Original article

Influence of resistance training in left ventricle of ovariectomized elderly rats

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Abstract

Several cardiovascular changes evoked by aging affect negatively the quality of people's lives, among them is the reduced functional capacity. Menopausal women, affected by the suspension of the ovarian hormones, suffer more intensely from metabolic and cardiovascular changes. It is known that physical training causes biochemical, electrical, morphological, and mechanical adaptations in the cardiac muscle, which together provide an improvement in the cardiac function. The goal of the present study was to evaluate the effect of resistance training in the left ventricle of ovariectomized rats through stereological analysis. We studied 15 Wistar female rats, 4 months- old, average weight 240 g. All animals were ovariectomized at 9 months of age and then divided into 3 groups of five individuals as follows: Ovariectomized Sedentary Adult (13 months) (OSA), Ovariectomized Sedentary Elderly (17 months) (OSE), and Ovariectomized Trained Elderly (17 months) (OTE). The rats were monitored for 13 months and subsequently underwent resistance training for 12 weeks. The stereological analysis was performed using light microscopy techniques and data obtained for each group were tabulated and statistically compared using ANOVA and post hoc Tukey tests ($p \leq 0.05$). It was verified that training decreased volume density of myocyte, interstitium and collagen fibers followed by increased volume density of capillaries, when compared with the ovariectomized sedentary elderly (OSE). Our data suggest that resistance training minimizes changes in the myocardium of elderly rats deprived of ovarian hormones.

Keywords: aging, menopause, resistance exercise, myocardium.

1 Introduction

Menopause is characterized by the progressive reduction of estrogens resulting to cessation of menses (MASTORAKOS, VALSAMAKIS, PALTOGLOU et al., 2010). Strategies to prevent cardiovascular disease (CVD) in this population should therefore be a principal objective for healthcare providers. Based on the above evidence, the hypothesis that estrogens have protective effect against atherosclerosis has been put forward. Studies investigating the role of age at menarche and the calculated total lifetime exposure to endogenous estrogen indicate that endogenous estrogens appear to play a protective role for the cardiovascular system (JANSEN, TEMME and SCHOUTEN, 2002; SALTIKI, DOUKAS, KANAKAKIS et al., 2006). These studies concluded that shorter lifetime exposure to endogenous estrogens is an important risk factor for the presence and the severity of coronary heart disease.

Early epidemiological studies indicated higher incidence of the of cardiovascular disease in postmenopausal women compared with women in reproductive age (GORDON and KANNEL, 1978; ROSENBERG, HENNEKENS, ROSNER et al., 1981; COLDITZ and WILLETT, 1987; MATTHEWS, MEILAHN, KULLER et al., 1989). It is known that estrogens exert several protective effects on the cardiovascular system (LIEBERMAN, GERHARD, UEHATA et al., 1994) and the menopause negatively impacts upon many traditional risk factors for CVD, including changes in body fat distribution, reduced glucose tolerance, abnormal plasma lipids, increased blood pressure, endothelial dysfunction, and vascular inflammation (ROSANO, VITALE, MARAZZI et al., 2007).

Countless studies show that physically active individuals enjoy a better quality of life and lower mortality rates, resulting in greater longevity (ZANESCO and ZAROS, 2009; MATSUDO and MATSUDO, 1992).

It has been demonstrated that Resistance Trainning (RT) exercise can be an effective substitute for hormone replacement therapy in preserving menopause related osteoporosis and sarcopenia (MADDALOZZO, WIDRICK, CARDINAL et al., 2007). In addition to increasing muscle mass and improving muscle function, RT has been reported to augment resting and total energy expenditure, and to induce decreases in total and abdominal fat (MAESTA, NAHAS, NAHAS-NETO et al., 2007).

In sedentary postmenopausal obese women, it has been suggested that RT has the potential to ameliorate/prevent the development of insulin resistance and may reduce the risk of glucose intolerance and non-insulin-dependent diabetes mellitus (RYAN, PRATLEY, GOLDBERG et al., 1996).

In ovariectomized rats, Corriveau and Paquette (2008) have shown that an 8-week program of resistance training in conjunction with a restrictive diet reduced intra- abdominal fat depot and plasma free fatty acid levels and prevented liver fat accumulation. They concluded that RT is an asset to minimize the deleterious effects of ovarian hormone withdrawal on abdominal fat and liver lipid accumulations in ovariectomized rats.

Leite, Prestes, Bernardes et al. (2009) recently indicated the potential benefits of resistance training as an alternative strategy to control the negative effects of ovariectomy.

Knowing that menopause decreases the protective action of estrogen in the female circulatory system, and that physical exercise has been considered an important non-pharmacological intervention to reduce risks of cardiovascular disease, the importance of this study is to analyze the structural changes in the left ventricle compared to these factors in order to contribute to the development of strategies that can minimize these changes and positively affect women's quality of life.

2 Material and methods

2.1 *Animals and groups*

The study was approved by the Research Ethics Committee of the São Judas Tadeu University (COEP-USJT) according to the protocol: A-00610. It was used 15 Wistar female rats, 4 months-old, weighting from 220-250 g. The animals were housed at room temperature controlled environment (22-24 °C) and light/dark cycle of 12/12 hours. The animals periodically received water and standard rat chow (NUVILAB CR1, Nuvital Nutrientes LTDA, Curitiba, PR) "*ad libitum*". At 9 months, elderly rats were ovariectomized and then randomly divided into three groups (n = 5), namely:

- Ovariectomized Sedentary Adult (OSA) – (sacrificed at 13 months);
- Ovariectomized Sedentary Elderly (OSE) – (sacrificed at 17 months);
- Ovariectomized Trained Elderly (OTE) – (sacrificed at 17 months).

2.1.1 *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 in the abdominal region. The ovaries were located, removed and the oviducts connected. The confirmation of the efficacy of ovariectomy was determined by analyzing the vaginal discharge for four consecutive days, and on the last day euthanasia of animals was carried out (MARCONDES, BIANCHI and TANNO, 2002).

2.1.2 *Training protocol*

Based on Duncan, David and Gordon (1998), the equipment used to carry out the animals' resistance training program was a wood made, 110 cm, vertical ladder (inclined 80°) with iron steps, and a box at the top, covered with newspaper to accommodate the animals.

The training program was based on the principle of overload number of repetitions and rest that comes closest to training in humans. Therefore, overload with lead weights was attached to the tail of animals weekly.

2.1.3 *Adaptation*

The OSA group did not undergo the training protocol, as they were sacrificed at 13 months. The animals in groups OSE and OTE adapted prior to the training protocol, climbed the equipment without load aiming to reach the rest area at the top. Five consecutive repetitions were carried out for five days.

2.1.4 *Load determination*

The initial loading was 75% of the animal's body weight according to the Heyward (1998) with respect to muscle strength in elderly people. As the increased overload was also related to the animal's body weight, every two weeks all animals were weighed and their loads adjusted.

2.1.5 *Training*

The OSE group did not perform the training protocol. They only climbed stairs once a week, five times a day without extra load until they were euthanized. This procedure was applied aiming to evoke similar stress to the OTE group.

The OTE group training was performed three times per week, with six consecutive repetitions a day allowing a rest interval of 45 seconds between repetitions. The training protocol was performed for twelve weeks.

2.2 *Euthanasia of the animals and tissue processing*

At the end of the protocol, the animals were weighed and euthanized by decapitation. Thoracotomy was performed in animals in which the hearts were removed. After washing the hearts with saline, they were weighed, the atria were sectioned with ventricles separated into left and right. The left ventricle samples were washed in phosphate saline (PBS) at 0.1 M and pH 7.4 weighed and fixed in buffered formaldehyde solution at 10%, for 24 hours, dehydrated in a growing series of alcohols, cleared in xylene, embedded in paraffin and sliced in 6 μm thick sections for analysis using a light microscope. The sections were stained with hematoxylin-eosin and Picrosirius.

Twenty photomicrographs per animal were captured with magnification of ×100 and ×400, totaling 100 images per group, and transferred to the image analysis software (Axio Vision, Zeiss and Image J).

2.3 *Stereological analysis*

For stereological analysis a test-system with 252 test-points was used. Values are expressed in percentage. Digital image processing system computer was used. The system consists of a light microscope (Zeiss) coupled to a Sony miniature Sony camcorder, which captures the images of the slides and transmits them to a computer equipped with Pentium IV and specific software for quantitative analysis (Axio Vision, Zeiss). The images were captured with magnification of 100× (for analysis of collagen fibers) and 400× (for analysis of myocytes, nuclei, interstitium and capillaries) using the Axio Vision software (Zeiss) and Image J (National Institute of Health, USA).

2.4 Statistical analysis

Data for each group were tabulated and statistically compared by oneway ANOVA. Then, the *post hoc* Tukey test was applied for multiple comparisons using a significance level of $p \leq 0.05$.

3 Results

Volume density of myocytes nuclei, shown in Figure 1, was decreased in 10% and in 16% in the trained elderly group when compared, respectively, with the sedentary elderly and the sedentary adult group.

There was no significant difference between groups for the volume density of myocytes. However, the volume density of intersttium (Vv [int]%) in the ovariectomized sedentary elderly group (OSE) showed significant increase (53%) in the interstitial volume density when compared with the OSA group, shown in Figure 2. It was also found that resistance training minimized the increase in volume density of collagen fibers compared with sedentary adult group (OSA).

It was found that the elderly group that performed resistance exercise obtained significant increase of 50% in volume density of capillaries compared with sedentary elderly group (Figure 3).

Resistance training evoked a significant decrease of 19% in volume density of collagen fibers compared with sedentary elderly animals (Figure 4).

4 Discussion

It is known that after menopause women show a greater incidence of cardiovascular diseases than men (SIMKIN-SILVERMAN, WING, BORAZ et al., 2003) and it is believed that estrogen deficiency, lipid profile changes, weight gain and sedentary lifestyle are the main factors associated with a higher prevalence of hypertension.

With aging, myocardial compensatory responses occur due to the increased work, such as connective tissue deposition, hypertrophy and cardiac remodeling, because myocardial hyperfunction induces myocardial injury and increased volume of cardiomyocytes, which occurs in parallel with the increased number of fibroblasts, collagen fibers and endothelial cells that will form new capillaries

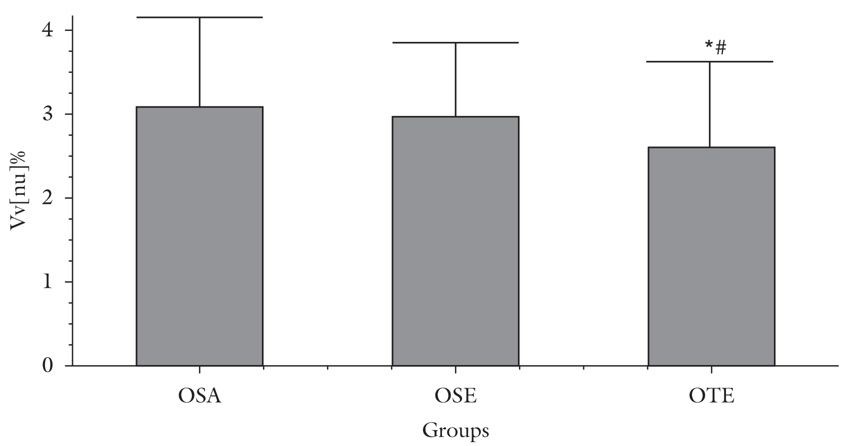

Figure 1. Volume density of myocyte nucleus (Vv [nu]%) of the left ventricle (Mean ± SD, n = 5). $*p < 0.01$; OTE *VS* OSA, $\#p < 0.05$; OTE *VS* OSE. Ovariectomized SedentaryAdult(OSA),OvariectomizedSedentaryElderly(OSE),and Ovariectomized Trained Elderly (OTE).

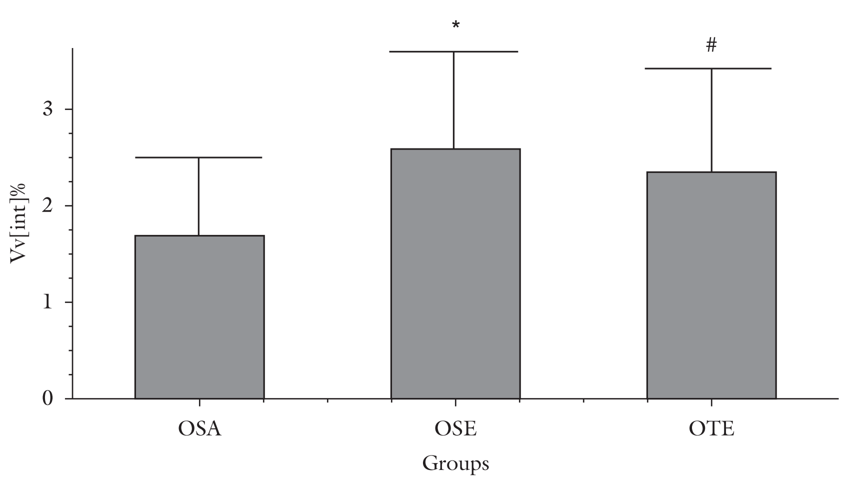

Figure 2. Volume density of interstituim (Vv[int] %) of the left ventricle. Mean ± SD. $*\#p < 0.05$ *VS* OSA. Ovariectomized Sedentary Adult (OSA), Ovariectomized Sedentary Elderly (OSE), and Ovariectomized Trained Elderly (OTE).

(PENPARGKUL, MALHOTHRA, SCHAIBLE et al., 1980; RUSSEL, SUZZANE, LESTER et al., 2000; MACHIDA, KARIYA, KOBAYASHI et al., 2000).

It is known that in aging there is a decrease in the number and an increase in cardiomyocytes hypertrophy. The chronic loss of myocytes reduces the elderly heart's ability to withstand variations of blood pressure and ventricular volume overload, favoring ventricular dysfunction and heart failure in this phase of life. This loss is favored by the existence of apoptosis, or programmed cell death – a process regulated actively by its own cells intended for destruction. This process occurs normally during cardiac development, postnatal maturation, in hypoxia, ischemia, in overload, and also in heart failure Chida, Ohkawa, Watanabe et al. (1994).

According to Leite, Gomes, Vassallo et al. (1995), the aging process determines degenerative changes of the cardiac muscle fibers. As a consequence, the number of cardiac fibers and capillaries decreases and the remaining ones increase in volume. The increased fibrous tissue, especially when there is deposition of Type I collagen, produces several consequences for the functioning of the myocardium. Initially, there tends to be an increased difficulty in the blood flow due to the result of reduced vascular compliance. Furthermore, the average capillary-myocyte distance increases. Therefore, fibrosis is a factor of the worsening of myocardial hypoxia, which is particularly harmful in cases where oxygen consumption by the myocardium is increased by arterial hypertension (GOLDSTEIN and SABBAH, 1994).

In the present study, it was found that resistance training minimizes the loss in volume density of cardiomyocytes in ovariectomized elderly rats. According to Colan (1997), the reduction in volume density of myocytes, resulting from resistance training is generated by pressure overload occurring in the left ventricular characterized by peak systolic pressure. In response to this hemodynamic overload, there is an increase in size of myocytes by the parallel addition of new sarcomeres, leading to an increase in thickness of the left ventricular wall.

It was also observed, in the present study, an increase in the volume density of capillaries when compared with sedentary elderly rats and a decrease in volume density of collagen fibers. Training probably promoted greater oxidative and nutritional needs of the tissue, and both induced the formation of angiogenic factors (ODEK-OGUNDE, 1982; AMARAL, ZORN and MICHELINI, 2000; MEDEIROS, MOTHÉ, AGUILA et al., 2005). Regarding the volume density of collagen fibers, the ovariectomized trained elderly group promoted a decrease in this parameter. One possible explanation

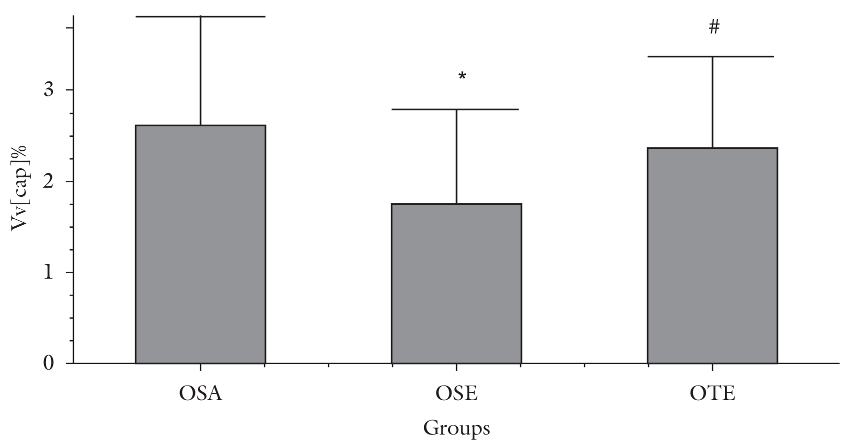

Figure 3. Volume density of capillaries of the left ventricle. Mean (± SD), $*p < 0.001$ *VS* OSA, $\#p < 0.001$ *VS* OSE. Ovariectomized Sedentary Adult (OSA), Ovariectomized Sedentary Elderly (OSE), and Ovariectomized Trained Elderly (OTE).

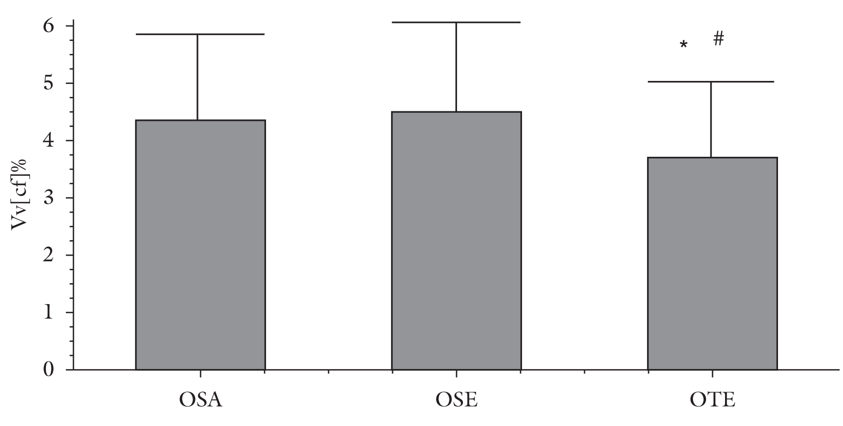

Figure 4. Volume density of capillaries of the left ventricle. Mean (± SD). $*p < 0.05$ *VS* OSA , $\#p < 0.001$ *VS* OSE. Ovariectomized Sedentary Adult (OSA), Ovariectomized Sedentary Elderly (OSE), and Ovariectomized Trained Elderly (OTE).

for the decrease of collagens fibers is that this promotes better modulation of growth factors activating the synthesis of collagen fibers, triggering a cardiac tissue remodeling in response to stimuli caused by mechanical or humoral agents released on myocardium (BRILLA, MATSUBARA and WEBER, 1996; SUZUKI, RUIZ-ORTEGA, LORENZO et al., 2003). This whole process leads to increased ventricular compliance, reducing the risk of ventricular dysfunction, thus improving its performance.

5 Conclusions

The present data suggest that resistance physical training minimizes changes in cardiac tissue in individuals deprived of ovarian hormones.

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