

Early ovarian hormone deprivation increases cardiac contractility in old female rats—Role of physical training

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ABSTRACT

Objectives: We investigated the effects of early ovarian hormones deprivation on morphology and cardiac function and the effects of aerobic training on these parameters, in old rats.

Methods: Female Wistar rats (N = 48) were divided into two groups, at 10 weeks of life: early ovarian hormones deprivation by ovariectomy (OVX; N = 24) and sham (SHAM; N = 24). Between weeks 62 and 82, 12 animals of each group underwent aerobic training (OVX-T and SHAM-T, N = 12). At the end of week 82, all were evaluated by echocardiography, cardiac function (Langendorff technique) and cardiac β -adrenergic receptor expression quantification.

Results: Echocardiography showed slight changes in morphology between OVX and SHAM groups. OVX group ($\Delta = 101 \pm 4.7$ mmHg) showed higher values for maximal left intraventricular pressure in response to dobutamine, when compared to SHAM group ($\Delta = 55 \pm 11.8$ mmHg). Both OVX-T ($\Delta = 70 \pm 4.0$ mmHg) and SHAM-T ($\Delta = 22 \pm 6.6$ mmHg) groups showed a reduction in this response. While, β -adrenergic receptor expression was not different between the untrained groups, SHAM-T (0.23 ± 0.02 AU) and OVX-T (0.29 ± 0.01 AU), showed decreased expression of these receptors.

Conclusion: Early ovarian hormones deprivation associated with aging, promotes discrete changes in cardiac morphology and increasing cardiac contractility. Aerobic training decreases β -adrenergic receptors expression, influencing the cardiac contractility.

1. Introduction

Physiological menopause occurs around 51 years of age. However, in some cases, menopause can happen early, that is, before the age of 40. Of the main consequences of early ovarian hormones deprivation or early menopause, one of the most alarming is the increased risk of cardiovascular diseases, contributing to an increase in the morbidity and mortality rate in these women (van der Schouw et al., 1996; Atsma et al., 2006; Lobo, 2007; Wellons et al., 2012).

This increased risk for cardiovascular diseases is due to the loss of the important cardioprotective action exerted by ovarian hormones, especially estrogens (Knowlton and Lee, 2012), resulting in different changes, such as those observed in cardiovascular autonomic regulation, as well as cardiac morphology and functionality (Vongpatanasin, 2009; Tezini et al., 2013a, 2013b; Felix et al., 2015). However, despite

advances in the understanding of the importance of ovarian hormones for the cardiovascular system, we know little about the long-term impairment resulting from the early deprivation of these hormones on different aspects of cardiac function, especially when associated with the aging process, since the majority of the studies are on animals that are still young. Thus, investigations of this condition on older animals may bring new and important information regarding the different cardiovascular aspects that are not presently being studied clinically.

Another important aspect addressed in the present study is related to the literature findings, where therapeutic approaches have been investigated in the last decades, that regardless of the timing of menopause, premature or physiological, lifestyle changes through regular physical exercise, especially aerobic ones, have been shown to not only decrease the occurrence and severity of cardiovascular diseases, but also improve quality of life and increase life expectancy. The

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cardiovascular benefits are most likely due to a number of adaptive processes that include improvement in cardiac morphology and function, cardiac autonomic control, and neurohumoral regulation (Goodman et al., 2005; Endlich et al., 2013; Ichige et al., 2016). However, the effects of aerobic physical training on the morphological and functional cardiac parameters, due to the early deprivation of ovarian hormones associated with aging, are still controversial, while other effects on specific parameters are not yet known, such as the effects on cardiac contractility and the expression of β -adrenergic receptors. Our hypothesis is that early deprivation of ovarian hormones, when associated with the aging process, promotes more adverse cardiac remodeling and greater damage to cardiac function, when compared to the physiological ovarian failure. In this case, aerobic physical training would play a key role in avoiding or even attenuating these effects, regardless of the age in which the ovarian failure occurred. Thus, the objectives of the present study were:

a) to investigate the effects of early deprivation of ovarian hormones on several cardiac morphological and functional parameters in 82 week-old female rats subjected to ovariectomy at 10 weeks of age, b) compare these effects to the physiological ovarian failure and c) to evaluate the use of aerobic training as a non-pharmacological therapeutic tool (weeks 62–82).

2. Methods

2.1. Experimental groups

Wistar rats ($N = 48$) were assigned to one of two groups: a group of elderly female rats aged 82 weeks old undergoing ovariectomy by the 10th week of life (OVX, $N = 24$) representing early ovarian failure; and a group of elderly female rats aged 82 weeks old undergoing sham surgery for pretend removal of the ovaries by the 10th week of life (SHAM, $N = 24$) representing physiological ovarian failure (Wolfe, 1943). The rats were housed in a room with a strictly controlled temperature ($21 \pm 1^\circ\text{C}$) and a 12-h light/dark cycle with unrestricted access to tap water and standard rat chow (Nuvilab CR-1, Nuvital, Brazil) until 82 weeks of life. In addition, half of each group underwent aerobic physical training between the 62nd and 82nd weeks.

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 # 167/2013).

2.2. Ovariectomy

The rats were anesthetized with ketamine and xylazine (80 mg/kg and 10 mg/kg, i.p., respectively) 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, 4000 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-week post-surgical recovery period was allowed. Next, the rats were housed in groups of 3 per cage ($60 \times 50 \times 22$ cm, Insight Ltda, Brazil). Daily vaginal smears were collected from all rats, as previously described (Marcondes et al., 2002). This procedure allowed for the phase of the oestrous cycle to be determined by daily analysis of the types of cells that sloughed off of the vaginal epithelium. With this approach, 4 different stages can be observed as follows: different stages: proestrus (nucleated epithelial cells), oestrus (cornified cells), metestrus (some cornified cells in addition to nucleated cells and a large number of leukocytes) and diestrus (leukocyte infiltration). Collected vaginal fluid was placed on glass slides and

examined by light microscopy ($40\times$). In the SHAM group, oestrous cycle regularity was confirmed by the presence of vaginal epithelial cells characteristic of each of the 4 stages described above. In the OVX groups, the absence of the oestrous cycle was confirmed by a permanent diestrus phase.

2.3. Physical Training

The rats in the training groups underwent a protocol of aerobic physical training that consisted of swimming sessions in a glass tank (100 cm long \times 80 cm wide \times 80 cm high), which allowed for the simultaneous training of 6 animals. The tank was filled with 50 cm of warm water ($30 \pm 2^\circ\text{C}$), which was changed after every group training session. The training program was conducted in 2 different stages over a total of 20 weeks (from 62 to 82 weeks of age). The first stage consisted of a 2-week adaptation period, during which the session length was gradually increased from 5 to 30 min per day, 5 times per week (in increments of 5 min per day). The second stage consisted of 18 weeks, with 30 min physical training sessions conducted 5 times per week. To evaluate physical training intensity, blood was collected from the tail vein of the animals at the 5th, 10th, 18th weeks immediately before and after the 30-min exercise sessions, 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 (Gobatto et al., 2001). 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% (Gobatto et al., 2001).

3. Experimental protocol

3.1. Echocardiography

At 82 weeks of age, the rats were evaluated by echocardiography via 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% O_2 and placed on a heated (37°C) platform. Electrocardiogram and platform 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 by a single individual who was blind to the characteristics of each group (Sahn et al., 1978).

The following parameters were obtained from the images: inter-ventricular 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: $\text{FS} (\%) = [(LVEDD - LVESD) \div LVEDD] \times 100$, and the ejection fraction (EF) was calculated according to Teichholz as follows: $[(LVEDV - LVESV) \div LVEDV] \times 100$ (Teichholz et al., 1976). The left ventricle mass (LV mass/final body weight) was obtained with the following formula: $1.047 \times [(LVEDD + PWT + IVST)^3 - (LVEDD)^3]$ and the relative wall thickness (RWT) was calculated as follows: $(2 \times PWT \div LVEDD)$ (Wichi et al., 2007). For the quantifications of left ventricular volumes, the following formula was used for Left ventricular end diastolic volume $LVEDV (\mu\text{L}) = (LVEDD^3 \times (7 \div 2.4 + LVEDD^3))$ and Left ventricular end systolic volume $LVESV (\mu\text{L}) = (LVESD^3 \times (7 \div 2.4 + LVESD^3))$ (Teichholz et al., 1976).

3.2. Recording of arterial pressure

Forty-eight hours after the echocardiographic exam, the animals were anesthetized with ketamine and xylazine (80 mg/kg and 10 mg/kg, i.p., respectively) and polyethylene catheter made in our laboratory (PE-50 soldered to PE-10; Intramedic, Clay Adams, Parsippany, NJ, USA) were implanted into the left femoral artery for the subsequent recording of hemodynamic parameters, systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP). 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 arterial pulse pressure was measured in conscious rats that were kept in a quiet environment. Arterial pressure (AP) was recorded with a pressure transducer (ADInstruments - MLT0380) and the amplified signal (ADInstruments - ML110) was fed to a computer acquisition system (LabChart 7 Pro). MAP was calculated from the arterial pulse pressure during 1 h.

3.3. Preparation of isolated hearts for perfusion - Langendorff modified technique

For the isolation and perfusion of hearts, the animals were anesthetized with ketamine and xylazine (80 mg/kg and 10 mg/kg, i.p., respectively), and heparin (5000 IU/kg). After decapitation, the hearts were perfused (8 mL/g) with a Krebs-Henseleit solution containing (mM/L): NaCl 118.4, KCl 4.7, CaCl₂ 2.5, MgSO₄·7H₂O 1.2, NaHCO₃ 25, KH₂PO₄ 1.2, and glucose 11.2 and pyruvic acid 2.0. The Krebs solution was gassed with a combination of 95% O₂ and 5% CO₂ at 37 °C (Skrzypiec-Spring et al., 2007).

The coronary perfusion pressure and the left intraventricular pressure were measured by cannulation of the aorta and via a latex balloon inserted in the left ventricle, respectively, two pressure transducers, previously calibrated, were used for the quantification of these parameters (MLT844, ADInstruments, Bella Vista, Australia). Once positioned in the heart, the balloon was inflated to 10–15 mmHg in order to preload/stretch myocardial fibers to obtain an optimal developed systolic isovolumetric pressure. Coronary perfusion pressure and intraventricular pressure were recorded using a data acquisition system (PowerLab 8/35, Chart8, ADInstruments, Bella Vista, Australia).

The experimental protocol lasted 1 h. Following 10 min of baseline stabilization, the evaluation of the reactivity in the coronary bed was initiated by increasing the basal flow rate gradually by 20% every 2 min, until it reached 200%. For each flow rate, the following parameters were recorded; coronary perfusion pressure, maximum left intraventricular pressure, maximal systolic contraction velocity (dP/dT_{max}) and maximal diastolic relaxation velocity (dP/dT_{min}).

Subsequently, after a 10 minute-stabilization, the evaluation of the β -adrenergic receptor sensitivity was performed, using the cardiac dose-response curve and the maximum intraventricular pressure response obtained by the administration of dobutamine (β ₁-adrenergic agonist) and salbutamol (β ₂-adrenergic agonist) increasing *in bolus* rate (0.25 to 100 nmoles of dobutamine, 0.25 to 15 nmoles of salbutamol) at 2-minute intervals.

3.4. Western Blot Analysis

To determine the amount of beta-adrenergic receptor 1 and 2 in the left ventricle homogenate of the hearts of all of the animals (N = 6 animals/group), were analyzed using immunoblotting after all the experimental protocols were completed. Frozen hearts (at –80 °C) were washed in cold phosphate buffered saline (PBS) and the left ventricle was isolated and homogenized in extraction buffer and protease inhibitor cocktail (Sigma-Aldrich, Saint Louis, MO, USA). Total heart protein (50 mg protein/well) was diluted in a 10% SDS-PAGE, and transferred to PVDF membrane (Immobilon®-P^{sq}, Millipore

Corporation, Billerica, MA, EUA). The membranes were blocked with 5% albumin/PBS-T for 24 h, and incubated overnight at 4 °C, with the primary antibodies (anti- β ₁ adrenergic receptor, 1:1000 Abcam, anti- β ₂ adrenergic receptor, 1:1000 Abcam and anti-GAPDH, 1:10000 Cell Signaling). The blots were washed and incubated with HRP-conjugated secondary antibodies for 1 h, at room temperature. The membranes were washed, developed using ECL (Amersham Pharmacia Biotech) and ChemiDoc XRS (BioRad). Analysis of the images was performed using the public domain Image J program (developed at the National Institutes of Health and available at <http://rbs.info.nih.gov/nih-image/>), using the 'Gel Analysis' functions. Result of the analysis is a value for each band, which is proportional to the integrated density value (IDV) of that band, which corresponds to arbitrary units (AUs). GAPDH was used to determine approximately equivalent loading conditions.

4. Statistical analysis

The results are shown as the mean \pm SEM (standard error of the mean). The effects of early ovarian deprivation and physical training were assessed by two-way analysis of variance (ANOVA). When appropriate, post hoc comparisons were performed using Student-Newman-Keuls's test. The reactivity of the coronary vascular bed and dose-response curves of cardiac contractility, after the administration of dobutamine and salbutamol, were analyzed with a multivariate repeated-measures model, and when appropriate, post hoc comparisons were performed. Differences were considered significant at $p < 0.05$. All statistical tests were performed using the BioEstat 5.0 and SigmaStat 3.5 software (Systat Software Inc., San Jose, CA, USA).

5. Results

5.1. Echocardiography-Cardiac Morphology

Table 1 shows the cardiac morphological parameters normalized by body weight. The OVX group showed higher values of interventricular septum thickness, when compared to the SHAM group. SHAM-T group showed higher values for cardiac weight, relative cardiac weight, posterior wall thickness, interventricular septum thickness, LV relative wall thickness and LV mass, and lower systolic diameter end of the VE, when compared to SHAM group. While OVX-T showed lower values for posterior wall thickness and interventricular septum thickness, when compared to OVX group, it showed lower values for cardiac weight, relative cardiac weight, wall thickness and LV mass, and higher values for LV end systolic diameter, when compared to SHAM-T.

5.2. Echocardiography-cardiac function

Table 2 shows the hemodynamic and functional cardiac parameters of all groups studied. The OVX group showed higher values for systolic blood pressure, when compared to the SHAM group. SHAM-T group showed lower values for final diastolic volume and final LV stroke volume, and higher values for ejection fraction and shortening fraction for when compared to SHAM group. While, OVX-T group showed higher values only for LV end-diastolic volume, when compared to OVX group, it showed higher values for systolic blood pressure, final diastolic volume and final systolic volume, and lower values for ejection fraction and shortening fraction, when compared to SHAM-T.

5.3. Langendorff Technique - Reactivity of the coronary bed- induced flow

Fig. 1A and B show the results of coronary perfusion pressure values and maximal left intraventricular pressure in response to the increase in coronary flow, respectively. The OVX group showed lower values for coronary perfusion pressure and higher values for maximal intraventricular pressure in response to flow increase, except for the basal flow, when compared to the SHAM group. Comparison between SHAM

Table 1

Cardiac morphology parameters normalized for body weight for sham (SHAM; N = 12) and ovariectomized (OVX; N = 12) groups, both untrained (SHAM and OVX; N = 06) and trained (SHAM-T and OVX-T; N = 06).

Parameters	SHAM (N = 12)		OVX (N = 12)		Ovariectomy factor	Physical training factor	Interaction
	Untrained	Trained	Untrained	Trained	P	P	P
Body weight, g	472 ± 24.5	479 ± 13.9	483 ± 20.0	485 ± 13.0	NS	NS	NS
Heart weight, g	1.35 ± 0.02	2.24 ± 0.10*	1.37 ± 0.01	1.43 ± 0.02 ^{*,+}	< 0.001	< 0.001	< 0.001
Relative heart weight, mg/kg	2.86 ± 0.14	4.67 ± 0.26*	2.83 ± 0.07	2.94 ± 0.06 ⁺	< 0.001	< 0.001	< 0.001
Cardiac morphology							
PWT, mm/kg	4.0 ± 0.25	4.9 ± 0.35*	3.8 ± 0.16	3.5 ± 0.23 ^{*,#,+}	0.008	NS	0.003
IVST, mm/kg	3.18 ± 0.18	4.95 ± 0.28*	4.14 ± 0.28*	3.16 ± 0.15 ^{#,+}	< 0.001	0.006	< 0.001
RWT, mm/kg	1.00 ± 0.08	1.33 ± 0.09*	1.02 ± 0.05	0.89 ± 0.08 ^{*,+}	0.01	NS	0.007
LVEDD, mm/kg	17.3 ± 0.8	15.3 ± 0.3	15.4 ± 0.8	16.5 ± 0.2	NS	NS	NS
LVEDS, mm/kg	11.6 ± 0.6	7.7 ± 0.1*	10.2 ± 0.6	11.2 ± 0.2 ⁺	NS	0.015	< 0.001
LV mass, mg/g	2.1 ± 0.11	2.9 ± 0.23*	2.3 ± 0.22	1.9 ± 0.04 ⁺	0.024	NS	0.004

All values are presented as the mean ± SEM. g, grams; mg, milligrams; kg, kilograms; mm, millimeters; PWT, posterior wall thickness; IVST, interventricular septum thickness; RWT, relative wall thickness; LVEDD, left ventricular end-diastolic diameter; LVEDS, left ventricular end-systolic diameter; LV, left ventricular. *P < 0.05 vs. untrained SHAM; #P < 0.05 vs. untrained OVX; and +P < 0.05 vs. trained SHAM. NS: not significant.

groups showed higher values only for perfusion pressure at baseline and maximal intraventricular pressure at flow values above 60% for the SHAM-T, when compared to untrained SHAM group. On the other hand, the comparison between the OVX groups showed that the two groups did not differ in relation to the coronary perfusion pressure, as opposed to the maximum intraventricular pressure, with the OVX-T group showing higher values when subjected to higher flow values and compared to untrained OVX group.

When the trained groups were compared, the OVX-T showed lower values for coronary perfusion pressure and higher values of maximal intraventricular pressure when subjected to higher flow values and compared to SHAM-T.

Fig. 2A and B show values for dP/dT_{max} and dP/dT_{min} in response to flow increase, respectively. Untrained OVX and OVX-T show higher values for dP/dT_{max} and dP/dT_{min} , when compared to untrained SHAM and SHAM-T.

5.4. Langendorff Technique - β -adrenergic receptor sensitivity

Fig. 3A and B show dose-response curves and left intraventricular pressure values (Δ variations) following administration of dobutamine and salbutamol, respectively, while Fig. 3C and D show the maximum intraventricular pressure responses. The untrained OVX group showed

higher left intraventricular pressure values from the 2.5 nmol dose of dobutamine and, consequently, greater maximal response of the left intraventricular pressure, when compared to the untrained SHAM group.

The comparison between the SHAM groups showed lower left intraventricular pressure values at all doses of the dose-response curve, and consequently lower maximum intraventricular pressure response after dobutamine administration for the SHAM-T group, when compared to untrained SHAM. Regarding salbutamol, the SHAM-T group also showed lower values for left intraventricular pressure and, consequently, lower maximum intraventricular pressure response, when compared to the untrained SHAM group.

Similarly, the comparison between the OVX groups showed lower left intraventricular pressure values at the 15, 30 and 100 nmol doses of the dobutamine dose-response curve, and consequently lower maximum intraventricular pressure response for the OVX-T, when compared to untrained OVX. Regarding salbutamol, there were no significant differences.

When the trained groups were compared, the OVX-T group showed higher left intraventricular pressure values, at all doses of the dobutamine dose-response curve, and consequently higher maximum intraventricular pressure response, when compared to SHAM-T. Regarding salbutamol, the OVX-T group showed higher values for left

Table 2

Hemodynamic parameters and values of cardiac function obtained in the sham (SHAM; N = 12) and ovariectomized (OVX; N = 12) groups, both untrained (SHAM and OVX; N = 06) and trained (SHAM-T and OVX-T; N = 06).

Parameters	SHAM		OVX		Ovariectomy factor	Physical training factor	Interaction
	Untrained	Trained	Untrained	Trained	P	P	P
Arterial pressure							
SAP, mmHg	123 ± 5	126 ± 1	140 ± 2*	137 ± 2 ⁺	< 0.001	NS	NS
DAP, mmHg	97 ± 4	91 ± 3	91 ± 2	94 ± 2	NS	NS	NS
MAP, mmHg	108 ± 4	105 ± 2	110 ± 1	111 ± 2	NS	NS	NS
Cardiac function							
HR, bpm	320 ± 10	315 ± 8	311 ± 9	316 ± 8	NS	NS	NS
LVEDV (μ L)	597 ± 69	411 ± 33*	469 ± 65	585 ± 47 ⁺	NS	NS	0.019
LVESV (μ L)	134 ± 16	36 ± 5*	101 ± 13	140 ± 12 ^{#,+}	0.017	0.026	< 0.001
Stroke volume, μ L	459 ± 61	376 ± 83	367 ± 53	445 ± 36	NS	NS	NS
Ejection fraction, %	76 ± 2.0	91 ± 0.8*	78 ± 1.0	76 ± 0.5 ⁺	< 0.001	< 0.001	< 0.001
Shortening fraction, %	32 ± 1.6	49 ± 1.4*	34 ± 1.0	32 ± 0.4 ⁺	< 0.001	< 0.001	< 0.001
Cardiac output, mL/min	146 ± 18	118 ± 9	114 ± 16	140 ± 12	NS	NS	NS
Cardiac index, mL/g	0.35 ± 0.03	0.24 ± 0.01	0.23 ± 0.03	0.28 ± 0.02	NS	NS	NS

All values are presented as the mean ± SEM. mmHg, millimeters of mercury; bpm, beats per minute; μ L, milliliter; %, percentage; mL, milliliters; min, minute; g, grams; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; HR, heart rate; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume. *P < 0.05 vs. untrained SHAM; #P < 0.05 vs. untrained OVX; and +P < 0.05 vs. trained SHAM. NS: not significant.

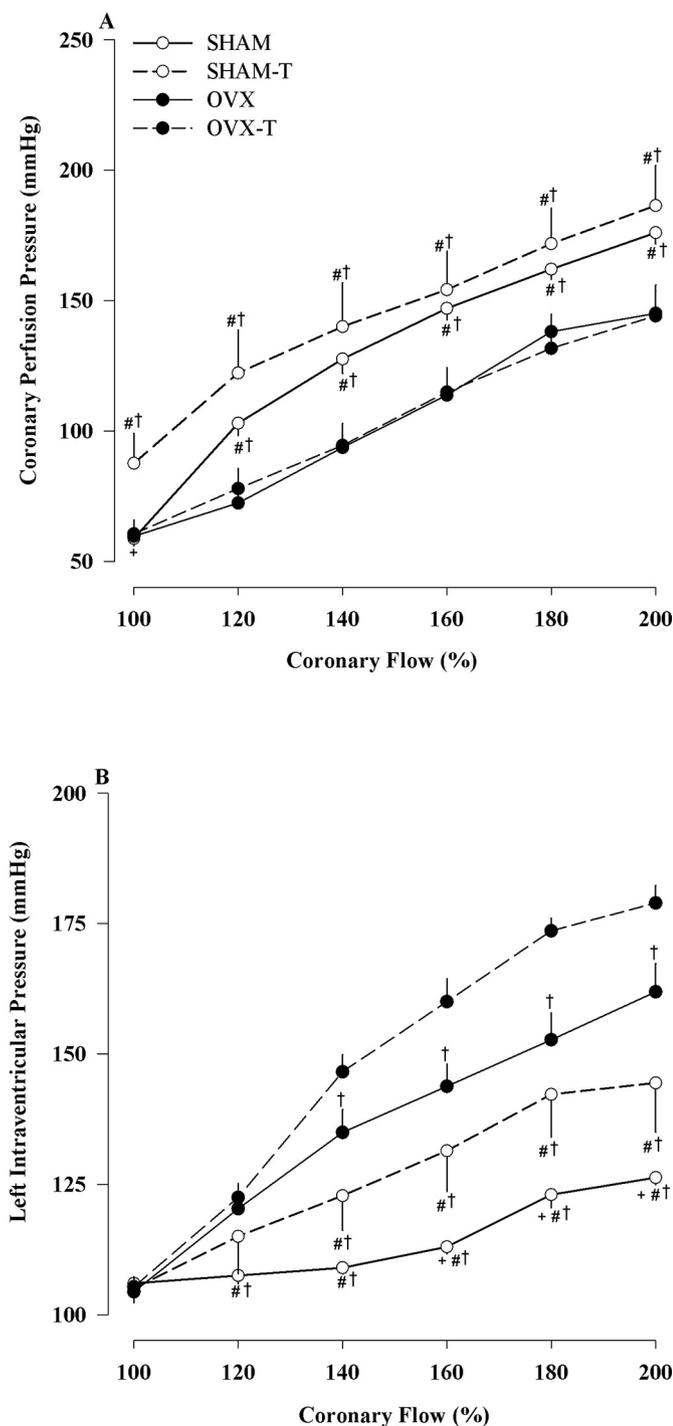


Fig. 1. Reactivity of the Coronary Bed (A) and the Left Intraventricular Pressure (B) flow-induced in the sham (SHAM; N = 12) and ovariectomized (OVX; N = 12) groups, both untrained (SHAM and OVX; N = 06) and trained (SHAM-T and OVX-T; N = 06). All values are presented as the mean \pm SEM. #P < 0.05 vs. untrained OVX; †P < 0.05 vs. SHAM-T; and ‡P < 0.05 vs. OVX-T.

intraventricular pressure at doses of 2.5 to 15 nmol, and consequently, higher maximum intraventricular pressure response, when compared to the SHAM-T group.

5.5. Western Blot- expression of cardiac β -adrenergic receptors

Fig. 4A and B show the results of cardiac β -1 and β -2 adrenergic receptor expression, respectively, as well as images representing the

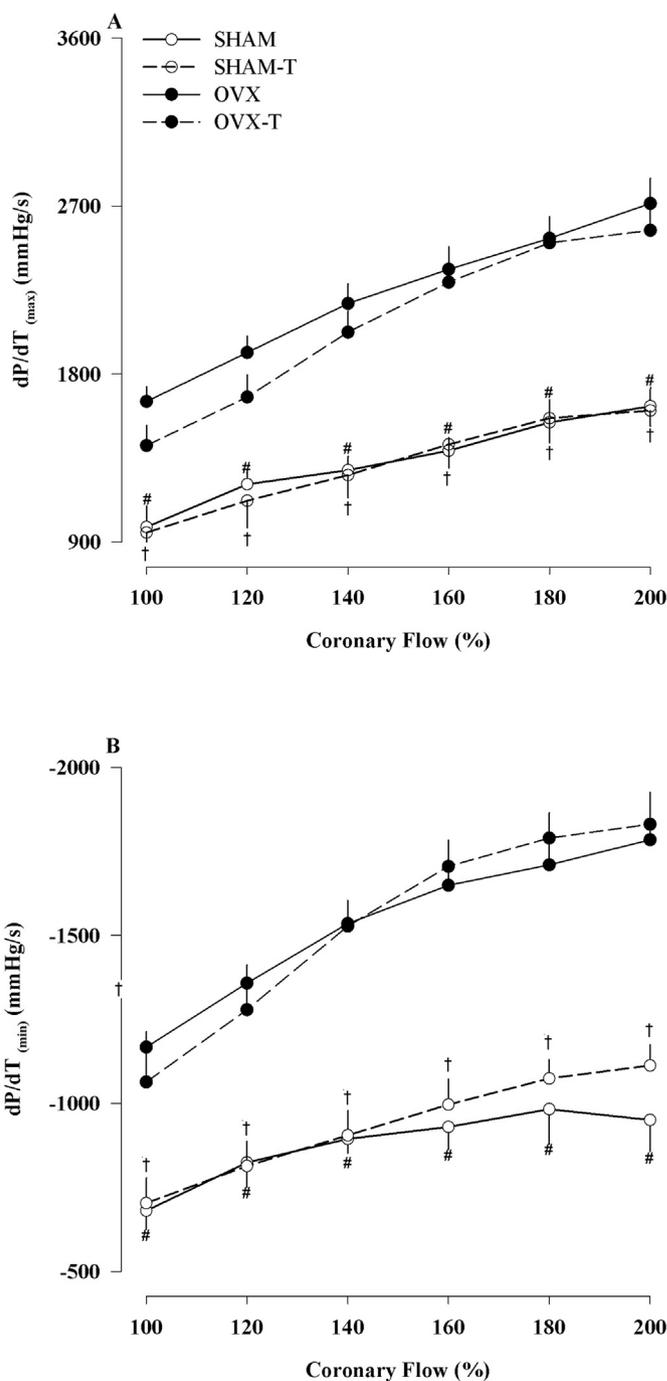


Fig. 2. Values of the dp/dT_(max) (A) and dp/dT_(min) (B) flow-induced in the sham (SHAM; N = 12) and ovariectomized (OVX; N = 12) groups, both untrained (SHAM and OVX; N = 06) and trained (SHAM-T and OVX-T; N = 06). All values are presented as the mean \pm SEM. #P < 0.05 vs. untrained OVX; †P < 0.05 vs. SHAM-T; and ‡P < 0.05 vs. OVX-T.

groups studied. As illustrated in Fig. 4A, the untrained groups, SHAM and OVX, showed similar values for cardiac β -1 adrenergic receptor expression and SHAM-T and OVX-T reduced β -1 adrenergic receptor expression, when compared to their respective untrained groups. SHAM-T group showed greater reduction. In turn, Fig. 4B shows that the untrained groups, SHAM and OVX, did not differ in the expression of β -2 adrenergic receptors. However, after the physical training, only the SHAM-T group showed reduction in the expression of β -2 adrenergic receptors.

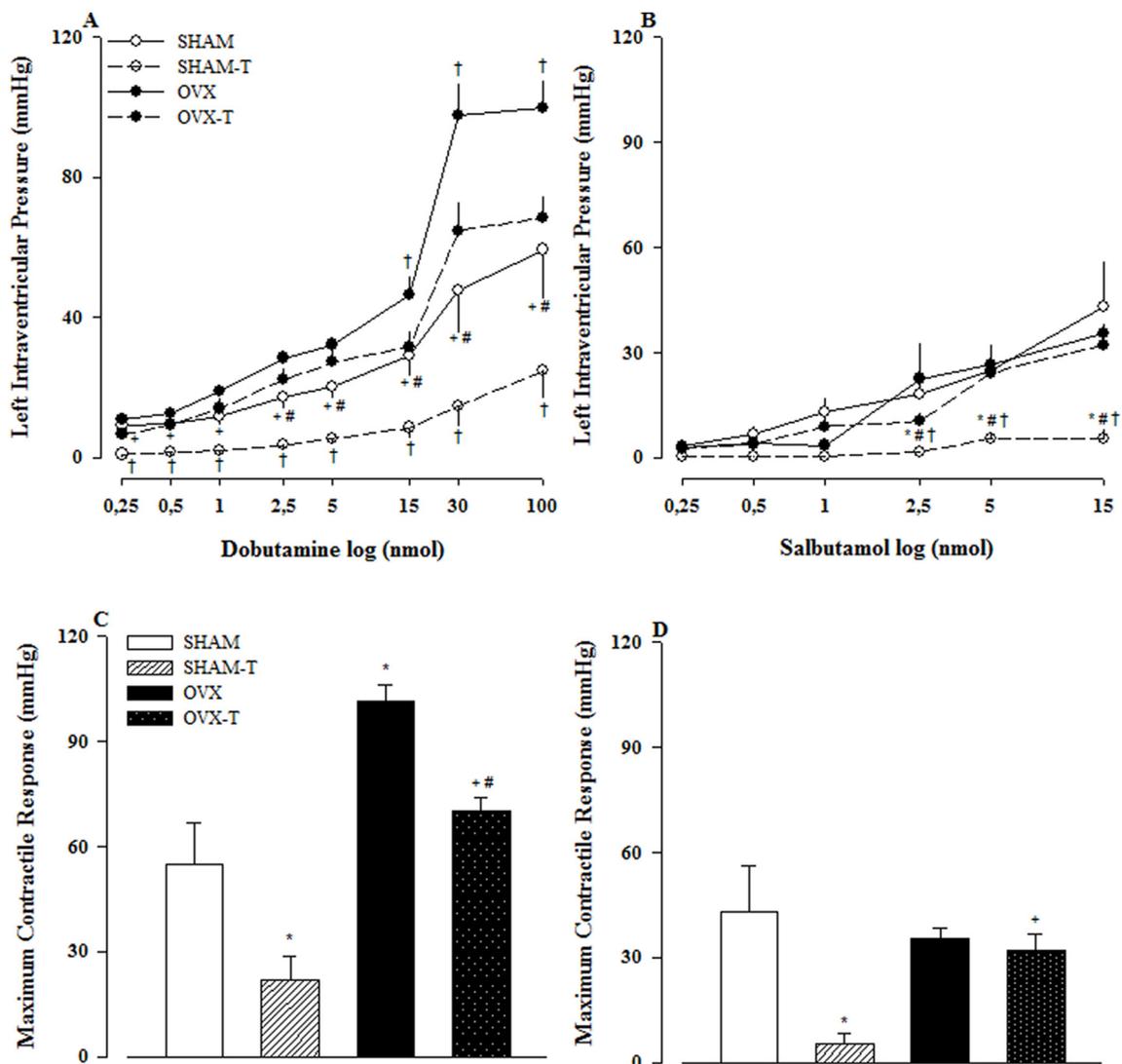


Fig. 3. Left intraventricular pressure dose response curves to Dobutamine (A) and Salbutamol (B). Maximum contractile response after Dobutamine (100 nmol) (C) and Salbutamol (15 nmol) (D), in the sham (SHAM; N = 12) and ovariectomized (OVX; N = 12) groups, both untrained (SHAM and OVX; N = 06) and trained (SHAM-T and OVX-T; N = 06). All values are presented as the mean \pm SEM. *P < 0.05 vs. untrained SHAM; #P < 0.05 vs. untrained OVX; †P < 0.05 vs. SHAM-T; and ‡P < 0.05 vs. OVX-T.

6. Discussion

Early deprivation of ovarian hormones associated with the aging process increased systolic arterial pressure and promoted a mild cardiac remodeling, when compared to physiological ovarian failure. However, there were no repercussions on the cardiac function evaluated through two-dimensional echocardiography. On the other hand, the aerobic physical training in the rats subjected to the early ovarian hormones deprivation lead to the reduction of thicknesses, whereas in rats with physiological ovarian failure it promotes increases of the cardiac weight, thicknesses of the walls and left ventricular mass, as well as changes in some functional parameters.

The higher values of systolic arterial pressure and interventricular septum thickness in the present study suggest that long-term deprivation of ovarian hormones is associated with the development of arterial hypertension and mildly adverse cardiac remodeling. These findings seem to corroborate with results from other studies that also used the same experimental model, however evaluating young rats (Hernández et al., 2000; Felix et al., 2015). In this case, the literature suggests that the deprivation of ovarian hormones promotes an increase in cardiac sympathetic drive and renin angiotensin system (RAS) activity, as well

as impairment of endothelial function, contributing to the elevation of BP levels, and consequently, development of cardiac hypertrophy (Vongpatanasin, 2009; Donaldson et al., 2009; Yung et al., 2011; Knowlton and Lee, 2012).

On the other hand, physical training in rats subjected to early deprivation of ovarian hormones did not reverse the high values of SBP, however, it reduced all cardiac thicknesses without altering the left ventricle mass, promoting the attenuation of adverse cardiac remodeling, due to deprivation of ovarian hormones. These results demonstrate the cardioprotective role of aerobic exercise training (Wisløff et al., 2002). However, after physical training, the animals subjected to physiological ovarian failure, showed very different morphological results, characterized by increase in cardiac weight, ventricular mass and cardiac thicknesses. In this case, there were no changes in the main functional parameters, such as ejection volume and cardiac index. The causes of these observations are not yet established and suggest a fundamental role of ovarian hormones in the cardiac tissue adaptations due to aging, as well as physical training, to the point that the cardiac morphological parameters of the OVX-T group are similar to the untrained SHAM group. These observations are very interesting, since clinical and experimental studies have shown that aerobic physical

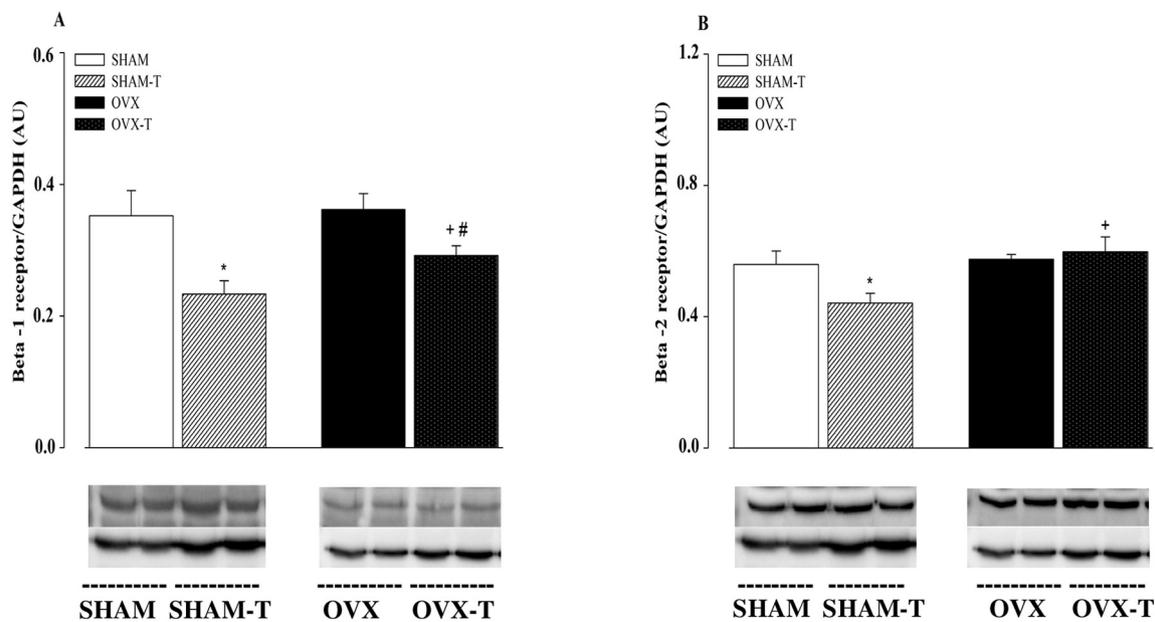


Fig. 4. (A) Protein expression of β -1 and (B) β -2 adrenergic cardiac receptors in the sham (SHAM; N = 12) and ovariectomized (OVX; N = 12) groups, both untrained (SHAM and OVX; N = 06) and trained (SHAM-T and OVX-T; N = 06). All values are presented as the mean \pm SEM. *P < 0.05 vs. untrained SHAM; †P < 0.05 vs. untrained OVX; †P < 0.05 vs. SHAM-T; and †P < 0.05 vs. OVX-T.

training promotes an increase in cardiac preload, inducing the thickening of the ventricular walls over time (Arbab-Zadeh et al., 2014), which corroborates the results found in the group with physiological ovarian failure, but not in the group subjected to early deprivation of ovarian hormones.

The results regarding the evaluation of isolated cardiac function, using the Langendorff technique, were very interesting. In this case, untrained rats subjected to early deprivation of ovarian hormones showed lower reactivity of the coronary bed and higher values for left intraventricular pressure, dP/dT_{max} and dP/dT_{min} as a result of the induced coronary flow increase. They also showed a greater maximal left intraventricular pressure in response to the administration of dobutamine. In turn, both trained groups showed higher values for left intraventricular pressure in response to the induced coronary flow increase, and lower left intraventricular pressure in response to dobutamine. However, only the group subjected to physiological ovarian failure showed reduction in these same parameters, after the administration of salbutamol.

The lower values for coronary perfusion pressure in response to induced coronary flow increase, in the untrained group subjected to early deprivation of ovarian hormones, suggest important changes in the reactivity of the coronary bed. Results from previous studies have shown that endothelial dysfunction is present in ovariectomized rats, characterized by increased coronary vascular lumen area (Borgo et al., 2016), as well as alterations in vascular relaxation in other vascular beds (Kang et al., 2011; Lamas et al., 2015). On the other hand, it is possible that the reduction of coronary reactivity to the induced increase in coronary flow in animals with early ovarian failure may be associated with a mechanism of self-regulation of the coronary circulation, with the objective of maintaining adequate blood flow, mainly due to of the increased cardiac work observed in these animals, evidenced by the higher values observed in dP/dT_{max} and dP/dT_{min} . However, we still need to identify how this mechanism is regulated. Likewise, the cause and mechanisms responsible for the higher values of left intraventricular pressure and dP/dT , in response to induced coronary flow increase in ovariectomized rats, are still unknown. In turn, it is possible that they are due to the effect of positive inotropic factors, for example of angiotensin II, which may be potentiated by the absence of estrogens (Yung et al., 2011). In addition, reinforcing this

hypothesis, early deprivation of ovarian hormones in untrained animals promoted an increase in the response of maximal left intraventricular pressure in response to the administration of dobutamine, an β 1-adrenergic agonist. These findings corroborate previous studies showing increased contractile response of cardiomyocytes after isoprenaline administration, in young ovariectomized rats. The authors associated this higher contractile response to the increase of β 1-adrenergic receptors (Wu et al., 2008). However, in the present study we did not observe differences in the expression of β 1-adrenergic receptors between untrained SHAM and OVX groups.

On the other hand, physical training promoted reduction of left intraventricular pressure in response to dobutamine in both groups, while salbutamol response was only reduced in the SHAM-T group. These lower values in the responses are associated with the reduction of β -1 and β -2 adrenergic receptors, respectively, and suggest a lower dependency on the sympathetic autonomic nervous system, especially in the SHAM-T group. In fact, while aging, regardless of early deprivation of ovarian hormones, there seems to be increased expression of β -adrenergic receptors (Tezini et al., 2013a), our study suggests that aerobic physical training reduces the expression of these receptors. However, the mechanisms are still not fully elucidated. All aerobic physical training leads to greater cardiac efficiency associated with a change in cardiac autonomic regulation, characterized by a reduction in sympathetic influence and/or an increase in vagal influence (Tezini et al., 2013b). In this case, it was observed that in central areas, such as the paraventricular nucleus of the hypothalamus (PVH), the nucleus of the solitary tract (NST) and the rostral ventrolateral medulla (RVLM), physical training promotes adjustments that can decrease both the sympathetic autonomic drive, implicating the participation of endothelial factors, such as nitric oxide (Zucker et al., 2004; Zheng et al., 2005; Higa-Taniguchi et al., 2007; Michelini and Stern, 2009; Martins-Pinge, 2011). Therefore, it is possible that the reduction in the sympathetic autonomic drive is related to the reduction of β -adrenergic receptors, mainly in the SHAM-T group.

However, as the trained group subjected to ovarian hormones (OVX-T) had a lower reduction of β -1 receptors, it suggests a greater dependency on the sympathetic autonomic nervous system for the maintenance of cardiac efficiency. Additionally, a previous study showed that old rats submitted to early ovarian failure had almost no

reduction of the sympathetic autonomic drive after a 10-week physical training protocol (Tezini et al., 2013a, 2013b).

In contrast, aerobic physical training promoted increased maximal intraventricular pressure in response to induced coronary flow increase in both groups. These results corroborate other studies showing beneficial effects of aerobic physical exercise on the heart, however using different methodologies (Wisløff et al., 2001; Andrews Portes et al., 2009). A possible explanation for this effect is that aerobic physical training promotes an increase in myocardial vascularization, mitochondrial increase and aerobic enzymatic activity and, therefore, an increase in aerobic metabolism (Cosmas et al., 1997; Brow et al., 2003).

In short, our study shows that early deprivation of ovarian hormones, when associated with the aging process, leads to mild cardiac morphological changes when compared to physiological ovarian failure. However, there was no repercussion on the cardiac function assessed by echocardiographic examination. On the other hand, the study of isolated cardiac function showed higher left intraventricular pressure associated with lower reactivity of the coronary bed in response to increased coronary flow, in old rats subjected to early ovarian hormones deprivation. On the other hand, physical training, even if performed in the last 20 weeks of life of these animals, promoted important cardiovascular alterations, characterized mainly by the increased cardiac performance with a lower dependency on the autonomic sympathetic nervous system, in both groups. This is evidence of the protecting effect of physical exercise, since adrenergic hyperactivity has been linked to the development of cardiovascular diseases (Schlaich et al., 2004). However, it is important to note that in the group of old rats with early ovarian hormones deprivation, this effect was less evident, suggesting the important role these ovarian hormones play as cardio protectors. It seems different physiological mechanisms are affected involving alterations in remodeling and increased cardiac fibrosis (Felix et al., 2015; Dutra et al., 2017), autonomic and hemodynamic losses (Tezini et al., 2013a, 2013b) and endothelial dysfunctions (Lamas et al., 2015).

These findings highlight the important long-term role of ovarian hormones and of aerobic physical training on vascular and cardiac function and of show the need for further studies to investigate how these physiological mechanisms are affected, since a significant proportion of women are subject to early ovarian failure.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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