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ORIGINAL ARTICLE

Hypertension induces additional cardiometabolic impairments and attenuates aerobic exercise training adaptations in fructose-fed ovariectomized rats

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We tested whether hypertension favors the development of additional cardiometabolic changes in fructose-fed ovariectomized rats and how it affects aerobic exercise training (ET) effects. All rats received fructose in drinking water (10%) beginning at weaning, were ovariectomized at 10 weeks of age and divided into the normotensive sedentary (NFOS) and trained (NFOT) and hypertensive sedentary (HFOS) and trained (HFOT) groups. ET was performed on a treadmill. Arterial pressure (AP) was directly recorded; heart rate and AP variabilities were analyzed. Lipoperoxidation (LPO) and antioxidant enzyme levels were measured in the left ventricle. In addition to increased AP levels, when compared with the NFOS group, the hypertensive groups had resting tachycardia, a reduction of 29% in the pulse interval variance (VAR-PI), 19% in RMSSD (root mean square of successive differences, a cardiac parasympathetic index) and 53% in the α-index (spontaneous baroreflex), while the systolic AP variance (VAR-SAP) and its low-frequency band (LF-SAP) were sharply increased. ET did not alter AP levels. Even in the presence of hypertension, ET induced resting bradycardia, decreases of 33% in VAR-SAP and 49% in LF-SAP, and an increase of more than 60% in VAR-PI and the α-index. However, some of these parameters were still impaired relative to those of normotensive rats. LPO was reduced and catalase was increased in both trained groups, with no difference between the normotensive and hypertensive groups. Negative correlations were obtained between LPO and RMSSD ($r=-0.60$, $P<0.05$) and α-index ($r=-0.63$, $P<0.05$). In conclusion, hypertension augmented the dysfunctions in fructose-fed ovariectomized rats and attenuated metabolic aerobic ET benefits. These changes may be related to cardiovascular autonomic and oxidative stress alterations.

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Keywords: autonomic nervous system; exercise training; menopause; metabolic syndrome

INTRODUCTION

Given the epidemic of obesity in both industrialized and Third World Countries, high dietary fructose has been regarded as a link to the development of metabolic and cardiovascular disorders.[1,2] Owing to the easy access to industrialized food, fructose overload may accompany eating habits throughout the different stages of life. Our group has previously demonstrated that normotensive female rats undergoing chronic fructose consumption had increased arterial pressure (AP), insulin resistance and reduced vagal tonus.[3] When hypertension and ovariectomy were combined with fructose overload, we also observed increases in triglycerides, insulin resistance, blood pressure levels and heart rate (HR), as well as significant cardiovascular autonomic modulation impairment.[4] Importantly, an association between the impairment of autonomic control of circulation and oxidative stress was demonstrated in both normotensive and spontaneously hypertensive rats (SHR).[5–7] In fact, a large number of studies have consistently demonstrated the role of increased oxidative stress in the pathogenesis of cardiometabolic disorders.[8–10] However, few studies have evaluated the changes in oxidative stress induced by fructose overload and whether there is an association between oxidative stress and cardiovascular autonomic dysfunction in this model.

Furthermore, it is important to highlight that in this scenario of cardiometabolic dysfunctions, hypertension has been shown to powerfully predispose patients to all of the major atherosclerotic cardiovascular events, and the tendency for risk factors to cluster in persons

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with hypertension has been found to increase in a stepwise manner with weight gain.[11–13] It should be stressed that a high proportion of hypertensive individuals have an increased prevalence of metabolic syndrome,[13] a cluster of conditions associated with a nearly twofold increase in cardiovascular events.[14] Moreover, given the increased risk of cardiometabolic disease observed after menopause, its association with this multiple disorder condition is of importance, and therapeutic alternatives have been sought for the management of this population. Thus, in this study, we first tested whether hypertension favors the development of additional cardiometabolic dysfunctions in fructose-fed ovariectomized rats, focusing on the cardiovascular autonomic and oxidative stress changes in this model.

Attempting to manage cardiometabolic disorders, we have shown that exercise training reduces AP and HR and improves the baroreflex sensitivity, which is correlated with a reduction in oxidative stress markers in ovariectomized Wistar rats.[5] Aerobic exercise training also attenuated the metabolic impairment, resting tachycardia, and cardiac and vascular sympathetic increases induced by fructose overload in hypertensive ovariectomized rats, although AP was not reduced.[4] Given this information, we also tested the hypothesis that hypertension may blunt some beneficial cardiovascular effects of exercise training in fructose-fed ovariectomized rats compared with normotensives. Therefore, the purpose of the present study was to assess the impact of hypertension on metabolic, cardiovascular, autonomic and oxidative stress effects in sedentary and trained ovariectomized rats undergoing fructose overload.

METHODS

Wistar and SHR female rats at 21 days of age were obtained from the Animals Facilities of the Institute of Cardiology of Rio Grande do Sul. As depicted in Figure 1, all rats underwent fructose overload and ovariectomy and were divided into four groups ($n=8$ each): normotensive sedentary (NFOS) or trained (NFOT) and hypertensive sedentary (HFOS) or trained (HFOT). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Ethics Committee of Sao Judas Tadeu University (Protocol: 01/2008).

Fructose overload

All the groups received D-fructose (100 g l^{-1}) in drinking water[3] from weaning until the end of protocol (19 weeks of treatment). Standard rat chow was freely offered to the studied rats (Nuvilab CR-1; fat = 4.5%, protein = 22%, carbohydrates = 55%, sodium = 2700 mg kg^{-1}). Chow and fructose consumption were measured weekly.

Ovariectomy

After 10 weeks of fructose overload, animals were anesthetized (80 mg kg^{-1} ketamine and 12 mg kg^{-1} xylazine), their oviduct was sectioned and the ovary removed as described in detail elsewhere.[5,15]

Aerobic exercise training

One week after ovariectomy, all animals were adapted to a treadmill (10 min per day; 0.3 km h^{-1}) for 5 days before beginning the exercise training protocol on the 11th week after fructose overload began. Rats underwent a maximal treadmill test, as described previously.[16] The tests were carried out at the 11th week of fructose overload and at the middle (15th week) and end of the protocol (19th week). The purpose of these tests was to determine exercise capacity and to prescribe an exercise training intensity.

Exercise training was performed on a motorized treadmill (Imbramed TK-01, Porto Alegre, Brazil) at low-to-moderate intensity (≅40–60% maximal running speed) for 1 h per day and 5 days per week for 8 weeks, with a gradual increase in speed from 0.3 to 1.2 km h^{-1}.[5]

Metabolic evaluations

At the time of ovariectomy (10 weeks of fructose overload) and at the end of the protocol (19 weeks of fructose overload), body weight and blood glucose and triglyceride concentrations were measured (Accucheck and Accutrend (Roche), respectively) after 4 h of fasting. Additionally, an intravenous insulin tolerance test was performed at these times. For this procedure, the animals were fasted for 2–3 h and anesthetized with sodium thiopental (40 mg kg^{-1} body weight, intraperitoneally). A drop of blood was collected from the tail for the blood glucose level measurement at baseline and 4, 8, 12 and 16 min after insulin injection (0.75 U kg^{-1}). The constant rate for blood glucose disappearance (KITT) was calculated from the slope of the least-squares analysis of the blood glucose concentrations during the linear phase of decline.[3,17]

Cardiovascular measurements

One day after the final metabolic evaluations, the rats were anesthetized with ketamine (80 mg kg^{-1}) and xylazine (12 mg kg^{-1}), and a polyethylene-tipped Tygon cannula filled with heparinized saline was implanted into the carotid artery for direct measurements of AP. During experiments, the rats received food and water with fructose *ad libitum*; they were conscious in their cages and allowed to move freely during the hemodynamic experiments. Twenty-four hours after the surgical procedures, an arterial cannula was connected to a transducer (Blood Pressure XDCR, Kent Scientific, Torrington, CT, USA), and blood pressure signals were recorded for a 30-min period using a microcomputer equipped with an analog-to-digital converter (CODAS, 2 kHz; DATAQ Instruments, Akron, OH, USA). The recorded data were analyzed on a beat-to-beat basis to quantify changes in systolic (SAP), diastolic (DAP) and mean AP (MAP) and HR.[5,15]

Autonomic measurements

Time-domain analysis consisted of calculations of the mean pulse interval (PI) and SAP, PI variability (PI-VAR) and SAP variability (SAP-VAR), the standard

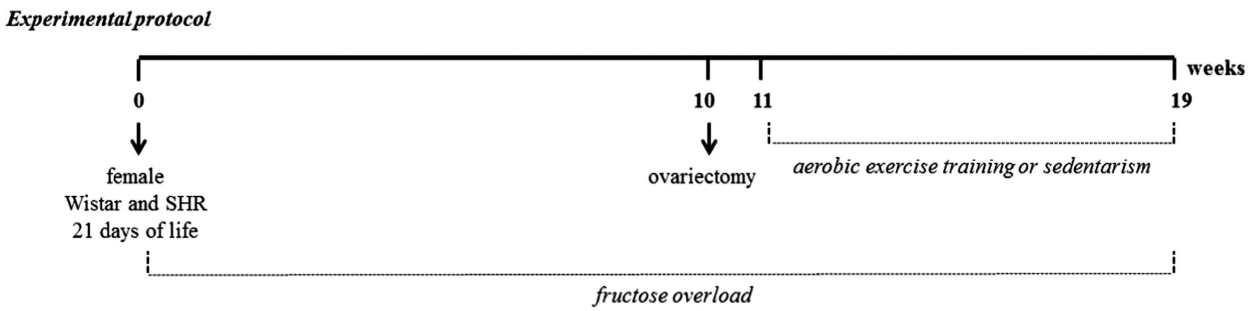

Figure 1 Experimental protocol. All rats began the protocol at 21 days of age. The four groups underwent fructose overload for 19 weeks. At the 10th week, the four groups underwent ovariectomy. At the 11th week, the groups were divided into sedentary or trained; aerobic exercise training was performed for 8 weeks (from the 11th to the 19th week).

deviation from PI and the sum of the squares of differences between adjacent PI (RMSSD (root mean square of successive differences)). For frequency domain analysis, the whole 20- min time series of PI and SAP were cubic-spline-interpolated (250 Hz) and decimated to be equally spaced in time. Following linear trend removal, the power spectral density was obtained by the fast Fourier transformation. The spectral power for low- (LF: 0.20–0.75 Hz) and high (HF: 0.75–4.0 Hz) frequency bands was calculated by means of power spectrum density integration within each frequency bandwidth, using a customized routine (MATLAB 6.0; Mathworks, Natick, MA, USA). Beat-to-beat values of SAP and PI were used to estimate the cardiac baroreflex sensitivity by spectral analysis, using the α-index for the LF band (0.20–0.75 Hz). Coherence between the PI and SAP variability signals was assessed by means of cross-spectral analysis. The α-index was calculated only if the magnitude of the squared coherence between the PI and SAP signals exceeded 0.5 (range, 0–1) in the LF band. After coherence calculation, the α-index was obtained from the square root of the ratio between PI and SAP variability in the two major bands of LF.[18]

Tissue collection

One day after cardiovascular measurements, rats were killed by decapitation. The hearts were rapidly excised, weighed and frozen in liquid nitrogen. The remainder of the heart (left ventricles) was homogenized with Ultra-Turrax (Wilmington, NC, USA) (1.15%, wt vol^{-1} KCl and 20 mmol l^{-1} phenylmethylsulfonyl fluoride). The homogenates were centrifuged at 600 *g* for 15 min at 4 °C (Sorvall RC 5B-Rotor SM 24, Waltham, MA, USA) to discard nuclei and cell debris. The obtained supernatant fraction was frozen at − 80 °C and used to determine the oxidative stress profile. Protein was measured by the method of Lowry *et al.*[19] using bovine serum albumin as the standard.

Oxidative stress evaluations

Lipid peroxidation was measured by the *tert*-butyl hydroperoxide-initiated chemiluminescence assay, as previously described by Gonzalez Flecha *et al.*[20] This assay was carried out with a Liquid Scintillation Spectrophotometer (TriCrab 2800TR; Perkin-Elmer, Waltham MA, USA) in the out-of-coincidence mode at room temperature. This method consists of the addition of a highly unstable organic synthetic hydroperoxide to the sample homogenates and the observation of the response. Hydroperoxides can react with membrane lipids, which initiate[s]a lipid peroxidation process, yielding singlet oxygen and excited carbonyls. These species are excited and can be stabilized by light emission. The heart supernatant fractions were diluted in 140 mmol l^{-1} KCl and 20 mmol l^{-1} phosphate buffer, pH 7.4, which were added to glass tubes placed in scintillation vials. Then, 3 mmol l^{-1} *tert*-butyl hydroperoxide was added, and chemiluminescence was examined using the maximal level of emission. The results were reported as counts per second (cps) per mg of protein.

Superoxide dismutase activity was measured spectrophotometrically in heart homogenates by the rate inhibition of pyrogallol auto-oxidation at 420 nm.[21] Enzyme activity was reported as U mg^{-1} protein. Catalase activity was measured by monitoring the decrease in H_2O_2 concentration at 240 nm, and the results are reported as pmol of H_2O_2 per mg protein.[22] Glutathione peroxidase activity was determined in cardiac samples by monitoring NADPH oxidation spectrophotometrically at 340 nm, and the results are reported as nmol min^{-1} per mg protein.[23]

Statistical analysis

Data are expressed as the means ± s.e.m. Two-way analysis of variance followed by the Student–Newman–Keuls test was used to compare groups. Pearson's correlation was used to study the association between variables. The significance level was established at $P<0.05$.

RESULTS

Maximal exercise test

Exercise capacity was evaluated through performance on a maximal treadmill test. As a common characteristic of the SHR strain, the exercise capacity was higher in the hypertensive groups at the beginning and at the end of the exercise training protocol than in the normotensive groups (Table 1). While the sedentary groups presented a similar loss in physical capacity throughout the studied period, the animals undergoing the exercise training protocol showed an increase in maximum running speed compared with the their initial values and to the respective sedentary group after 8 weeks of training.

Metabolic evaluations

Metabolic evaluations are shown in Table 1. The SHR groups (HFOS and HFOT) had lower body weight than the normotensive rats (NFOS and NFOT). All groups showed higher body weight at the end of the protocol compared with the respective values before ovariectomy. Exercise training reduced blood glucose in the NFOT and HFOT groups and triglycerides in the NFOT group. The hypertensive groups presented a decrease in insulin sensitivity after 10 weeks of fructose overload compared with that of the normotensive groups. At the end of the protocol (19 weeks of fructose overload), the NFOS group also showed reduced KITT in relation to its initial values, and no difference was observed between the NFOS, HFOS and HFOT groups at this time. Exercise training increased insulin sensitivity only in the NFOT group compared with the NFOS group (Table 1).

Table 1 Metabolic evaluations after 10 and 19 weeks of fructose overload

Parameters	*NFOS (8)*	*NFOT (8)*	*HFOS (8)*	*HFOT (8)*
Physical capacity (km h^{-1})				
10 weeks	1.71 ± 0.38	1.70 ± 0.15	2.73 ± 0.08†	2.79 ± 0.08†,‡
19 weeks	1.55 ± 0.21*	2.05 ± 0.23†,*	2.30 ± 0.12†,*	3.03 ± 0.03†,‡,§,*
Body weight (g)				
10 weeks	246 ± 8.9	248 ± 4.9	192 ± 11.3†	191 ± 8.9†,‡
19 weeks	365 ± 10.8*	329 ± 15.7*,†	257 ± 9.6*,†	245 ± 10.8*,†,‡,§
Blood glucose (mg dl^{-1})				
10 weeks	88 ± 2.63	88 ± 1.15	88 ± 1.50	92 ± 2.87
19 weeks	94 ± 2.06	80 ± 3.30*,†	88 ± 1.59	80 ± 1.54*,†,§
Triglycerides (mg dl^{-1})				
10 weeks	136 ± 9.39	156 ± 8.10	162 ± 8.59	163 ± 14.32
19 weeks	161 ± 12.81	94 ± 7.26*,†	145 ± 6.09	147 ± 6.49‡
Insulin tolerance test KITT (% min^{-1})				
10 weeks	5.30 ± 0.37	4.93 ± 0.38	3.97 ± 0.18†	4.00 ± 0.17†
19 weeks	3.34 ± 0.16*	4.55 ± 0.22†	3.82 ± 0.17	4.05 ± 0.19

Abbreviations: HFOS, hypertensive submitted to fructose overload, ovariectomized and sedentary group; HFOT, hypertensive submitted to fructose overload, ovariectomized and trained group; KITT, the rate constant for blood glucose disappearance after insulin tolerance test; NFOS, normotensive submitted to fructose overload, ovariectomized and sedentary group; NFOT, normotensive submitted to fructose overload, ovariectomized and trained group.
Data are reported as mean ± s.e.m. Ten weeks of fructose: after 10 weeks of fructose overload/before ovariectomy and exercise training; 19 weeks of fructose: after 19 weeks of fructose overload, at the end of the exercise training period.
*$P<0.05$ *vs.* 10 weeks in the same group; $^{\dagger}P<0.05$ *vs.* NFOS; $^{\ddagger}P<0.05$ vs NFOT; $^{\S}P<0.05$ *vs.* HFOS.

Hemodynamic assessments

As shown in Table 2, the hypertensive rats (HFOS and HFOT groups) showed higher AP than the normotensive rats (NFOS and NFOT groups). Additionally, the HFOS group showed resting tachycardia (*vs.* NFOS). However, compared with sedentary animals (NFOS and HFOS groups), the trained animals (NFOT and HFOT groups) presented resting bradycardia.

Cardiovascular autonomic modulation

Hypertension induced a reduction in SD-PI, RMSSD and VAR-PI relative to the values in the NFOS and HFOS rats. Exercise training induced an increase in RMSSD and VAR-PI in the NFOT group compared with NFOS and HFOT groups; however, VAR-PI was higher in the HFOT group than in the HFOS group (Table 2).

Table 2 Hemodynamics and heart rate variability evaluations

Parameters	*NFOS (8)*	*NFOT (8)*	*HFOS (8)*	*HFOT (8)*
SAP (mm Hg)	129 ± 1.7	129 ± 2.6	206 ± 3.8†	210 ± 10.9†,‡
DAP (mm Hg)	93 ± 2.4	96 ± 1.5	153 ± 2.5†	154 ± 1.8†,‡
MAP (mm Hg)	109 ± 1.9	111 ± 1.4	179 ± 2.9†	180 ± 1.6†,‡
HR (b.p.m.)	358 ± 7	334 ± 6†	394 ± 11†	348 ± 6§
SD-PI (ms)	7.26 ± 0.56	10.03 ± 0.85	6.80 ± 0.43†	6.29 ± 0.45†,‡
RMSSD (ms)	4.56 ± 0.23	5.64 ± 0.48†	3.70 ± 0.15†	3.45 ± 0.17†,‡
VAR-PI (ms^2)	41.35 ± 4.44	77.66 ± 11.21†	29.49 ± 4.07†	51.22 ± 6.53‡,§
LF-PI (ms^2)	4.37 ± 1.44	4.66 ± 0.74	1.74 ± 0.25†	1.42 ± 0.21†,‡
HF-PI (ms^2)	6.49 ± 0.99	8.51 ± 0.82	4.96 ± 0.54	4.14 ± 0.64‡

Data are reported as mean ± s.e.m.
Abbreviations: DAP, diastolic arterial pressure; HFOS, hypertensive submitted to fructose overload, ovariectomized and sedentary group; HF, high-frequency band; HFOT, hypertensive submitted to fructose overload, ovariectomized and trained group; HR, heart rate; LF, low-frequency band; MAP, mean arterial pressure; NFOS, normotensive submitted to fructose overload, ovariectomized and sedentary group; NFOT, normotensive submitted to fructose overload, ovariectomized and trained group; PI, pulse interval; 10 wks of fructose, after 10 weeks of fructose overloadday before ovariectomy; RMSSD, root mean square of successive differences; SAP, systolic AP; s.d., standard deviation; VAR, total variance.
†$P<0.05$ vs NFOS; ‡$P<0.05$ vs NFOT; §$P<0.05$ vs HFOS.

The LF band of PI showed an exacerbated reduction in both hypertensive groups in comparisons with both normotensive groups. The HF band of PI was lower in the HFOT group than in the NFOT group (Table 2).

Hypertensive rats showed an increase in systolic AP variance (VAR-SAP), although exercise training attenuated this dysfunction, as demonstrated by the fact that VAR-SAP was lower in the HFOT group than the HFOS group (NFOS: 23.13 ± 3.38; NFOT: 23.65 ± 2.09; HFOS: 74.16 ± 6.63; HFOT: 49.17 ± 4.86 mm Hg^2) (Figure 2a). The LF band of SAP was higher in the HFOS group than in the NFOS and NFOT groups (NFOS: 9.24 ± 1.19; NFOT: 6.10 ± 0.54; HFOS: 15.54 ± 2.35; HFOT: 7.97 ± 1.69 mm Hg^2). However, exercise training was effective at normalizing this variable in the HFOT group (Figure 2b).

The sedentary SHR animals showed a reduction in α-index relative to that of sedentary normotensive rats (HFOS: 0.34 ± 0.16 *vs.* 0.73 ± 0.08 ms mm Hg^{-1} in NFOS). Exercise training induced an increase in spontaneous baroreflex sensitivity in both trained groups relative to their respective sedentary groups (NFOT: 1.06 ± 0.09 *vs.* NFOS: 0.73 ± 0.08 ms mm Hg^{-1} and HFOT: 0.56 ± 0.07 *vs.* HFOS: 0.34 ± 0.16 ms mm Hg^{-1}). However, the α-index was higher in NFOT rats than in HFOT and HFOS rats (Figure 2c).

Oxidative stress profile

As shown in Table 3, cardiac (left ventricle) lipoperoxidation, which was analyzed by chemiluminescence, was lower in the NFOT and HFOT groups than in the NFOS and HFOS groups.

In this same table, no differences were found in superoxide dismutase among the studied groups. However, the cardiac catalase concentration was higher in both trained groups than in their respective sedentary pairs.

Negative correlations were obtained between RMSSD and lipoperoxidation ($r = -0.60$, $P<0.05$) (Figure 3a) and α-index and lipoperoxidation ($r = -0.63$, $P<0.05$) (Figure 3b).

DISCUSSION

Our results showed the adverse cardiometabolic effects of regular fructose consumption throughout life, a very common condition observed in the contemporary eating habits of industrialized countries, which was associated with genetically determined hypertension, the main etiology of hypertension, in an experimental model with similar dysfunctions observed in metabolic syndrome. Given that this syndrome is more prevalent in women experiencing climacterium, the ovarian hormone deprivation model used in this study enabled us to evaluate whether the metabolic syndrome characteristics were associated with hypertension in the absence of ovarian hormones. Finally, the use of aerobic training also enabled us to clearly demonstrate the beneficial effects of this non-pharmacological approach on the cardiometabolic profile. Interestingly, some adaptations were attenuated when several cardiovascular risks factors were added, as observed in HFOT rats.

The experimental model of menopause induced by ovariectomy has been extensively used in relatively young and old female rats for short periods (3–9 weeks). Although some results may differ according to the age of the rat at ovariectomy, this model promotes metabolic and hemodynamic alterations similar to those observed in postmenopausal women.[5,15,24–27] Although this model may not be appropriate to determine long-term changes in cardiovascular regulation after menopause, the ovariectomy procedure is an effective way to simulate menopause status as it promotes increased body weight, blood glucose and blood pressure, together with a reduction in baroreflex sensitivity and impairment in cardiovascular autonomic modulation compared with the condition for rats of the same age with intact ovaries.[5,15,24–26,28]

In normotensive ovariectomized rats undergoing fructose overload, exercise training (NFOT group) improved exercise capacity and reduced blood glucose, triglycerides and insulin resistance and was associated with resting bradycardia and increased RMSSD, PI-VAR and α-index (spontaneous baroreflex sensitivity). This increase in cardiac parasympathetic modulation (RMSSD and PI-VAR) might be associated with the improved α-index, as well as the decreased cardiac lipid peroxidation and increased catalase concentration.

In fact, several physiological alterations may be associated with both fructose consumption and the onset of menopause, for example, increased body weight, reduction in insulin sensitivity, hypertriglyceridemia and high blood pressure.[1,2,3,5,29] However, differences in body weight have not been found after fructose overload in SHR[30] and some studies failed to demonstrate alterations in body weight, in both trained male[31] and female[32] SHR animals. Indeed, in the present study, the weight gain in SHR was smaller than in Wistar rats at the end of the protocol, a difference that can be attributed to the strain characteristics.

Although the blood glucose level was not different in the sedentary groups by the end of the protocol, exercise training similarly decreased this value in both normotensive and hypertensive rats. However, triglyceride levels were increased and insulin tolerance was decreased in normotensives at the 19th week. Consistent with this finding, it has been demonstrated that the exposure of the liver to large quantities of fructose promotes a rapid stimulation of lipogenesis, which contributes to triglyceride accumulation and leads to a decreased number of insulin receptors and, consequently, to decreased insulin sensitivity.[33] Indeed, a causative link between elevated circulating triglyceride and impaired insulin action has been observed in fructose-fed rats in other studies.[1,2,34] Moreover, the sustained elevation of plasma triglyceride concentrations after fructose ingestion suggests that chronic consumption of fructose could also contribute to cardiovascular disease.[3,4,35]

Importantly, we also observed in this study that exercise training reduced both blood glucose and triglyceride levels along with insulin resistance in normotensive ovariectomized rats undergoing fructose overload (NFOT group). However, it should be emphasized that these metabolic benefits were sharply attenuated in hypertensive rats, as only

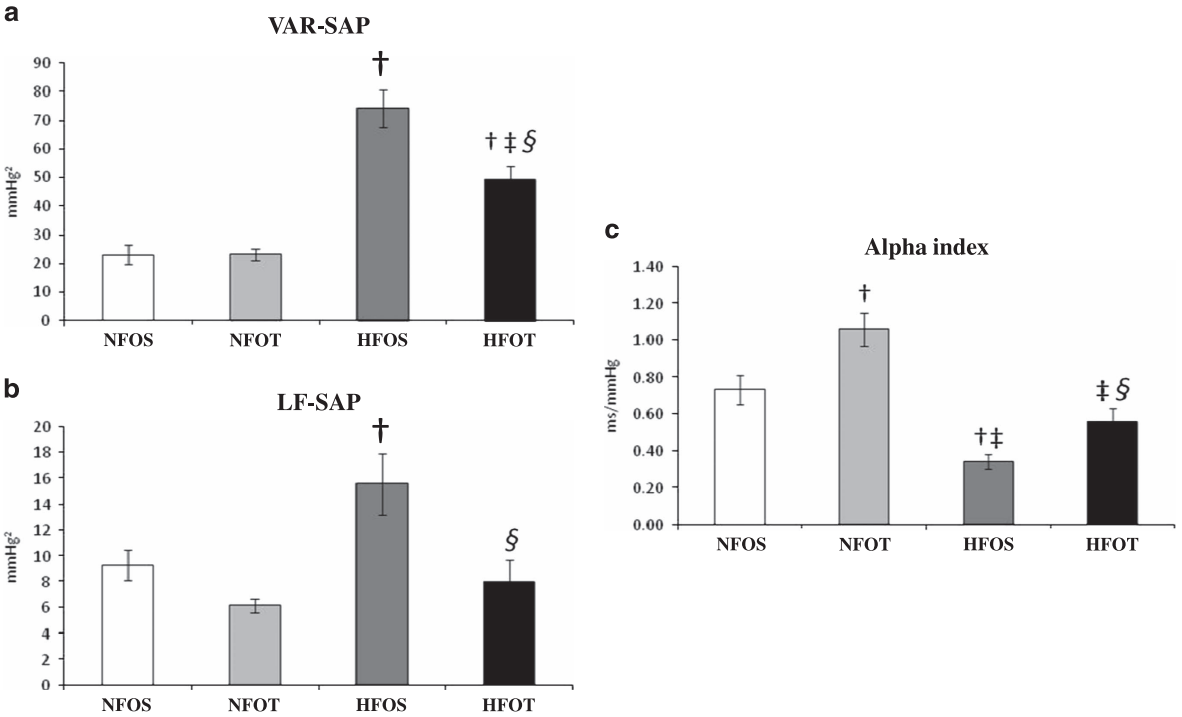

Figure 2 (**a**) Total variance of systolic arterial pressure; (**b**) low-frequency band of systolic arterial pressure; and (**c**) spontaneous baroreflex sensitivity evaluated by the α-index in the studied groups ($n=8$ in each group). $^{\dagger}P<0.05$ vs NFOS, $^{\ddagger}P<0.05$ vs NFOT, $^{\S}P<0.05$ vs HFOS. HFOS, hypertensive rats subjected to fructose overload, ovariectomy and a sedentary status; HFOT, hypertensive rats subjected to submitted to fructose overload, ovariectomy and training; NFOS, normotensive rats subjected to fructose overload, ovariectomy, and a sedentary status; NFOT, normotensive rats subjected to fructose overload, ovariectomy and training.

Table 3 Cardiac oxidative stress evaluations

Parameters	*NFOS (8)*	*NFOT (8)*	*HFOS (8)*	*HFOT (8)*
Lipoperoxidation by CL (cps per mg protein)	15 432 ± 1010	9270 ± 1438†	14 914 ± 2159‡	11 183 ± 818†,§
SOD (USOD per mg protein)	12.56 ± 2.09	12.04 ± 0.94	10.53 ± 0.65	10.72 ± 0.75
GPx (nmol per mg protein)	14.14 ± 1.58	12.68 ± 0.80	15.61 ± 1.29	14.26 ± 1.34
Catalase (pmol per mg protein)	52.39 ± 4.8	80.17 ± 8.27†	60.79 ± 4.97	82.35 ± 4.58§

Abbreviations: CL, chemiluminescence; GPx, glutathione peroxidase; HFOS, hypertensive submitted to fructose overload, ovariectomized and sedentary group; HFOT, hypertensive submitted to fructose overload, ovariectomized and trained group; NFOS, normotensive submitted to fructose overload, ovariectomized and sedentary group; NFOT, normotensive submitted to fructose overload, ovariectomized and trained group; SOD, superoxide dismutase.
Data are reported as mean ± s.e.m.
$^{\dagger}P<0.05$ vs NFOS; $^{\ddagger}P<0.05$ vs NFOT; $^{\S}P<0.05$ vs HFOS.

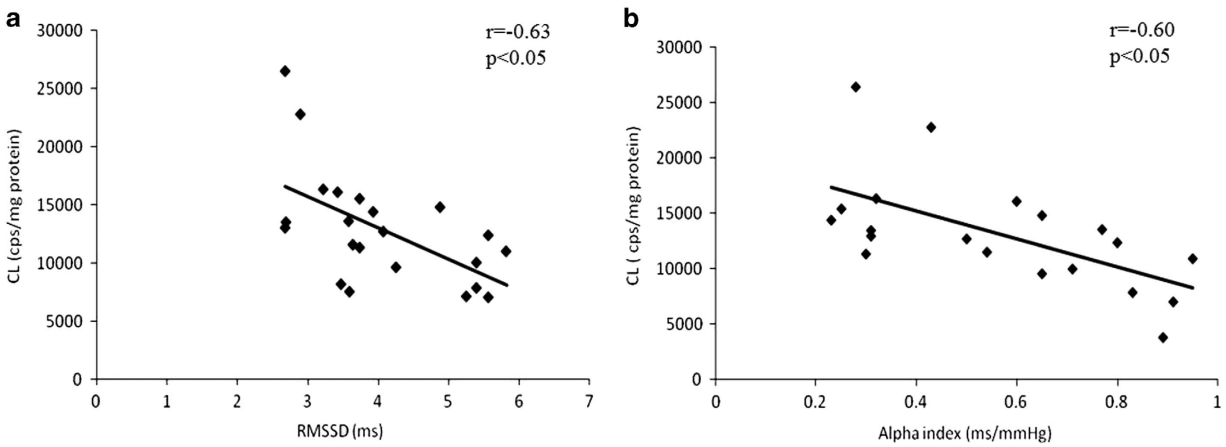

Figure 3 Correlations obtained by linear regression of the studied groups between (**a**) root mean square of successive differences (RMSSD) and lipoperoxidation evaluated by chemiluminescence (CL); and (**b**) α-index and lipoperoxidation evaluated by CL.

reduced glycemia was observed in the HFOT group compared with the HFOS group. A probable mechanism for the exercise training-induced improvement in fructose-induced hypertriglyceridemia would be the suppression of the conversion of fructose into triglycerides at the liver and the promotion of triglyceride muscle utilization, as trained rats have higher uptake, activation and oxidation of fatty acids in muscle tissue.[33] In this regard, the higher velocity obtained in the maximal exercise tests after 4 and 8 weeks of exercise training in the trained groups is a marker of the effectiveness of the exercise training protocol used in the present study.[5,16,26,28]

One of the most desirable effects of exercise training in hypertension is the decrease in blood pressure levels. In fact, several studies demonstrated that aerobic exercise training significantly reduced systolic and diastolic pressure.[7,31,36] Nevertheless, in the present study, when hypertension was concomitantly associated with other cardiovascular risk factors, such as estrogen deprivation and chronic and excessive fructose intake, blood pressure was not diminished after exercise training, thus corroborating the study of Sanches *et al.*[4] In our study situation, blood pressure was impacted by multiple disorders (hypertension, fructose overload and ovariectomy), with the consequent effects on several mechanisms of AP control at the same time. As cardiac output and peripheral resistance are the key elements of blood pressure, we have to consider that although a slight improvement in autonomic modulation was observed, other mechanisms, such as endothelium-dependent relaxation, the renin–angiotensin system, vascular oxidative stress, and inflammation, may have not been sufficiently modified, thus contributing to the maintenance of blood pressure within hypertensive levels.[37–39] Moreover, HFOT rats presented decreased values of parasympathetic parameters compared with those of normotensive rats (RMSSD, HF-PI); therefore, even with a partial reduction in LF-SAP, the vagal modulation was still low, a factor that may also have contributed to the unchanged blood pressure.

Although the aerobic exercise training in this protocol did not provoke all of the expected results when hypertension, ovariectomy and fructose intake were concomitantly associated, it is noticeable that this exercise is still a widely recommended tool to minimize menopause-related perturbations.[5,26] In a condition with several cardiovascular risk factors, as evidenced in this study, a low-to-moderate aerobic exercise training alone seemed to be not sufficient to evoke all of the beneficial alterations from exercise training practice. However, in the study of Conti *et al.*,[40] using the same model, a combination of resistance and aerobic training was effective in attenuating the cardiometabolic dysfunctions, including a blood pressure decrease. This finding indicates that for a blood pressure decrease, a combination of resistance and aerobic exercise training may be more effective than only aerobic training when several cardiovascular risk factors are combined.

Although the causes of higher cardiovascular morbidity and mortality in postmenopausal women remain poorly understood, they may involve changes in AP regulation after estrogen deprivation.[41–43] Moreover, low baroreflex sensitivity, a marker of autonomic control, is associated with severity of cardiovascular disease.[44,45] In these sense, fructose overload promotes an increase in AP, which may be attributed to increased VAR-SAP and LF-SAP[46] and a reduction in spontaneous baroreflex sensitivity[47] in male mice.

In the present study, the hypertensive ovariectomized rats undergoing fructose overload (HFOS group) showed a well-established systolic and diastolic hypertension accompanied by resting tachycardia, reduced cardiac parasympathetic modulation (SD-PI, RMSSD, VAR-PI), increased vascular sympathetic modulation (LF-SAP band and SAP-VAR) and impairment in spontaneous baroreflex sensitivity (α-index) relative to those of normotensive rats undergoing fructose overload (*vs.* NFOS group). We also observed an exacerbated reduction in the LF band of PI, which has been associated with cardiac sympathetic overactivity in heart failure. It should be noted that although exercise training did not reduce AP among hypertensive rats, this approach induced attenuation of the resting tachycardia, a reduction in vascular sympathetic modulation (LF-SAP band, SAP-VAR) and increases in VAR-PI and the spontaneous baroreflex (α-index) in hypertensive rats (HFOT *vs.* HFOS).

In this study, exercise training promoted an improvement in the spontaneous baroreflex sensitivity in both trained groups (NFOT and HFOT), but hypertensive rats (HFOT group) showed a reduction in this parameter relative to that of the normotensive group (NFOT group). The reduced α-index in hypertensive groups (*vs.* normotensive) may be related to the inefficiency of exercise training in lowering blood pressure and normalizing cardiac and vascular autonomic modulation in the HFOT group (*vs.* HFOS). The mechanism involved in baroreflex sensitivity improvement after exercise training in the present study can be associated with a reduction in the LF-SAP band and in VAR-SAP in the HFOT group (*vs.* HFOS), as well as an improvement in VAR-PI. In fact, we have reported improved baroreflex sensitivity associated with improved sympathovagal cardiac balance in infarcted[48] or diabetic ovariectomized rats undergoing exercise training.[28]

Beyond alterations in the efferent branch of the baroreflex, there is a general agreement that the afferent pathway of this reflex is important for the activation of neurovascular adjustments to exercise training, as improved baroreflex sensitivity in trained SHR rats has been related to a significant increase in aortic depressor nervous activity.[49] furthermore, we may speculate that after exercise training, arterial compliance would be improved in the vascular network,[50,51] including the aorta and carotid arteries, thus facilitating the mechanical transduction at baroreceptors and, consequently, baroreflex control. In this regard, Bertagnolli *et al.*[6] have reported a significant improvement in the oxidative stress of the aorta in trained SHR, which was associated with an increase in baroreflex sensitivity in trained rats. Finally, we cannot rule out the possibility that the changes in the central nervous system may be related to the improvement in baroreflex sensitivity after exercise training. In fact, Felix *et al.*[52] observed that exercise training normalizes the central high angiotensinogen RNAM levels in SHR.

Previous studies have shown that the consumption of a high fructose diet negatively affects the balance between reactive oxygen species production and antioxidant defense in rats, leading to increased lipid susceptibly to peroxidation.[53,54] Lipoperoxidation indicates oxidative damage, particularly in the membrane lipids, and its association with decreased antioxidant defense determines oxidative stress status.[20] Female ovariectomized rats have shown a higher susceptibility to lipid peroxidation after sucrose intake.[53] Bertagnolli *et al.*[6,7] have shown that trained male SHR showed reduced cardiac lipoperoxidation correlated with autonomic improvement. In our study, both trained groups presented reduced cardiac lipoperoxidation and increased catalase, while no differences were found in the superoxide dismutase and glutathione peroxidase activities among the studied groups. Girard *et al.*[30] have demonstrated similar results in the cardiac tissue of male Wistar rats and SHR undergoing fructose overload. Moreover, our data reinforce the role of exercise training in the increase in antioxidant defenses, which leads to improvement in the oxidative stress profile, as previously observed in trained male SHR and ovariectomized wistar rats.[5,6]

It should be stressed that although hypertension adversely affects the exercise training benefits in rats undergoing fructose consumption, our study demonstrated correlations between training-induced cardiovascular autonomic improvement and oxidative stress reduction, thus indicating that rats presenting higher RMSSD (a parasympathetic index) or baroreflex sensitivity also presented reduced cardiac lipoperoxidation. Although we cannot exclude the possibility that oxidative stress may also induce a decrease in parasympathetic function, current evidence indicates that parasympathetic function may modulate the inflammatory and oxidative stress in pathophysiological conditions.[55] Consequently, we hypothesize that in the studied model, aerobic exercise training promoted cardiovascular autonomic control improvement, which would, in turn, trigger oxidative stress changes and prompt clinical cardiovascular benefits in this experimental model of metabolic syndrome.

In conclusion, hypertension promoted additional hemodynamic and autonomic dysfunctions in fructose-fed ovariectomized rats and attenuated some aerobic exercise training benefits. Autonomic and oxidative stress parameters were improved in both normotensive and hypertensive groups; however, most of these parameters were still impaired in hypertensive rats relative to normotensive rats. These alterations may be related to the changes in cardiovascular autonomic modulation associated with oxidative stress.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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