggest that ovarian hormone deprivation (OS) did not promote changes in the RV. In the ovariectomized trained animals (OT), we observed myocyte hypertrophy, characterized by increased myocyte area. Corroborating our results, Matsubara, Narikawa, Ferreira et al. (2006) studying RV and LV of animals with volume overload due to aortocaval fistula, observed right ventricular hypertrophy secondary to volume overload, and the diastolic stretch was the main stimulus for RV hypertrophy.

Studies carried out in our laboratory, in the LV of animals from this same model, showed myocyte hypertrophy and increase in interstitium in the sedentary group with ovarian hormone deprivation (BRIANEZZI, 2011). We did not find such alterations in the RV of this same group, suggesting that the RV does not change with ovarian hormone deprivation. Moreover, Brianezzi (2011) found that trained ovariectomized animals had myocyte hypertrophy and decrease in interstitium, characterized by a decrease in collagen. In our study we also observed, in the RV, myocyte hypertrophy and increased interstitium, probably due to hemodynamic interdependence between the ventricles.

Although the RV has a mass of approximately 15% of the LV, both ventricles generate similar cardiac output. This is due to the fact that the pulmonary vascular resistance corresponds to one tenth of the systemic vascular resistance (DELL'ITALIA, 1991; LEE, 1992). The two ventricles work in series, which creates a hemodynamic interdependence between them. There is also the interdependence mediated by the interventricular septum and the pericardium around them (BARNARD and ALPERT, 1987). This makes hemodynamic or volume alterations that have occurred in a ventricle to have consequences in the contralateral ventricle.

5 Conclusion

Our data suggest that moderate exercise promoted right ventricular remodeling with cardiomyocyte hypertrophy and increase in the connective tissue, to fit the alterations promoted by exercise in the left ventricle.

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Received January 1, 2012

Accepted May 23, 2012