Original article

Effects of resistance exercise on ascendent aorta on ovariectomized elderly rats

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Abstract

Studies have shown a strong relationship between menopause, diet, physical inactivity and presence of risk factors causing endothelial and tissue damages, leading to increased risk of developing cardiovascular disease. The aim of this study was to investigate the influence of resistance training on the effects of estrogen deprivation in aortic collagen and elastic tissue in aging. Fifteen Wistar female rats, 4 months-old, average weight 240 g were studied. All animals were ovariectomized at 6 months of age, after divided into 3 groups (n = 5): Sedentary adult (13 months), Sedentary old and Trained old (both with 17 months). All animals were ovariectomized at 6 months of age. The animals were observed for 8 months after its ovariectomy and then submitted to resistance training protocol during 12 weeks. At the end of the experiment the animals were euthanized. Samples of the ascending aorta were sectioned, fixed, processed and stained for examination by light microscopy. Photomicrographs were used for stereological study and analyzed the following parameters: body weight, volume density of collagen fibers and elastic lamellae. No significant difference was found between the initial and final weights in the studied groups. Resistance training attenuates the increase in volume density of elastic lamellae (21%) and collagen fibers (16%), when compared with the sedentary older group.

Keywords: ascendent aorta, resistance training, ovariectomy, stereology, collagen fibers and elastic lamellae.

1 Introduction

Cardiovascular diseases (CVD) remain the leading causes of morbidity and mortality in modern societies, and age is the major risk factor for CVD (LAKATTA and LEVY, 2003; LLOYD-JONES, ADAMS, BROWN et al., 2010). This effect of aging on CVD risk is primarily the result of pathophysiological changes to arteries that lead to vascular dysfunction and disease (LAKATTA and LEVY, 2003).

Epidemiological studies have indicated that postmenopausal women have higher risk of developing heart disease (PERELLA, BERCO, CECUTTI et al., 2003; SOWERS, 1998; IRIGOYEN, MOREIRA, MOREIRA et al., 1995) when compared with men of the same age and with other risk factors (STAMPFER, COLDITZ, WILLETT et al., 1991).

These observations suggest that the physiological hormonal changes associated with menopause might be responsible for the higher risk for CVD. The possible atherosclerotic protection of hormone replacement therapy in postmenopausal women has also become a popular research topic (BAKER, MELDRUM, WANG et al., 2003)

It happens because, in this phase, women lose cardiovascular protection by decreasing the production of estrogen hormone, which confers antioxidant properties (DEBING, PEETERS, DUQUET et al., 2007).

Decreased estrogen promotes the appearance of central obesity, it may cause metabolic complications such as dyslipidemia. Studies have shown that increased serum total cholesterol concentrations (TC), particularly the cholesterol fraction of the low density lipoprotein (LDL-C), and the decrease of high density cholesterol fraction (HDLc) are associated with an increased incidence of cardiovascular events , including coronary artery disease (CAD) (OLIVEIRA, IRIGOYEN and MOREIRA, 1992; COELHO, CAETANO, LIBERATORE et al., 2005).

During the aging period the arteries lose elasticity, their walls become stiffer, thicker, there is a loss of elastic tissue, accumulation of connective tissue and calcium deposits. These factors lead to a lack of distensibility of the vessel wall, rise of systolic level and ventricular increase of the wall (LAKATTA and LEVY, 2003; O'ROURKE and HASHIMOTO, 2007).

The regular practice of physical activity through resistance exercise envolving large muscle groups produces cardiovascular adaptations that increase the skeletal muscle strength capacity. This regular practice prevents the development of coronary artery disease and reduces the symptoms in patients with established cardiovascular disease. The number of studies reporting the benefits of resistance training reducing the risks of chronicle diseases, improving the daily functions and increasing the mass and muscle strength has grown (SILVA, MARANHÃO and MATOS VINAGRE, 2006; PHILLIPS, 2007).

The present study aims to expand the knowledge on this topic by verifying whether resistance exercise have influence on the effects of estrogen deprivation in aortic collagen and elastic tissue in aging.

2 Material and methods

2.1 *Samples*

The study was approved by the Ethics Committee in Research of São Judas Tadeu University , according to the protocol number: A-00610. A total 15 Wistar female rats, 4 months-old, average weight 235 g. The rats 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): adult ovariectomized sedentary (OvxSA), old ovariectomized sedentary (OvxSE) and old ovariectomized trained (OvxTE).

2.2 *Ovariectomy*

The ovariectomy was performed at 6 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 uterine tubes were tied (IRIGOYEN, MOREIRA, MOREIRA et al., 1995). 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 (MARCONDES, BIANCHI and TANNO, 2002).<0}

2.3 *Exercise training*

The equipment used to carry out the resistance training program was a vertical ladder made of wood with iron steps with height of 110 cm (43 inches) and an inclination of 80°, and has a box at the top to accommodate the animals. The overload was performed using lead weights attached to the tail of the animal with adhesive tape. OvxSA group did not perform the training protocol, as it was euthanized at 13 months. OSE group underwent the exercise without the load once a week, until its euthanasia; with the aim of causing stress similar to the OvxTE group.

Exercise training started seven month after the ovariectomy surgery; the trained groups underwent a training protocol for 12 weeks on a climbed the ladder with progressive load (6 times a day/3 days a week). The resistance training was of moderate intensity. After the training period the animals were euthanized. The animals were adapted to the ladder during five days before the start of training.

The overload was established from a percentage of body weight of each animal. It was initially assumed a value of 75% by body weight, adjusted every two weeks, plus one addition of 15% more, so as to cause a progressive increase in training load.

2.4 *Tissues preparation*

After the 12 weeks of training which lasted the same time for the sedentary group, the animals were euthanized by decaptation. Thoracotomy was performed for exposure and removal of ascendant aorta

The ascending aorta was sectioned at its root, located in the region adjacent to the base of the heart. Then the samples were washed (PBS 0.1M, 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 µm thick and collected on glass slides for analysis under light microscopy. The sections were stained with Picrosirius to verify the volume density of collagen fibers and with Verhoeff-Van Gieson to verify the volume density of elastic lamellae. Images were captured in 4 points at 0°, 90°, 180° and 270°, with a 10× and 40× objective, and transferred to the image analysis program (Axio Vision Software, Zeiss). For quantitative analysis, the photomicrographs of the aorta were analyzed by a stereological test system with 252 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 $p < 0.05$.

3 Results

No significant difference was found between the initial and final weights in the groups studied (Table 1).

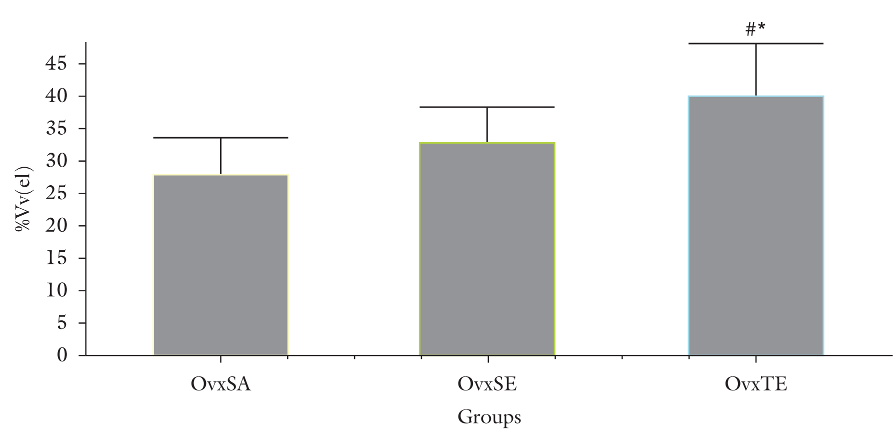

Figure 1. Graph representing the volume density of elastic lamellae (Vv [el]%) of the ascending aorta in both groups. (M ± SD) #p < 0.001 vs. OvxSA; *p < 0.05 vs. OvxTE.

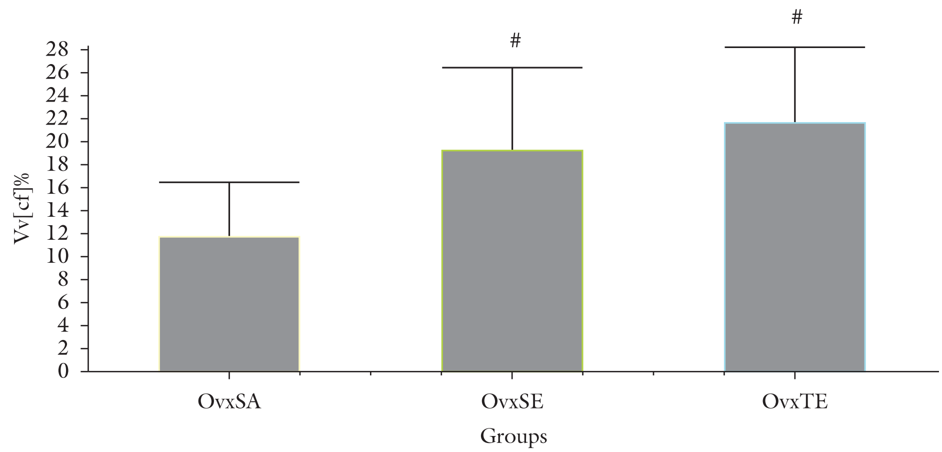

Figure 2. Graph representing the volume density of collagen fibers (Vv [cf]%) of the ascendant aorta in both groups. (M ± SD) #p < 0.001 vs. OvxSA.

Table 1. Mean values of initial weight (IW), final weight (FW) and the difference between final and initial weights (FW-IW) (M ± SD).

	OvxSA	OvxSE	OvxTE
Initial weight (g)	235 ± 21.5	244 ± 16.7	221 ± 12.4
Final weight (g)	331 ± 10.8	327 ± 19.2	341 ± 33.9
FW-IW (g)	96 ± 22.8	83 ± 30.3	120 ± 30.6

Analyzing the volume density of elastic lamellae (Figure 1), we observe that elderly rats which performed resistance training (OvxTE) had a significant increase (48%) compared to adult sedentary (OvxSA) and that training attenuates this increase (21%) compared OvxSE to the group.

As for the volume density of collagen fibers (Figure 2), we found that the aging (OvxSE) caused a significant increase (58%) when compared to the adult sedentary group of rats (OvxSA). We also observed that resistance training downplayed this increase when compared with the elderly sedentary group.

4 Discussion

The endothelium is considered an active endocrine organ that, in response to humoral, neural and mechanical stimulus, synthesizes and releases substances that participate in the process of modulation of vascular tone, regulation of coagulation, thrombosis, vascular remodeling and inflammatory response (BEHRENDT and GANZ, 2002).

These attacks to the vessel may lead to endothelial dysfunction and thus the impairment of vascular reactivity. The vascular response to these aggressive agents involves interaction of several cell groups such as monocytes, lymphocytes, platelets and vascular smooth muscle cells, triggering a series of events that culminate in the long run, with the formation of atherosclerotic plaque (BEHRENDT and GANZ, 2002).

The exercise is a physiological stress that causes an imbalance in homeostasis by promoting an increase in energy demand of the muscles exercised and hence the organism as a whole. Thus, numerous integrated physiological responses are triggered integrated, notably in the cardiovascular system and particularly endothelial function. The endothelial responses contribute to the decrease in peripheral vascular resistance and increased blood flow to active muscles and the coronary bed.

The collagen fibers and elastic lamellae are major constituents of the extracellular matrix of blood vessels where the elastic fibers give resilience, and consequently, the compliance, and collagen is related to arterial wall stiffness, giving limit to large vessels' extensibilty (CATTEL, ANDERSON and HASLETON, 1996).

The reduction in the amount of elastic fibers and increased collagen content in senescence were noted as a regular phenomenon due to the aging process (HUANG, RABOLD, SCHOFIELD et al., 2007).

Aguila and Mandarim-de-Lacerda (2003) add that any change in the amount or quality of extracellular matrix components, can directly affect the aorta. The smooth muscle cells, endothelial cells, fibroblasts and collagen have function of resisting the changes resulting from cellular diseases or injuries. However, depending on the stimulus, the collagen fibers are important modulators and can accumulate in the tissues.

Seals, Moreau, Gates et al. (2006) demonstrated that physical exercise improves the compliance of large arteries in postmenopausal women, and improve body composition by reducing adipose tissue, lower LDL cholesterol and raise HDL cholesterol

Concerning collagen fibers, our study shows that aging enhances significantly the increase in volume density of collagen fibers (58%) and that resistance training did not cause quantitative changes in volume density of collagen fibers compared with elderly sedentary rats. As for the elastic lamellae we found that the resistance training minimized significantly increased (21%) of the volume density of elastic lamellae compared with the old sedentary group (OSE).

Our findings corroborate Kallikazaros, Tsioufis, Zambaras et al. (2002), where the authors relate the decrease of the estrogen hormone and inactivity with arterial stiffening and diverge with Mariotti, Liberti, Maifrino et al. (2011) and Moraes-Teixeira, Félix, Fernandes-Santos et al. (2010) probably by the type of exercise used.

According to Lakatta and Levy (2003), during the aging period, the aortic elastic system is related to its thickness, this change suggests proliferation of smooth muscle cells, increased intercellular matrix, and increased fragmentation of collagen tissue elastic fibers. Safar and Frohlich (1995), hypertensive subjects studied, related increasing the thickness of the arterial wall with morphological changes such as increased muscle cells and extracellular matrix, and functional.

After the data analysis we concluded that resistance exercise attenuates age-related changes in the arterial wall composition of elastin and collagen, suggesting that resistance training reduced arterial stiffness. Decreasing arterial stiffness is important to maintain appropriate vascular peripheral resistance, to diminish pressure on the aorta wall, and to allow appropriate blood flow through the vascular bed.

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