Moderate Resistance Training Attenuates the Increase in Blood Pressure and Decreases the Cardiomyocyte Nuclei Number in Hypertensive Rats

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Abstract

Background: Resistance training (RT) is a strategy that decreases blood pressure (BP) in patients with stage 1 hypertension and attenuates BP progression in hypertensive rats (stage 3). The influence of RT in cardiac remodeling in uncontrolled hypertension is not yet established.

Objective: The aim of this study was to evaluate the long-term effects of RT in moderate intensity in cardiac remodeling in spontaneously hypertensive rats (SHRs) without treatment.

Methods: Ten male SHRs with stage 3 hypertension (systolic BP ≥ 180 mmHg) and five normotensive Wistar-Kyoto (WKY) rats (systolic BP < 120 mmHg) were divided into three groups: sedentary normotensive (SED-WKY), sedentary hypertensive (SED-SHR), and RT hypertensive (RT-SHR). The RT was conducted in a vertical ladder (3 days/week for 12 weeks, on nonconsecutive days) at 70% of the maximum load. BP and heart rate were recorded. At the end of the protocol, the animals were euthanized and had their left ventricles sectioned for histological analysis (hematoxylin and eosin and picrosirius red).

Results: RT did not attenuate the absolute and relative weight gains of the heart and left ventricle in the RT-SHR group compared with the sedentary group (SED-SHR). There was no difference in nuclear cardiomyocyte volume and collagen content between groups. However, RT decreased the number of nuclei in the cardiomyocytes and attenuated the increase in systolic BP in the RT-SHR when compared with the SED-SHR group. The Δ of the rate-pressure product was lower in the RT-SHR group compared with the groups SED-WKY and SED-SHR.

Conclusions: The data obtained demonstrate that RT did not attenuate the cardiac hypertrophy, but reduced the nuclear proliferation in cardiomyocytes and the BP progression in SHRs with severe hypertension. (Int J Cardiovasc Sci. 2016;29(6):492‐499)

Keywords: Hypertension; Rats; Hypertrophy, Left Ventricular; Myocytes, Cardiac

Introduction

The left ventricle (LV) is a target organ for hypertension, responding with hypertrophy to constant overload pressure in the myocardium. Although hypertension is considered a strong determinant of LV hypertrophy (LVH), blood pressure (BP) can only explain limited interindividual variations in LV mass. Other important structural changes in the myocardium, such as cardiomyocyte hypertrophy and increase in fibroblast content and interstitial elements, are directly linked to LVH in spontaneously hypertensive rats (SHRs).

In rodents, LVH is characterized by changes in cardiomyocyte nuclei volume and number and collagen deposition. These changes induce cardiac fibrosis and stiffness, leading to reduced cardiac contractility, which progresses to cardiac insufficiency.
Aerobic training (AT) is an effective intervention to protect against cardiovascular diseases, decreasing the occurrence of risk factors and myocardium damage and improving cardiac function in hypertensive rats. Patients with severe hypertension undergoing AT show a decline in BP levels and LVH. The suggested mechanisms between AT and LVH is a reduction in interstitial collagen deposition, which prevents cardiac remodeling in humans and rodents. On the other hand, resistance training (RT) has also been reported to be an effective intervention to decrease cardiac risk factors and BP in hypertensive patients, hindering BP progression in SHRs with stage 3 hypertension (BP levels ≥ 180 mmHg). Still, little is known about the effects of RT on LVH in hypertensive patients. Although studies have shown a hypotensive effect of RT in normotensive and hypertensive patients, as well as in hypertensive rodents, no studies have investigated the association of RT with BP decrease or control and LVH in systolic hypertension.

In addition, there is no consensus about the cardiac morphological adaptations promoted by RT and their possible effects on risk factors in hypertensive patients. Thus, the aim of this study was to analyze the cardiac morphological adaptations in an experimental model of hypertension submitted to moderate RT. Our hypothesis was that RT would attenuate the development of pathologic LVH induced by hypertension in untreated SHRs.

Methods

Animals

The study included five male Wistar-Kyoto (WKY) rats and 10 male SHRs. The animals were 17 weeks old, and their mean body weights were 268.4 ± 32.1 g (WKY) and 327.0 ± 17.5 g (SHR). The mean systolic BP levels of the animals were 119.4 ± 3.6 mmHg and 205.8 ± 10.9 mmHg, respectively. The animals were obtained from the Federal University of São Paulo (UNIFESP). The study was approved by the institution’s Ethics Research Committee (n.922985/2014 omittido pela revisão).

The animals were maintained in plastic cages with five rats in each cage at a temperature of 22 ± 2 °C and relative humidity of 55 ± 10%, with ad libitum standard rodent chow (Nuvital® CR1, São Paulo, Brazil) and water. The rats were divided into three groups with five animals in each group: WKY sedentary (SED-WKY), SHR sedentary (SED-SHR), and SHR submitted to RT (RT-SHR). A small number of rodents was used in this study and a satisfactory reproducibility of the BP data was achieved, with variation coefficients of 2 ± 1% (SED-WKY), 1 ± 1% (SED-SHR), and 2 ± 1% (RT-SHR), demonstrating that the sample had good homogeneity and reliability.

Resistance training conditioning

Before the RT, all animals were conditioned to the RT apparatus (vertical ladder), as previously described by Neves et al. The animals underwent three sessions of RT per week on nonconsecutive days for 2 weeks without any load, as previously described. All training sessions occurred between 6:00 and 8:00 p.m. The animals in the sedentary groups were maintained in a box of the same size of the box in the RT group. This procedure was performed in order to generate the same conditions of stress in all animals.

Maximum carried weight

Two days after the conditioning procedure, the maximum carried weight (MCW) of the animals in the training group was determined. For the initial climb, the animals carried 75% of their body weights. After that, 30 g of load were added, until the maximum load was reached when the rat could no longer climb the entire length of the ladder in 4 – 9 attempts. Failure was determined when the animal was unable to climb the ladder after three consecutive stimuli in the tail (using tweezers), with a 60 s rest period between each climb. The heaviest load that the animal successfully carried over the entire length of the ladder was considered the rat’s MCW for that test session. Then, the next test session consisted of a ladder climb with 50%, 75%, 90%, and 100% of the rat’s previous MCW with a resting interval of 60 s between each climb. For the subsequent ladder climbs, a 30 g load was added until a new MCW was determined; the recovery period between each climb was 120 s. This procedure was applied in the first week and repeated every 15 days throughout the 12 weeks to adjust the training intensity.

Resistance training protocol

Following the MCW, the animals were submitted to the RT protocol, in which they completed 36 sessions...
divided into three sessions per week in nonconsecutive days, between 6:00 and 8:00 p.m. for 12 weeks, with each of the session comprising 6 – 8 climbing sets with 10 – 12 repetitions, 1 min pause between sets, and a mean duration of each training session of ~10 – 12 min. The training load was progressively adjusted (30 to 70% of the MCW), as described by Hornberger & Farrar,20 using 50-mL conical tubes with weights inside and fixed to the proximal part of the animal’s tail with a coast lock snap swivel and Scotch rubber tape (Scotch 3M, São Paulo, Brazil), as described by Neves et al.15

**Blood pressure measurement**

The systolic BP (SBP) was measured using the tail-cuff method with PowerLab System (ADInstruments Inc., Sydney, Australia) with the rats conscious. To calculate the change in BP, the SBP was measured in all animals before start of the training period (T0) and at the end of 12 weeks of RT, 48h after the last exercise session at the same time each day (between 6:00 – 8:00 p.m.) to allow the animals to adapt to the procedure. The rate-pressure product (RPP) was calculated as the product of the heart rate and the SBP. The SBP, heart rate, and body weight measurements were assessed on a weekly basis by the same examiner.15

**Tissue collection**

The rats were euthanized by decapitation 48 hours after the last training session. Their hearts were collected and weighed immediately. The LV was isolated and weighed, and then sliced transversally in the major axis and immersed in 10% formaldehyde (PBS buffer 10 mM, pH 7.4) for subsequent paraffin inclusion. During histological processing, 5 µm slices were obtained and stained with hematoxylin and eosin (H.E.) for identification of the nuclear volume and number per area and stained with picrosirius red for collagen quantification.7

**Morphological and morphometric analyses**

Photomicrographs were obtained using a light microscope (Leica DM 1000, Wetzlar, Germany, 40X objective and 10X ocular). For analysis of the nuclear cardiomyocyte volume, eight pictures were obtained from each LV, totaling 40 images per group. We measured the greatest and smallest diameter of 12 nuclei per image and evaluated the cellular activity. The values obtained were applied to the formula: \[ V = \frac{A^2 \times B}{1.91} \], in which A is the smallest diameter and B is the greatest diameter of the nucleus, and 1.91 is a constant.7 The number of nuclei was evaluated in the same photomicrograph area, and the number of nuclei were subsequently calculated in a 0.016 mm² area, as described by Cabanelas et al.7 To analyze the nuclear volume and number per area, we used the software AxioVision Rel. 4.8 (Carl Zeiss, IL, USA). To quantify the amount of collagen in the LV, we obtained eight pictures from each animal, totaling 40 images per group. These photomicrographs were analyzed with the software Image J®, and the collagen quantification was expressed in percentage per area.7

**Statistical analysis**

All data are expressed as median (interquartile range). The Shapiro-Wilk and Levene tests were used to evaluate the normality and homoscedasticity of the data. Subsequently, the Kruskal-Wallis nonparametric test was used to compare the morphologic, morphometrical, and hemodynamic data, while Dunn’s post-test was used to evaluate the difference between groups. P values < 0.05 were considered significant. The statistical analyses were conducted using the software GraphPad Prism, 6.0 (GraphPad Software, OH , USA).

**Results**

Both the SED-SHR and RT-SHR groups showed increases in heart absolute and relative weights when compared with the SED-WKY group. A similar result pattern occurred in regards to the LV absolute and relative weights. No difference in nuclear volume was observed between groups. The number of nuclei per area was reduced in the RT-SHR group when compared with the SED-SHR, but no difference was observed between the SED-WKY and RT-SHR groups in this regard. Similarly, no difference was observed in the volume of collagen between the groups. Body weight gain was reduced in the RT-SHR group compared with the SED-WKY group, but no significant difference in this variable was observed between the hypertension groups (SED-SHR and RT-SHR). All results are presented in Table 1.

The ΔSBP was higher in the SED-SHR group compared with the RT-SHR group, but no difference was observed between the SED-WKY and RT-SHR
groups. No changes in heart rate were observed among the groups. The ΔRPP in the RT-SHR group was reduced when compared with that in the SED-WKY and SED-SHR groups, as presented in Table 2. The SBP was higher in the hypertensive groups when compared with the normotensive group both before training (SED-WKY: 122 mmHg [116 – 122 mmHg], SED-SHR: 202 mmHg [198 – 2016 mmHg], and RT-SHR: 208 mmHg [193 – 218 mmHg]) and after training (SED-WKY: 127 mmHg [126 – 137 mmHg], SED-SHR 222 mmHg [219 – 232 mmHg] and RT-SHR 202 mmHg [191 – 205 mmHg]). As presented in Figure 1, post-training SBP progression was attenuated in the RT-SHR group when compared with the SED-SHR group.

Figure 2 shows representative photomicrographs of LV slices stained with H.E. and picrosirius red in all groups.

**Discussion**

The present study analyzed the morphological adaptations of the myocardium of SHRs with severe hypertension submitted to 12 weeks of RT. Our hypothesis was that RT could attenuate the LVH, decreasing the progression of cardiac morphological changes induced by the overload pressure of hypertension. However, our results demonstrated that RT was unable to reverse the LVH in this experimental model of severe hypertension. The elevated BP levels (≥ 180 mmHg) of the animals and the absence of pharmacological treatment could have also influenced these results.

We are aware that to achieve the greatest reductions in BP, it is necessary to combine exercise, diet, and antihypertensive drugs to decrease the afterload and change biochemical mechanisms, including a reduction in the renin-angiotensin system activity, leading to a regression of the LVH in severe hypertension.21-23

On the other hand, a relevant result of our study was the finding on the histomorphometric analysis of a reduction in cardiomyocyte nuclear number (CNN) in SHRs submitted to RT (RT-SHR group) when compared with sedentary SHRs (SED-SHR group). This decrease

| Table 1 – Morphological analysis of the animals' hearts after 12 weeks of resistance training |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variables                                    | SED-WKY (g)     | SED-SHR (g)     | RT-SHR (g)      | p value         |
| Body Weight                                 | 54 (35 - 68)    | 25 (22 - 28)    | 23 (17 - 25)    | 0.0031          |
| Heart Weight                                 | 1.24 (1.14 - 1.29) | 1.65 (1.54 - 1.83) | 1.83 (1.67 - 2.05) | 0.0019          |
| LVW (g)                                      | 0.84 (0.79 - 0.85) | 1.09 (1.06 - 1.18) | 1.13 (1.03 - 1.21) | 0.0089          |
| HW/BW (mg/g)                                 | 3.80 (3.63 - 3.99) | 4.70 (4.29 - 5.19) | 5.20 (5.04 - 5.77) | 0.0036          |
| LVW/BW (mg/g)                                | 2.62 (2.47 - 2.67) | 3.04 (2.95 - 3.39) | 3.24 (2.99 - 3.54) | 0.0018          |
| NV (µm³)                                     | 34 (29 - 46)    | 46 (31 - 50)    | 41 (31 - 61)    | 0.6200          |
| NN / 0.016 mm²                               | 9.0 (7.1 - 10.8) | 12.1 (11.2 - 14.3) | 6.3 (5.6 - 8.9) | 0.0205          |
| Collagen Volume (%)                          | 8.0 (7.1 - 9.1) | 8.7 (8.2 - 11.1) | 10.6 (8.3 - 11.5) | 0.1330          |

Data are expressed as median (interquartile range [25th – 75th percentile]). Δ, Delta, post-training – pre-training (12th week – 1st week); BW, body weight; HW, heart weight; LVW, left ventricular weight; HW/BW, heart weight to body weight rate; LVW/BW, left ventricular weight to body weight rate; NV, nuclear volume; NN / 0.016mm², number of nuclei limited per area of 0.016 mm². * Difference versus SED-WKY; * difference versus SED-SHR.
Table 2 - Hemodynamic parameters after 12 weeks of resistance training

<table>
<thead>
<tr>
<th>Variables</th>
<th>SED-WKY</th>
<th>SED-SHR</th>
<th>RT-SHR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>9 (5 - 18)</td>
<td>20 (15 - 22)</td>
<td>-13 (-22 - 11)</td>
<td>0.0115</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>23 (5 - 68)</td>
<td>14 (-37 - 55)</td>
<td>-25 (-92 - 11)</td>
<td>0.0534</td>
</tr>
<tr>
<td>RPP (mmHg•bpm)/100</td>
<td>86 (61 - 175)</td>
<td>135 (-16 - 207)</td>
<td>-145 (-187 - -27)</td>
<td>0.0111</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range [25th – 75th percentile]). Δ, Delta, post-training – pre-training (12th week – 1st week); BP, blood pressure; RPP, rate-pressure product. a Difference versus SED-WKY; b difference versus SED-SHR.

Figure 1 – Pre- and post-training systolic blood pressure monitoring.
Data are expressed as median (interquartile range [25th – 75th percentile]). The values were compared within each group and between groups.

Regarding the occurrence of fibrosis, our results showed no significant changes in total collagen volume in SED-SHR and RT-SHR when compared with SED-WKY animals. These data corroborate those of Pagan et al., who submitted SHRs to AT on a treadmill (12 m/min, 30 min/day, 5 days/week) for 4 weeks and observed no decrease in total collagen I volume. We verified in our study that the RT did not attenuate the cardiac hypertrophy evaluated by the gain in absolute and relative heart weight in SHRs. However, the RT applied in this study may have
attenuated the progression of SBP in the RT-SHR group (Δ = -7 mmHg) at the end of the 12 weeks. In contrast, the sedentary animals presented an increase in SBP: SED-SHR (Δ = +19 mmHg) e SED-WKY (Δ = +11 mmHg).

Although some studies point to a significant decrease in BP levels associated with RT in patients with stage 1 hypertension, as well as in animals, it is possible that in severe hypertension (≥ 180 mmHg), a more expressive BP reduction is required to prevent LVH progression. Moreover, pharmacological treatment with antihypertensive medications should be started right after the diagnosis of hypertension. Along this line, a recent study in rats with hypertension induced by L-NG-nitro arginine methyl ester (L-NAME) has demonstrated that treatment with a beta-blocker (e.g., nebivolol) associated with AT for 4 weeks was more effective in decreasing the cardiac collagen percentage than each of these interventions alone.

This present study was limited by a small sample size in each group (n = 5), which may have restricted our capacity to obtain statistically significant results. Furthermore, we could have used Wistar rats instead of WKY ones, since unlike Wistar rats, the occurrence of LVH in WKY rats is independent of the animals’ BP levels. In future studies, it would be relevant to investigate the cardiac function and biochemical and molecular parameters as well, in order to elucidate the role of RT in ventricular remodeling in untreated SHRs.

In addition, moderate intensity RT was effective in reducing the RPP in SHRs with stage 3 hypertension. This result has an important clinical significance since it reflects a reduction in cardiac work. The RPP is considered a good marker to evaluate the consumption of oxygen by the myocardium during rest or effort, and is effective in estimating the work of the myocardium in response to the RT. Additionally, we observed no changes in heart rate; therefore, the RPP decrease may be attributed to the significant BP decrease observed.

Conclusion

Moderate RT was unable to reduce the LVH in an experimental model of severe hypertension without pharmacological treatment but was effective in attenuating the increase in BP levels in hypertension and decrease in cardiomyocyte nuclear proliferation in SHRs. Still, we believe that the decline in BP is an important factor for LVH. In the future, it would be interesting to analyze the effects of RT with mild-to-moderate intensity exercise as a support to pharmacological treatment in humans and rodents with severe hypertension.
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Author contributions

Conception and design of the research and Writing of the manuscript: Neves RVP, Rosa TS, Souza MK, Passos CS, Carbonel AAF, Teixeira CP, Navarro F, Simões RS, Moraes MR; Acquisition of data, Analysis and interpretation of the data and Critical revision of the manuscript for intellectual content: Neves RVP, Rosa TS, Souza MK, Passos CS, Carbonel AAF, Teixeira CP, Navarro F, Simões RS, Moraes MR; Statistical analysis and Obtaining financing: Neves RVP, Rosa TS, Moraes MR.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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