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

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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].

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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