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Resistance training prevents the cardiovascular changes caused by high-fat diet

Guilherme F. Speretta [a], André A. Silva [a], Regina C. Vendramini [b], Angelina Zanesco [c], Maria A. Delbin [d], José V. Menani [a], Mirian Bassi [a], Eduardo Colombari [a], Débora S.A. Colombari [a,*]

[a] Department of Physiology and Pathology, School of Dentistry, São Paulo State University (UNESP), Araraquara, SP, Brazil
[b] Department of Clinical Analysis, School of Pharmaceutical Sciences, UNESP, Araraquara, SP, Brazil
[c] Department of Physical Education, Institute of Bioscience, UNESP, Rio Claro, SP, Brazil
[d] Department of Structural and Functional Biology, Institute of Biology, University of Campinas (UNICAMP), Campinas, SP, Brazil

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ABSTRACT

Aims: Aerobic exercise is indicated for prevention and treatment of obesity-induced cardiovascular disorders. Although the resistance training (RT) may also produce effects similar to aerobic exercise, this is not completely clear yet. In the present study, we tested if RT in moderate intensity might prevent alterations in blood pressure (BP), sympathetic modulation of systolic blood pressure (SBP), baroreflex function and the changes in renin-angiotensin system (RAS) and cytokines mRNA expression within the nucleus of the tract solitary (NTS) in rats fed with high-fat diet (HFD).

Main methods: Male Holtzman rats (300–320 g) were divided into 4 groups: sedentary with standard chow diet (SED-SD); sedentary with high-fat diet (SED-HFD); RT with standard chow diet (RT-SD); and RT with high-fat diet (RT-HFD). The trained groups performed a total of 10 weeks of moderate intensity RT in a vertical ladder. In the first 3 weeks all experimental groups were fed with SD. In the next 7 weeks, the SED-HFD and RT-HFD groups were fed with HFD.

Key findings: In SED-HFD, BP and sympathetic modulation of SBP increased, whereas baroreflex bradycardic responses were attenuated. RT prevented the cardiovascular and inflammatory responses (increases in tumoral necrosis factor-α and interleukin-1β) produced by HFD in SED rats. The anti-inflammatory interleukin-10, angiotensin type 2 receptor, Mas receptor and angiotensin converting enzyme 2 mRNA expressions in the NTS increased in the RT-HFD compared to SED-HFD.

Significance: The data demonstrated that moderate intensity RT prevented obesity-induced cardiovascular disorders simultaneously with reduced inflammatory responses and modifications of RAS in the NTS.

1. Introduction

Excessive fat accumulation, particularly in the visceral adipose tissue, is associated with chronic inflammation in different tissues, including the brain, causing metabolic changes which favor the development of degenerative diseases such as cardiovascular disorders [10,14,47]. In this regard, impairment of baroreflex sensitivity, increased sympathetic nerve activity (SNA), increase in the renin angiotensin system (RAS) and high blood pressure (BP) have been described in this situation [7,15,29,49].

The RAS components are found in the main areas of the brain involved in cardiovascular control, including the nucleus of the tract solitary (NTS) [31], the primary site of baroreceptor afferents in the dorsal hindbrain [9]. It has been shown that the activation of AT_1 receptors in the NTS attenuates baroreflex sensitivity [32,36] and angiotensinergic mechanisms in the NTS contribute to the development of different models of hypertension [5,18,22,54]. In young obese rats, it was demonstrated that AT_1 receptors are augmented in the NTS [13]. Additionally, chronic inflammation in the NTS is suggested to be involved in the etiology of neurogenic hypertension [53,54] and the microinjection of the pro-inflammatory cytokine interleukin-6 (IL-6) in the NTS blunts the baroreflex function in rats [50]. Interestingly, recent studies have described an interaction between inflammation and the RAS in the brain as a mechanism involved with different models of cardiovascular disorders including those produced by obesity [1,11,44,48].

Aerobic exercise is an important non-pharmacological intervention to prevent or treat hypertension [28,37]. In animals, the obesity-associated mild hypertension decreased after aerobic exercise [38] and the full development of hypertension was prevented with aerobic exercise in pre-hypertensive spontaneously hypertensive rats (SHR) [1,18].

* Corresponding author at: Department of Physiology and Pathology, School of Dentistry, São Paulo State University — UNESP, Rua Humaitá, 1680, Araraquara 14801-903, SP, Brazil.
E-mail address: deborac@foar.unesp.br (D.S.A. Colombari).

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Aerobic exercise also decreases the mRNA levels of angiotensinogen in the NTS in hypertensive SHR [18].

Similar to aerobic exercise, resistance training (RT) also produces positive effects on the cardiovascular system of hypertensive rats [16,29,45]. High intensity RT attenuated the increase in adiposity and the development of mild hypertension [29], and ameliorated the changes of metabolism in obese rats [47], which suggests that similar to aerobic exercise, RT could be an intervention to treat the deleterious changes in metabolism and cardiovascular system found in obese rats. Therefore, the purpose of the present study was to test if moderate intensity RT prevents the alterations in BP, sympathetic modulation of systolic blood pressure (SBP) and baroreflex function in HFD feeding rats. In addition, no study has investigated in HFD feeding rats the formation of pro- and anti-inflammatory cytokines or the RAS components in the NTS, a key area of the brainstem for cardiovascular control [5,22,32,36]. Then, we also tested if the positive effects of RT in the cardiovascular system were accompanied by alterations in RAS and cytokines mRNA expression in the NTS.

2. Materials and methods

2.1. Animals

A total number of 40 male Holtzman rats weighing 300–320 g were used. The animals were maintained in collective polypropylene cages (2 or 3 animals per cage) with food (please see composition bellow) and water provided ad libitum in a room with controlled temperature (23 ± 2 °C) and humidity (55 ± 10%). Lights were on from 7:00 am to 7:00 pm. Ethics Committee for Animal Care and Use of the Dental School of Araraquara, UNESP, approved the experimental protocols used in the present study (protocol number CEUA15/2013) and it has been carried out in accordance with the EU Directive 2010/63/EU for animal experiments.

2.2. Experimental design

The experimental groups received standard rat chow diet (Biobase, Águas Frias, SC, Brazil), named standard diet (SD) or high-fat diet (HFD). Bromatological analysis (Engeali, São José do Rio Preto, SP, Brazil) determined that SD contained 22 g of protein, 48 g of carbohydrates, 4 g of total fat, 8 g of fiber and 200 mg of sodium per 100 g of diet. HFD was composed of standard rat chow plus peanuts, milk chocolate, and sweet biscuits in a proportion of 3:2:2:1 as previously described [47] and contained 13 g of protein, 40 g of carbohydrate, 19 g of total fat, 4 g of fiber and 73 mg of sodium per 100 g of diet. The caloric values of the diets were approximately 2.25 kcal/g for the SD and 3.82 kcal/g for HFD. Body weight and food intake were recorded 3 times a week.

Rats were divided into four experimental groups balanced to ensure equal initial body weight and maximum voluntary carrying capacity (MVCC) across groups: sedentary (SED) with SD (SED-SD); SED with HFD (SED-HFD); resistance training (RT) with SD (RT-SD); and RT with HFD (RT-HFD). Trained groups performed 10 weeks of RT on ladder at 50–60% of MVCC, 3 days a week (Mondays, Wednesdays and Fridays). Each training session consisted of 15–20 ladder climbs with a 30 s rest interval between climbs. The maximal voluntary carrying test was repeated every 2 weeks until the week 8. Sedentary rats also performed MVCC tests at the same time intervals as indicated for the trained animals, but otherwise remained in their cages and did not undergo to the training protocol. All groups were fed with standard chow diet until the end of the week 3 of RT to guarantee an increase in physical fitness observed by MVCC in the trained groups (RT-SD and RT-HFD) before offering the HFD. After that both HFD (SED-HFD and RT-HFD) groups were fed with HFD for 7 weeks.

24 h after the last RT session, the rats were submitted to 8 h of fasting to conduct insulin tolerance test (ITT) and blood glucose levels measurement. Two days later, mean arterial pressure (MAP) and heart rate (HR) were recorded in conscious freely moving rats. Baroreflex tests started at least 20 min after connecting the arterial cannula to the recording system. In the next day, the rats were decapitated after 12 h of fasting for collection of blood sample to analyze the levels of insulin, total cholesterol, triglycerides (TGL) and high-density lipoprotein (HDL). The brains were removed to analyze mRNA expression and the adipose tissue to analyze relative weight.

2.3. Resistance training

The RT protocol was adapted from Hornberger & Farrar [26]. The rats were adapted to the RT protocol by climbing a vertical ladder (1.1 m; 0.18 m, 2-cm grid, 80° inclination) with a load apparatus without weight. The load apparatus was fixed to the tail by wrapping its proximal portion with a self-adhesive foam strip. With the load apparatus fixed to the tail, each rat was placed at the bottom of the ladder and familiarized with the climbing procedure. If necessary, a stimulus with tweezers was applied to the animal's tail to initiate movement. When the rats reached the top of the ladder (house chamber), they were allowed to rest for 60 s. This procedure was repeated until they would voluntarily climb the ladder for three consecutive turns without any stimuli. This procedure was repeat for two no consecutive days.

Two days after the adaptation procedures, each animal performed a test in order to evaluate its MVCC that consisted of climbs with progressive heavier loads. The initial climb was performed with 75% of the animal's body mass and additional 30-g weight loads was added in the next climbs until the rat could not climb the entire length of the ladder. The highest load that the animal successfully carried the entire length of the ladder was considered the MVCC for that training session. Failure was determined when the animal could not progress up the ladder after three successive stimuli to the tail.

Two days after the MVCC test, RT protocol was performed as described above in the Experimental Design.

2.4. Drugs

Phenylephrine [5 μg/kg of body weight (wt.)] and sodium nitroprusside (30 μg/kg of body wt.) purchased from Sigma Chem. Co. (St. Louis, MO, USA) were injected i.v. for the baroreflex tests. The drugs were diluted in saline.

2.5. Arterial pressure recording and baroreflex function

MAP and HR were recorded in conscious freely moving rats. 48 h after the last RT session the rats were anesthetized with ketamine (80 mg/kg of body wt.); Cristália, Itapira, SP, Brazil] and xylazine (7 mg/kg of body wt.; Agener Union, Embu, SP, Brazil) and under aseptic conditions the femoral artery and vein were isolated and cannulated with polyethylene tubes (PE-10 connected to a PE-50) filled with saline. The catheters were exteriorized between the scapulae and fixed on the back of the animal to allow MAP and HR recording in freely moving animals. The femoral vein catheter was used to drugs administrations.

To record pulsatile arterial pressure, MAP and HR in conscious unrestrained, freely moving animals, the arterial catheter was connected to a Statham Gould (P23 Db; El Segundo, CA, USA) pressure transducer coupled to a pre-amplifier (model ETH-200 Bridge Bio Amplifier, Chicago, IL, USA) that was connected to a Powerlab computer data acquisition system (model Powerlab 16SP, ADInstruments, Colorado Springs CO, USA). After a baseline period of cardiovascular recordings, rats received i.v. injections of phenylephrine (5 μg/kg of body wt.) or sodium nitroprusside (SNP; 30 μg/kg of body wt.) to test the HR reflex responses to pressor and depressor stimuli, respectively. We analyzed the one-second mean HR values in response to 10 mm Hg incremental changes in MAP, starting in 5 mm Hg up to a maximal change of 35 mm Hg. The values were plotted and a linear regression was performed for each

animal, and the slope of each linear regression was used to calculate the differences between groups.

2.6. Cardiovascular variability

The pulse interval (PI) and systolic blood pressure (SBP) variability analysis were performed using a custom computer software (CardioSeries v2.4 — http://www.danielpenteado.com), as described previously [5,39,42]. Briefly, beat-by-beat series obtained from pulsatile arterial pressure recordings were converted to data points every 100 ms using cubic spline interpolation (10 Hz). The interpolated series was divided into half-overlapping sequential sets of 512 data points (51.2 s). Before calculation of the spectral power density, segments were visually inspected and non-stationary data were not taken into consideration. A Hanning window was used to attenuate side effects and the spectrum was computed using a direct FFT algorithm for discrete time series. The spectra were integrated in low-frequency (LF; 0.2–0.75 Hz) and high-frequency (HF; 0.75–3 Hz) bands, and results are expressed in absolute for SBP (mm Hg^2) and normalized units (nu) for PI. The normalized values were achieved by calculating the percentage of LF and HF power with regard to the total power of spectrum minus very low frequency band (VLF; <0.2 Hz) power [4,52]. To assess the sympathovagal balance, LF/HF ratio of PI variability was calculated [33]. LF of SBP is an index of the sympathetic vasoconstrictor tone, whereas LF and HF of PI are index of sympathetic and parasympathetic modulation of the PI.

2.7. Insulin tolerance test

Insulin sensitivity was measured by insulin tolerance test (ITT) and the rate constant for plasma glucose disappearance (Kitt) was calculated using the formula 0.693/biological half-life ($t_{1/2}$). The plasma glucose $t_{1/2}$ was calculated from the slope of the least square analysis of the plasma glucose concentration during linear phase of decline [6,12]. Rats were submitted to an ITT after 8 h of fasting. Human recombinant insulin (Novolin R, Novo Nordisk, Montes Claros, MG, Brazil) was administered at dose of 2.0 U/kg, i.p. and blood samples were collected at 0, 5, 10, 15, 20, 30 and 60 min from tail vein and blood glucose was measured using standard test strips (One Touch UltraMini/Johnson & Johnson, Milpitas, CA, USA).The ITT is expressed as Kitt (%/min), meaning the percentage of plasma glucose concentration decline per minute [12].

2.8. Blood and tissue collection

The animals were anesthetized with halothane (4% in 100% O_2) and immediately decapitated. Blood was collected in EDTA coated tubes for plasma insulin analyses and non-coated tubes for lipid profile and glucose measurements. Blood was centrifuged (1200 g for 10 min at 4 °C). Insulin levels were analyzed using commercially available ELISA kits, following the instructions of the manufacturer (ALPO, Salem, NH, USA). The triglycerides (TGL), total cholesterol, and high-density lipoprotein (HDL) concentrations were determined enzymatically (Labtest, Lagoa Santa, MG, Brasil), using automation equipment Labmax 240 (Hirose Electronic System CO, Nishinasuno, Japan). After blood collection, the brains were quickly removed, immediately frozen on dry ice and stored at −80 °C for subsequent mRNA extraction (described below). Finally, retroperitoneal (RET), epididymal (EPI) and mesenteric (MES) white adipose tissues were removed and weighed.

2.9. qRT-PCR analyses in the NTS

We evaluated the mRNA levels in the NTS of tumor necrosis factor α (TNF-α; Rn99999017_m1), interleukin-1β (IL-1β; Rn99999009_m1), interleukin-6 (IL-6; Rn01410330_m1); interleukin-10 (IL-10; Rn99999012_m1), angiotensin type-1 receptor (AT_1; Rn01435427_m1), angiotensin type-2 receptor (AT_2; Rn00560677_s1), Mas receptor (Mas; Rn00562673_s1), angiotensin-converting enzyme (ACE; Rn00561094_m1), angiotensin-converting enzyme 2 (ACE2; Rn01416293_m1). The NTS was removed by micropunch with the aid of a surgical microscope using the area postrema and *calamus scriptorius* as reference sites. Total RNA was extract using b-mercaptanol and isolated using a PureLink® RNA mini kit (Life technologies, Grand Island, USA). The isolated RNA was converted in cDNA using a high-capacity cDNA reverse transcription kit (Life technologies, Grand Island, USA) and samples were run in duplicate using a StepOne Real-time PCR system, Taqman Universal Gene Expression Master Mix and validated Taqman probes (Applied Biosystems, Foster City, CA, USA). Expression patterns of genes of interest were normalized to constitutively-expressed 18 s (Hs99999901_s1) and relative expression was quantified using the $2^{\Delta\Delta Ct}$ method [30].

2.10. Statistical analysis

Data are expressed as means ± SEM. One or two-way ANOVA followed by Student–Newman–Keuls post hoc were used for comparisons as appropriate. Differences were taken as significant at $P < 0.05$.

3. Results

3.1. Body weight and MVCC in rats on high-fat diet performing resistance training

Body weight was measured weekly and the MVCC was adjusted every two weeks to guarantee that the intensity of exercise would be maintained between 50 and 60% during the 10 weeks of RT and to compare the relative intensity between SD and HFD groups. Baseline body weight before the commencing of the experimental protocol was similar in all experimental groups and all rats had a similar increase of body weight during the first 8 weeks of RT (5 weeks of HFD). At week 9 (6 weeks of HFD) the SED-HFD group showed increased body weight compared to SED-SD, and at week 10 (7 weeks of HFD) both HFD groups (SED-HFD and RT-HFD) showed similar increased body weight compared to their control groups (SED-SD and RT-SD, respectively) [$F(3352) = 16.94$; $P < 0.0001$] (Fig. 1A).

The MVCC was similar among all groups at the beginning of the training protocol (week 0). RT increased the MVCC similarly in SD and HFD groups from week 4 until the end of the experimental period [$F(3,80) = 72.18$; $P < 0.0001$] (Fig. 1B).The number of climbs per session was not different between RT groups (RT-HFD: 16.8 ± 0.3 vs. RT-SD: 16.9 ± 0.3 climbs/session) [$F(1,232) = 1.054$; $P = 0.3057$].

3.2. Adiposity and metabolic changes in rats on high-fat diet performing resistance training

Considering that high intensity RT reduces body weight and adiposity [29,47], the impact of moderate RT on adiposity and metabolic changes in HFD feeding animals were evaluated. After 10 weeks (7 weeks of HFD), both HFD groups exhibited increased relative weight of epididymal, retroperitoneal and mesenteric adipose tissue [$F(3,24) = 7.07$, $P = 0.0014$; $F(3,24) = 16.90$, $P < 0.0001$; and $F(3,24) = 9.04$, $P = 0.0003$, respectively] (Table 1).Total cholesterol [$F(3,16) = 8.24$; $P = 0.0015$] and triglycerides [$F(3,16) = 5.86$; $P = 0.0067$] also increased in sedentary HFD group. RT prevented the increase in total cholesterol in HFD rats (Table 1).

In sedentary HFD group, fasting blood glucose [$F(3,28) = 3.20$, $P = 0.0385$] and blood insulin levels increased [$F(3,20) = 8.34$, $P = 0.0009$], whereas insulin sensitivity was reduced [$F(3,31) = 4.84$, $P = 0.0071$]. RT prevented the increase in blood glucose levels and the decrease in insulin sensitivity and slightly decreased the concentration of insulin (not statistically different) in HFD rats (Table 1).

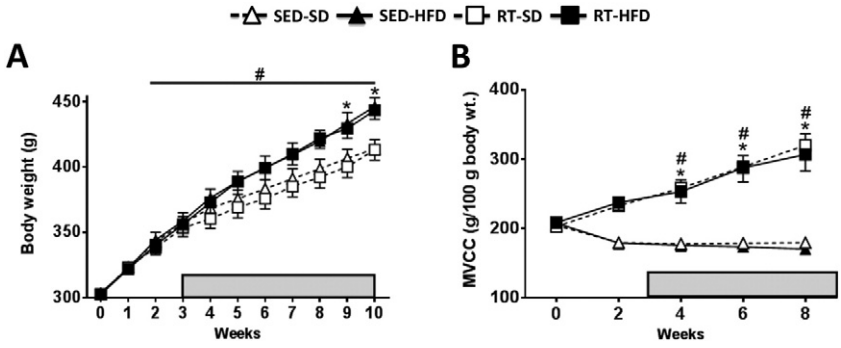

Fig. 1. (A) Body weight and (B) maximal voluntary carrying capacity (MVCC) of sedentary (SED) and resistance trained (RT) rats fed with standard chow diet (SD) or high-fat diet (HFD) for 7 weeks. The results are presented as means ± SEM. The bar indicates the period of HFD administration. Two-way ANOVA followed by the Student–Newman–Keuls test. *Different from SD groups; #different from week 0; $P < 0.05$. In (A) the number of animals was 9 in all groups and in (B) the number of animals was 5 in all groups.

3.3. MAP, HR and baroreflex in rats on high-fat diet performing resistance training

To test if RT could prevent the cardiovascular changes induced by HFD, MAP, HR, baroreflex function and cardiovascular variability were assessed. After 10 weeks (7 weeks of HFD), MAP increased in HFD sedentary rats (119 ± 1, vs. SED-SD: 107 ± 2 mm Hg) (Fig. 2A). RT prevented the increase in MAP produced by HFD (109 ± 2 mm Hg), without changing MAP in rats with SD (106 ± 4 mm Hg) [$F(3,28) = 4.46$; $P = 0.0110$] (Fig. 2A). HR also increased in sedentary HFD rats (370 ± 7, vs. SED-SD: 341 ± 3 bpm), an effect prevented by RT (336 ± 11 bpm) [$F(3,28) = 3.73$; $P = 0.0225$]. RT did not change HR in SD rats (339 ± 12, vs. SED-SD: 341 ± 3 bpm; $P > 0.05$) (Fig. 2B).

The bradycardic response to baroreflex activation was impaired in sedentary rats fed with HFD (SED-HFD slope: −1.24 ± 0.15, vs. SED-SD slope: −2.38 ± 0.22 bpm/mm Hg). RT prevented these alterations in HFD fed rats (RT-HFD slope: −2.76 ± 0.23 bpm/mm Hg), without changing baroreflex bradycardia in SD rats (RT-SD slope: −2.41 ± 0.19 bpm/mm Hg), [$F(3,24) = 10.02$; $P = 0.0002$] (Fig. 2C). The tachycardic response to baroreflex activation was similar between groups [$F(3,24) = 0.46$; $P = 0.71$] (Fig. 2C).

In the sedentary HFD group, the spectral analysis of SBP showed increased LF of SBP (8.49 ± 0.59, vs. SED-SD: 4.96 ± 0.37 mm Hg^2), [$F(3,26) = 4.70$; $P = 0.0094$], LF of PI [45 ± 2, vs. SED-SD: 22 ± 1 normalized unit (nu)], [$F(3,26) = 14.98$; $P < 0.0001$] and LF/HF ratio of PI (0.77 ± 0.07, vs. SED-SD: 0.29 ± 0.02), [$F(3,26) = 18.13$; $P < 0.0001$] and reduced HF of PI (57 ± 2, vs. SED-SD: 78 ± 2 nu), [$F(3,26) = 6.91$; $P < 0.0014$]. RT prevented the changes in the LF of SBP in HFD group (RT-HFD: 6.29 ± 1.06 mm Hg^2), LF of PI (RT-HFD: 34 ± 3 nu), LF/HF ratio of PI (RT-HFD: 0.58 ± 0.03) and HF of PI (RT-HFD: 69 ± 5 nu) (Fig. 3).

3.4. RAS components and cytokines mRNA expression in the NTS in rats on high-fat diet performing resistance training

Seeking the mechanisms for positive effects induced by RT on cardiovascular system, we analyzed the alterations in the RAS components and cytokines mRNA expression in the NTS, a key area in the brainstem involved with cardiovascular regulation. In HFD sedentary rats, AT_1 receptor mRNA expression increased (3.10 ± 0.12, vs. SED-SD: 1.00 ± 0.06 fold change), [$F(3,13) = 25.69$; $P < 0.0001$] and AT_2 receptor mRNA expression decreased in the NTS (0.68 ± 0.11, vs. SED-SD: 1.00 ± 0.07 fold change), [$F(3,13) = 4.97$; $P = 0.016$]. RT prevented the decrease in AT_2 receptor expression (RT-HFD: 1.01 ± 0.05 fold change), without changing the increase in AT_1 receptor expression (RT-HFD: 3.68 ± 0.42 fold change). In trained rats fed with HFD, Mas mRNA expression in the NTS also increased (RT-HFD: 1.55 ± 0.19 fold change) compared to sedentary rats fed with HFD (SED-HFD: 0.70 ± 0.11), [$F(3,13) = 4.72$; $P = 0.019$]. In both trained groups, ACE2 mRNA expression in the NTS increased compared to their control groups (RT-HFD: 2.62 ± 0.23, vs. SED-HFD: 0.89 ± 0.06; RT-SD: 3.45 ± 0.64, vs. SED-SD: 1.00 ± 0.06; fold change), [$F(3,13) = 13.49$; $P = 0.0003$]. There was no significant change in ACE mRNA levels in the NTS of all groups [$F(3,13) = 1.27$; $P = 0.32$] (Fig. 4).

At the end of experimental protocol, sedentary HFD rats presented increased TNF-α (SED-HFD: 1.90 ± 0.26, vs. SED-SD: 1.00 ± 0.07 fold change), [$F(3,13) = 4.07$; $P = 0.030$] and IL-1β mRNA expression in the NTS (SED-HFD: 2.31 ± 0.13, vs. SED-SD: 1.00 ± 0.10 fold change), [$F(3,13) = 7.53$; $P = 0.0036$] and reduced IL-10 (SED-HFD: 0.50 ± 0.04, vs. SED-SD: 1.00 ± 0.10 fold change), [$F(3,13) = 11.30$; $P = 0.0006$] mRNA expression levels in the NTS. RT prevented the changes in TNF-α (RT-HFD: 1.15 ± 0.25 fold change), IL-1β (RT-HFD: 1.10 ± 0.27 fold change) and IL-10 mRNA expression in the NTS (RT-HFD:

Table 1
Adiposity and plasma measurements in sedentary rats fed with standard chow diet (SED-SD), sedentary rats fed with high-fat diet (SED-HFD), resistance training rats fed with standard chow diet (RT-SD), and resistance training rats fed with high-fat diet (RT-HFD).

	SED-SD	SED-HFD	RT-SD	RT-HFD
Epididymal adipose tissue (g/100 g body wt.)	0.40 ± 0.05	0.68 ± 0.09*	0.35 ± 0.01	0.62 ± 0.05*
Retroperitoneal adipose tissue (g/100 g body wt.)	0.45 ± 0.04	1.02 ± 0.14*	0.49 ± 0.14	1.20 ± 0.08*
Mesenteric adipose tissue (g/100 g body wt.)	0.40 ± 0.05	0.65 ± 0.08*	0.41 ± 0.03	0.76 ± 0.05*
Total cholesterol (mg/dl)	55 ± 3.4	107 ± 14.1*	64 ± 3.1	80 ± 5.6#
HDL (mg/dl)	31.4 ± 2.8	31 ± 3.0	34.8 ± 3.7	32.8 ± 3.2
TGL (mg/dl)	46 ± 1.3	70 ± 4.5*	57 ± 4.5	56 ± 4.6
Blood glucose (mg/dl)	94 ± 3	106 ± 2*	96 ± 4	95 ± 2#
Kitt (%/min)	4.62 ± 0.33	3.35 ± 0.26*	5.01 ± 0.46	5.17 ± 0.35#
Insulin (ng/ml)	0.63 ± 0.17	1.45 ± 0.11*	0.61 ± 0.13	1.13 ± 0.13*

All values are presented as means ± SEM. One-way ANOVA followed by the Student–Newman–Keuls test. HDL: High-density lipoprotein; TGL: triacylglycerol. Kitt (%/min): percentage of plasma glucose concentration decline per minute. Total cholesterol, HDL, TGL and insulin levels were measured after 12 h fasting and glucose levels after 8 h fasting.

* Different from SD groups.

\# Different from SED-HFD; $P < 0.05$ ($n = 5$–7/group).

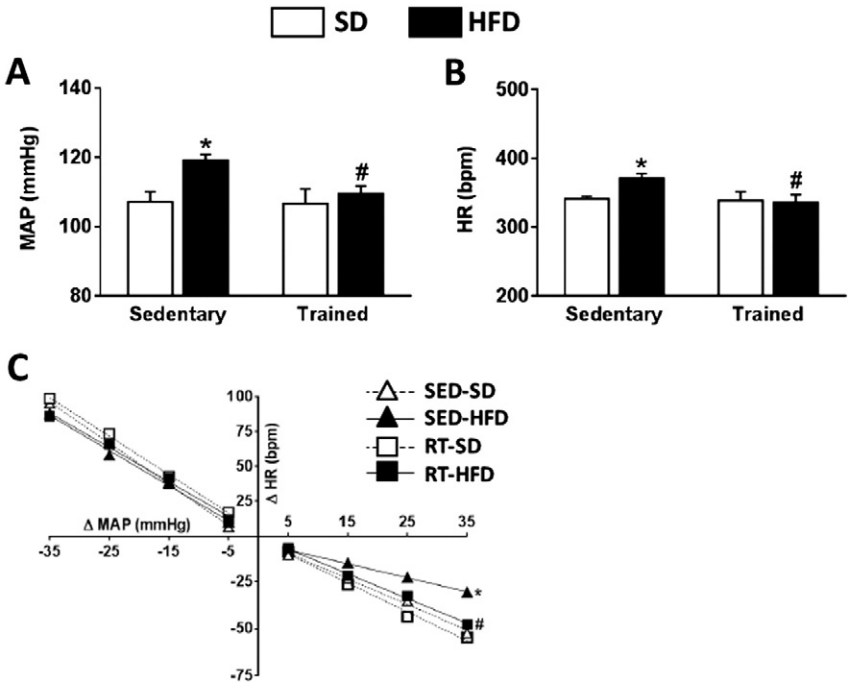

Fig. 2. (A) Mean arterial pressure (MAP), (B) heart rate (HR) and C) baroreflex function in sedentary (SED) and resistance trained (RT) rats fed with standard chow diet (SD) or high-fat diet (HFD) for 7 weeks. The results are presented as means ± SEM. One-way ANOVA followed by the Student–Newman–Keuls test. *Different from SD groups; #different from SED-HFD; $P < 0.05$. SED-SD, n = 9 rats; SED-HFD, n = 9 rats; RT-SD, n = 8 rats and RT-HFD, n = 7 rats.

1.04 ± 0.69 fold change). The IL-6 mRNA expression in the NTS was similar in all groups [$F(3,13) = 1.58$; $P = 0.24$] (Fig. 5).

4. Discussion

The present results demonstrated that moderate intensity RT prevented the cardiovascular changes induced by HFD including increase in MAP, sympathetic modulation of SBP and impaired baroreflex sensitivity. These responses were associated with an improvement in the protective axis of the RAS components (AT_2 and Mas receptors and ACE2) and a reduction in inflammatory responses in the NTS, without changing the increased AT_1 receptors mRNA expression in this area.

HFD increased body and adipose tissue weights that are associated with metabolic alterations like increased blood insulin and glucose levels, reduced insulin sensitivity as well as alterations in lipid profile in sedentary animals [13,15,29,47]. The present results showed that RT prevents cardiovascular and metabolic disorders produced by HFD independently of the increase in body weight and adiposity. Contrasting with the present study, it was previously demonstrated that RT performed on ladder, attenuated the increase in body weight induced by

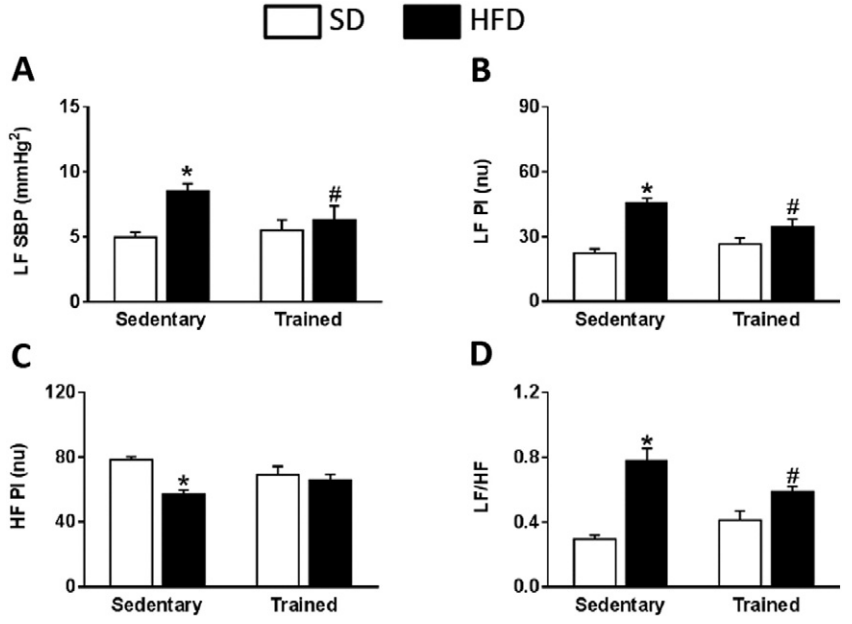

Fig. 3. (A) Low frequency (LF) of systolic arterial pressure (SBP), (B) LF of pulse interval (PI), (C) High frequency (HF) of PI and (D) LF/HF ratio of PI in sedentary (SED) and resistance trained (RT) rats fed with standard chow diet (SD) or high-fat diet (HFD) for 7 weeks. The results are presented as means ± SEM. One-way ANOVA followed by the Student–Newman–Keuls test. *Different from SD groups; #different from SED-HFD; $P < 0.05$. SED-SD, n = 9 rats; SED-HFD, n = 7 rats; RT-SD, n = 7 rats; RT-HFD, n = 7 rats.

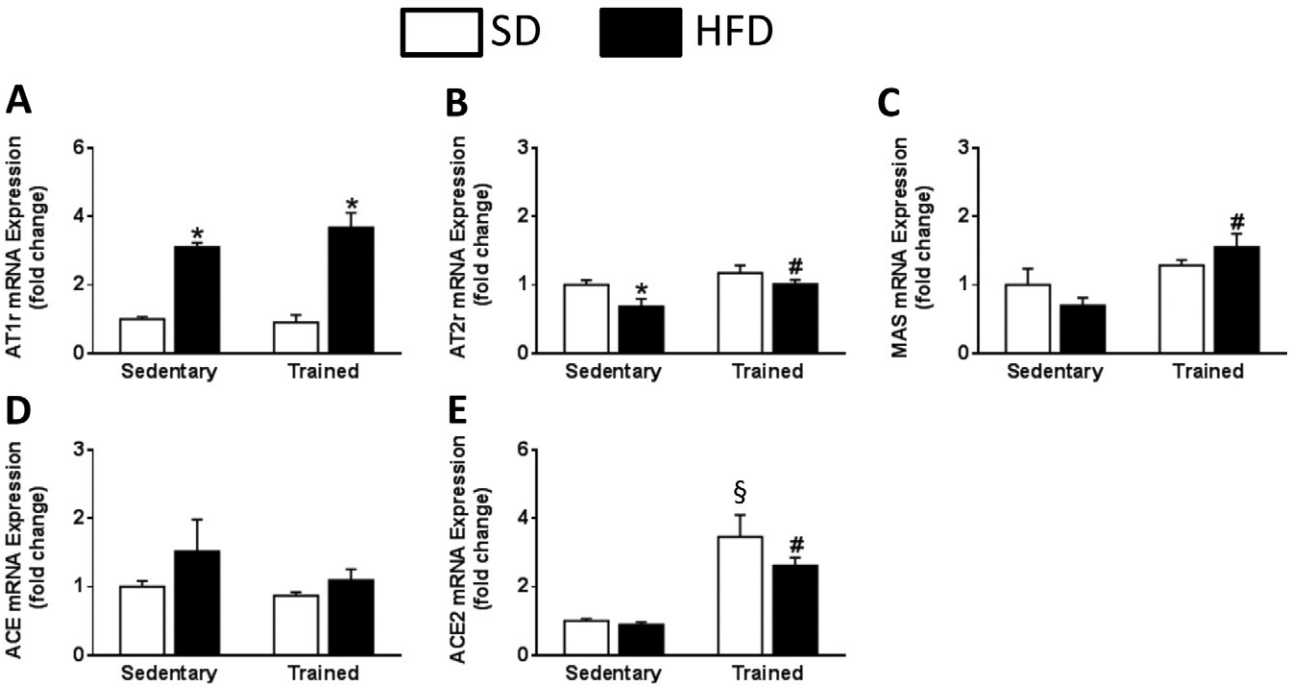

Fig. 4. Fold change in mRNA expression levels of (A) angiotensin II type 1 receptor (AT_1), (B) angiotensin II type 2 receptor (AT_2), (C) Mas receptor, (D) angiotensin converting enzyme (ACE) and (E) angiotensin converting enzyme 2 (ACE2) in sedentary (SED) and resistance trained (RT) rats fed with standard chow diet (SD) or high-fat diet (HFD) for 7 weeks. The results are presented as means ± SEM. One-way ANOVA followed by the Student–Newman–Keuls test. *Different from SD groups; #different from SED-HFD; §different from SED-SD; $P < 0.05$. SED-SD, n = 4 rats; SED-HFD, n = 4 rats; RT-SD, n = 4 rats and RT-HFD, n = 5 rats.

HFD [29,47]. These differences may rely on the different intensity of exercise used in the present and previous studies. Resting energy expenditure and fat oxidation post RT seems to occur in an intensity-dependent manner in overweight individuals [17]. Therefore, the moderate intensity exercise (all the climbs with 50–60% of MVCC) in contrast to the high intensity exercise used in previous studies (first climb with 50% of MVCC and last climb with at least 100% of MVCC) may explain the difference between studies [29,47].

We also observed that RT prevented the decrease in insulin sensitivity and the rise in glucose blood levels, effects that might be associated to increased glucose transporter type 4 (GLUT4) protein expression in the skeletal muscle of trained animals [51]. RT also prevented the

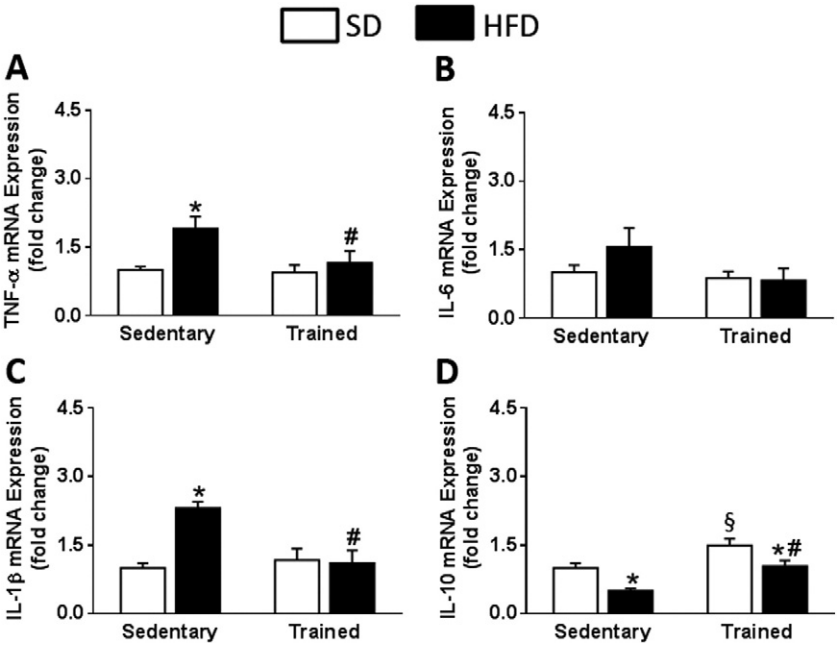

Fig. 5. Fold change in mRNA expression levels of (A) tumoral necrosis factor-α (TNF-α), (B) interleukyn-1β (IL-1β), (C) interleukyn-6 (IL-6) and interleukyn-10 (IL-10) in sedentary (SED) and resistance trained (RT) rats fed with standard chow diet (SD) or high-fat diet (HFD) for 7 weeks. The results are presented as means ± SEM. One-way ANOVA followed by the Student–Newman–Keuls test. *Different from SD groups; #different from SED-HFD; §different from SED-SD; $P < 0.05$. SED-SD, n = 4 rats; SED-HFD, n = 4 rats; RT-SD, n = 4 rats and RT-HFD, n = 5 rats.

alterations in lipid profile in rats fed with HFD. These results are consistent with a clinical study that demonstrated decreases in the SBP, improvement in the lipid profile and decreases in plasma inflammatory cytokines without weight loss or waist-to-rip reduction after aerobic exercise training in overweight patients with type II diabetes [27]. Changes in plasma levels of pro-inflammatory cytokines are associated with insulin resistance [19,24] and low circulating levels of pro-inflammatory cytokines are associated with a regular physical activity [20]. Although not tested in the present study, a decrease in plasma levels of pro-inflammatory cytokines might also account for the improvement of the metabolic alterations found in RT-HFD feeding rats.

Evidence shows that RT also promotes cardiac remodeling and increases in the endothelium-mediated vasodilatation which were effective for the treatment of obesity-induced hypertension [29] and the hypertension in SHRs, a neurogenic model of hypertension [16]. The present study demonstrated that baroreflex impairment present in sedentary HFD fed rats was improved after RT. In addition, the increase in the sympathetic modulation SBP (an index of vasomotor sympathetic activity) was prevented by RT. HFD increases the activity of the renin-angiotensin system [7,11] and the peripheral blockade of AT_1 receptors with losartan seems to reduce obesity-induced hypertension [7]. In the brain, it is well know that Ang II acting on the AT_1 receptors in the NTS blunts the baroreflex sensitivity [32,36] and angiotensinergic mechanisms in the NTS is involved with the maintenance of different forms of hypertension [5,22,44,54]. In spite of the improvement of the baroreflex and the attenuation of the sympathetic modulation of the SBP, the increased AT_1 mRNA expression in the NTS detected in HFD sedentary rats was not modified by RT, which is similar to a previous study that demonstrated that aerobic exercise promoted falls in BP in SHR in spite of the increased AT_1 mRNA expression in the NTS [18].

The activation of AT_2 receptors can oppose the actions of Ang II on AT_1 receptors in the cardiovascular system [23]. Recently, we demonstrated that the increased expression of AT_2 receptors in the NTS attenuated the renovascular hypertension, decreasing the sympathetic modulation of SBP and improving baroreflex function in these animals [5]. In the present study, RT increased mRNA levels of AT_2 and Mas receptors and ACE2 in the NTS. ACE2 is an enzyme that has a high catalytic activity converting Ang II to angiotensin-(1-7) [21], and angiotensin-(1-7), acting in the NTS, increases baroreflex sensitivity and decreases arterial pressure [8].Considering that AT_2 receptors and the ACE2/Ang-(1-7)-Mas receptors are part of the protective axis of the RAS, the increased mRNA levels of AT_2 and Mas receptors and ACE2 in the NTS might be the reason for the reduction of arterial pressure and improvement of the baroreflex in HFD trained rats, in spite of the high levels of AT_1 receptors. Indeed, preliminary data from our laboratory demonstrated that although AT_2 overexpression in the NTS was not able to blunt the mild-hypertension induced by HFD, it was effective to improve the baroreflex in this condition. Thus, it is possible that the increase in the expression of AT_2 receptors in the NTS produced by RT might be involved with some of the cardiovascular improvement observed in HFD feeding rats. However, it seems this is not sole mechanism. Thus, it is possible that changes in other components of the protective RAS pathway such as Mas receptors and ACE2 and/or cytokines in the NTS might also be important for the alterations in blood pressure in HFD feeding rats submitted to RT.

The reduction in the vasoconstrictor axis towards the protective axis of the RAS was also found in other tissues in animals submitted to aerobic exercise [25,46]. For instance, it was demonstrated that aerobic exercise caused a shift in RAS in skeletal muscle towards the ACE2-Ang-(1–7)-Mas axis in rats with congestive heart failure [25] and reduced the vasoconstrictor axis of RAS in renal arteries of SHRs [46]. Together, these data demonstrated that aerobic exercise and RT are modulators of the RAS components in the tissues producing either an increase in the protective axis and/or a reduction in the hypertensive axis.

The mRNA expression of the pro-inflammatory cytokines TNF-α and IL-1β increased and the mRNA expression of the anti-inflammatory cytokine IL-10 decreased in the NTS of HFD sedentary rats, suggesting a chronic low-grade inflammation in this nuclei, a response prevented by RT. Our data are in line with a previous study that showed increases in IL-10 mRNA expression in the hypothalamus of HFD feeding rats performing a single session of moderate intensity aerobic exercise [41]. Hypertensive stimuli like HFD activate astrocytes and microglia (central nervous system immune resident cells) that are involved in the production and release of pro-inflammatory cytokines in the brain [11]. Microinjection of the pro-inflammatory cytokine interleukin-6 (IL-6) in the NTS blunts the baroreflex function in rats [50] and sustained increases in systemic levels of IL-10 decrease pro-inflammatory cytokines in the heart and kidney decreasing blood pressure in SHR and Dahl salt-sensitive rats [34,35]. Thus, decreased levels of inflammatory and increased levels of anti-inflammatory cytokines observed in the NTS may have beneficial effects in the baroreflex sensitivity and sympathetic modulation of SBP, preventing the increase in MAP in RT-HFD fed rats.

An interaction between inflammation and the RAS in the brain as a mechanism involved with different models of cardiovascular disorders, including those produced by obesity has been suggested [1,11,44,48]. For instance, intracerebroventricular infusion of minocycline, an anti-inflammatory antibiotic, resulted in attenuation of the pressor response to chronic Ang II infusion and reduction of microglia activation and IL-1β, IL-6 and TNF-α mRNA expression in the paraventricular nucleus of hypothalamus (PVN) [44]. Gene expression of pro-inflammatory cytokines in the PVN in HFD mouse is attenuated in mouse with deletion of AT_1 receptor specifically at this site [10]. In contrast, administration of the AT_2 receptor agonist, Compound 21, has been shown to increase IL-10 levels in the stroked hemisphere of rats with ischemic stroke [2]. Thus, it is possible that changes in RAS in the NTS observed in RT-HFD rats in the present study affect the cytokines expression at the same site. However, this is still an open question to be tested in future studies.

It is well established that aerobic exercise decreases resting HR [18,43] due to increases in the parasympathetic modulation and decreases in the sympathetic modulation on heart [43]. However, it seems that RT has no effect in resting HR [3,40] as also showed in the present study. On the other hand, RT prevented the increase in HR observed in HFD fed rats. This protective effect might be due to the combined reduction of sympathetic modulation and increased parasympathetic modulation in the PI found in these animals. Similarly to our results, hypertensive ovariectomizaded rats submitted to RT showed a decrease in HR associated to a lower sympathetic tone [45].

5. Conclusions

Although aerobic exercise has been recommended as primary intervention for prevention and treatment of different forms of hypertension [18,37,38], more recently RT has been shown to reduce the arterial pressure in hypertensive rats [29,45]. The present study also demonstrates that moderate intensity RT prevents obesity-induced mild hypertension, the increased sympathetic modulation of the arterial pressure and the impairment of baroreflex in HFD feeding rats. The possible mechanisms for these responses may involve reduction of inflammatory responses and modifications of RAS in the NTS. The cardiovascular protection in obese rats pre-conditioned with RT suggests that this type of exercise may have beneficial effects preventing chronic diseases even in individuals with increased risks. However, considering that the usual is to start an exercise program after gaining weight, a question that remains for future studies is if starting RT later would have beneficial effects on obesity related cardiovascular disorders.

Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the author(s).

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