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Regular Aerobic Exercise as a Therapeutic Strategy for Inflammation and Toxicity in the Right Ventricle of Hypertensive Rats

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Abstract

Purpose: Hypertension is a major public health problem worldwide and is the most common cardiovascular disease (CVD) risk factor. The present study was designed to investigate the protective effects of regular aerobic training on inflammatory and toxicity markers in right ventricle tissue of male wistar rats exposed to chronic nitro-L-arginine-methyl ester(L-NAME)-induced hypertension.

Material and Methods: Thirty two adult, male, Wistar rats were randomly classified into 4 groups; aerobic training, L-NAME, saline, and baseline groups. Hypertension was induced by administration of L-NAME (10 mg/kg) 6 sessions a week and for 8 weeks. Aerobic training was performed between 25 to 64 minutes and at the speed of 15 to 22 m/min, 5 sessions a week and for 8 weeks.

Results: Chronically administration of L-NAME caused a significant increase in angiotensin converting enzyme (ACE), interleukin 6 (IL-6) levels, and a significant decrease in superoxide dismutase (SOD) and nitric oxide (NO) levels, as compared to saline and baseline groups. In contrast, 8 weeks of aerobic training caused a significant increase in SOD, and NO and a significant decrease in IL-6 and protein carbonyl (PC), as compared to L-NAME and saline groups.

Discussion and Conclusion: These findings suggest that aerobic training could have a protective effect against inflammation and toxicity caused by hypertension the in right ventricle tissue through up-regulating antioxidant systems and down-regulating the inflammatory and vasoconstrictor factors in hypertensive rats.

Keywords: Oxidative injury, Endurance exercise, Cardiovascular disease, Inflammation, Hypertension

Introduction

Hypertension is responsible for half of the coronary heart diseases (CHD) and about two thirds of cerebrovascular accidents. By 2030, 23 million cardiovascular deaths are projected to have hypertension, with about 85% occurring in low and middle-income countries [1]. Prevention of hypertension is possible, and its early detection and treatment can reduce the incidence of complications including stroke, CHD, heart failure, and kidney disease [1]. Pulmonary hypertension (PH) is a hemodynamic disorder with pulmonary vasculature remodeling, causing progressive right heart insufficiency leading to right heart failure and ultimately to death [2]. PH is defined as a mean pulmonary arterial pressure of over 25 mmHg at rest or greater than 30 mmHg during exercise, and is characterized by a progressive and sustained increase in pulmonary vascular resistance that eventually leads to right ventricular failure and death [3]. Regardless of the etiology, several pathological processes play pivotal roles in the development and progression of PH, primarily including endothelial dysfunction, pulmonary vasoconstriction, and inflammation and remodeling of the pulmonary vessels [3].

Accumulating evidence suggests that inflammation is an important stimulus for the pathologic changes seen in various types of PH in both human and animal models [4]. Furthermore, Oxidative stress can significantly contribute to the pathogenesis of atherosclerosis, heart failure, ventricular hypertrophy, respiratory distress, ischemia-reperfusion injury, and pulmonary and systemic hypertension [5]. Because ROS may promote vasoconstriction, smooth muscle cell proliferation, and vascular remodeling, they are likely to play a critical role in many forms of PH [5].

It has been consistently shown that exercise

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training is a powerful non-pharmacological strategy to reduce blood pressure (BP) levels of hypertensive patients and to improve functional capacity, quality of life, and ventricular ejection fraction of heart failure (HF) patients [7]. Ruivo et al reported aerobic exercise to be almost completely free of secondary effects, and a useful adjunctive therapy in treating hypertension [8]. Meirelles mentioned the beneficial effects of exercise training in hypertension, and explained that they were the result of reduction in inflammatory responses and restoration of d NO synthesis in platelets.[9]. Although, there is a partial list of proposed mechanisms for exercise-induced protection, much controversy exists concerning the effects of aerobic training on the inflammation, toxicity and antioxidant defense systems of the right ventricle tissue, particularly during chronic Nitro-L-arginine-methyl ester (L-NAME)-induced hypertension. Furthermore, despite the knowledge that hypertension can induce oxidative stress, limited data is available with respect to the effects of regular aerobic training on inflammatory and toxicity markers in right ventricle tissue, particularly during chronic L-NAME-induced hypertension [6]. The other property of the present study is the application of non-pharmacological strategies, with no serious undesirable toxic side effects on the right ventricle tissue.

The proposed hypothesis was that if the hypertension and PH pathogenesis involved various factors including toxicity, stress oxidative, anti-oxidative and inflammatory biomarkers, and on the other hand, hypertension was related to free radical formation, oxidative stress and inflammation, an enhancement in antioxidant/oxidation ratio after regular aerobic exercise may have protective effects against L-NAME - induced hypertension. Thus, the purpose of the present study was to determine the protective effects of 8 weeks of aerobic training on the inflammatory marker (interleukin 6, [IL-6]), vascular dysfunction (nitric oxide [NO] and angiotensin-converting enzyme [ACE]) and oxidative/antioxidant (superoxide dismutase [SOD] and protein carbonyl [PC]) markers of right ventricle tissue in the rats exposed to chronic L-NAME-induced hypertension.

Material and Methods

Animals and the experimental environment

Thirty two male, Wistar rats (8 weeks of age, 240 ± 20 g weight), were obtained from Laboratory of Animal Bearing and Multiplying of the Pasture Institute of Iran. All the experiments involving the animals were conducted according to the guiding procedures in the Care and Use of Animals, prepared by the Council of the American Physiological Society, were performed in accordance with the guidelines outlined by the Experimental Animal Laboratory, and were approved by the Department of Physiology, University of Mazandaran. . Rats were housed in standard cages of polycarbonate (20 × 15 × 15 cm), in a large air-conditioned room with a controlled temperature of 22 ± 2°C, light- dark cycles of 12:12 hours and humidity of 50 ± 5%. The pollutant standard index (PSI) was in the acceptable range as determined by the Iranian Meteorological Organization. Rats were fed with a standard rat chow provided by Pars Institute for animals and poultry with a daily regimen of 10 /100 gr of body weight for each rat. Water was available ad libitum.

Experimental procedures and exercise training

The familiarization protocol was performed once a day for 10 min/session, at a speed of 10 m/min and a slope of 0◦. An electric stimulus (30 V, 0.5 A) was manually turned on for less than 2 s when the animals stayed on the electric grid for longer than 10 s. Following this familiarization period, the rats were randomly assigned into four experimental groups. Rats in all the groups were adapted to the treadmill running for 5 days. The groups were defined as follows: Group1– the baseline group; the animals in this group were not exposed to any variables. Group 2– the saline group; these rats received NACL solution injection (0.1 mg/kg), intraperitoneally, in the same manner and for the same duration of time as the other groups. Group 3– N(ω)-nitro-L-arginine methyl ester (L-NAME); the animals in this group were exposed to L-NAME solution (10 mg/kg) , intraperitoneally,6 days weekly and for 8 weeks, in order to induce the hypertension [10,11]. Group 4 – aerobic exercise; the rats in this group received L-NAME, and in addition performed a progressive running exercise of 15 to 22 m/min for 25 to 64 min, 5 times a week [12]. As the running on a slope may damage the body cell membranes [13]; the training program was conducted on the even surface.

N(ω)-nitro-L-arginine methyl ester administration

According to some previous researches, introduction of N(ω)-nitro-L-arginine methyl ester

(L-NAME) caused hypertension in rats [14], thus hypertension was induced by administration of the soluble analogue of L-arginine at a concentration of 10 mg/kg intraperitoneally,6 days a week and for 8 weeks.

Tissue collection and analyses of markers

After 8 weeks of treatment for each group followed by a 24 h of rest and after 10-12 h of overnight fast, the animals were anesthetized with an intra-peritoneal injection of a mixture of xylazine and katamin . Blood samples were collected 24 hours after the last dose of treatment. These blood samples were initially centrifuged by a refrigerated centrifuge at 3000 rpm for 15 minutes within 30 minutes of collection and were then stored at -80ºC for subsequent assay of angiotensin-converting enzyme (ACE) and nitric oxide (NO).

Then, the thoracic cavity was opened and the right ventricle tissues were quickly excised from the aortic root. Right ventricle tissue was weighed and cleaned in Petri dishes containing cold isolation medium(0.1 mol/L K_2HPO_4, 0.15 mol/L NaCl, pH 7.4). They were then frozen immediately in liquid nitrogen and stored at -80ºC for subsequent analysis of protein carbonyl(PC), superoxide dismutase(SOD) and interlukin-6(IL-6). Right ventricle tissue was squashed in liquid nitrogen, homogenized in a lysis buffer (5 ml/g of tissue) with a protease inhibitor cocktail for mammalian cell and tissue extracts (Sigmae Aldrich, St. Louis, U.S.A) 100 ul/1 ml, and 10 m Mtris base (Sigma-Aldrich, St. Louis, U.S.A), pH 7.4 and was centrifuged at 1600 g at 4 ºC for 15 min. Right ventricle tissue supernatant was diluted 1:30. Plasma was diluted 1:10. Protein carbonyls (PC) were analyzed according to Dabidi Roshan *et al* (2011)[15]. Homogenate samples were centrifuged at 500 g and 4°C for 3 min. Aliquots containing 900 µl of the resulting supernatant were incubated for 15 min with 0.9% streptomycin sulphate and 0.1% Triton X-100, and centrifuged at 12,000 g for 10 min at 4°C. Aliquots of 0.5 ml supernatant were incubated for 60 min in the dark, with 2 ml of 2.5 M HC1, or with 2 ml of 10 mm/2, 4-dinitrophenylhydrazine (DNPH) in 2.5 M HC1, and were shaken every 10 min. Protein concentration in the samples was determined by measuring the absorbance at 280 nm using a bovine serum albumin standard curve in 6 m guanidine hydrochloride and 20 mM potassium phosphate buffer (pH 2.3).

Superoxide dismutase (SOD) activity was determined spectrophotometrically using the method described by Dabidi Roshan *et al* (2011). In brief, for total SOD (tSOD) activity the adequate amount of protein (2 mg tissue wet weight) was incubated at 25 ºC with 1 mMN, Nbis(2-(bis(carboxymethyl)amino)-ethyl) glycine (DTPA) in 50 m MTris_HCl, pH 8.2, in 1 rate was recorded at 420 nm. Also, IL-6 was measured using a highly sensitive Quantikine assay, as described by Bruunsgaard *et al* (1997) [16]. All the samples were processed in the same assay in order to avoid interassay variations in statistical comparisons. The serum NO concentration was determined through reducing the nitrate to nitrite using nitrate reductase (Sigma). Plasma levels of angiotensin-converting enzyme (ACE) were measured using a sandwich enzyme-linked immune sorbant assay (ELISA).

Statistical analysis

Statistical analysis was performed using a commercial software package (SPSS version 16.0 for Windows). All data have been expressed as mean ± standard deviation (SD). One-way ANOVA and Tukey post hoc tests (Statistical software, Stat Soft, Inc., Tulsa, OK,) were performed to identify the differences between the groups. Differences were considered statistically significant at $p\text{-value}<0.05$.

Results

Changes in biomarkers related to toxicity consisting of superoxide dismutase (SOD) and protein carbonyl (PC) in rats exposed to N(ω)-nitro-L-arginine methyl ester (L-NAME)-induced hypertension and rats in the control group are summarized in figures 1 and 2, respectively. Intra-peritoneal administration of L-NAME (10 mg/kg) caused an insignificant increase (12%) in the level of PC in right ventricle tissue and a significant decrease (23%) in SOD level, as compared to saline group. In contrast, 8 weeks of aerobic exercise resulted in a significant increase (17%) in the levels of SOD in right ventricle tissue, as compared to the saline group. Moreover, an insignificant difference was detected in the PC level between the aerobic exercise group and the saline group (17%). On the other hand, 8 weeks of aerobic exercise caused a significant increase (52%) in the levels of SOD and a significant decrease (26%) in PC levels, as compared to L-NAME group (figure 1, 2). However, no significant differences were detected in SOD and PC levels between the baseline group and the saline group.

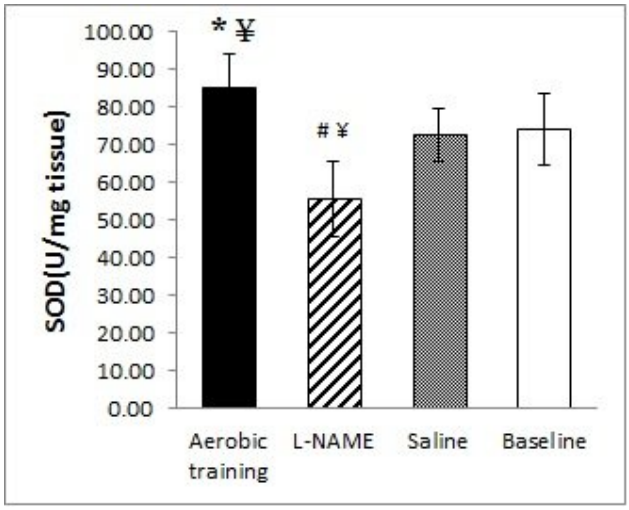

Figure 1: Changes of superoxide dismutase (SOD) levels following 8 weeks of aerobic training in rats during chronic exposure to L-NAME. Data are presented as the mean ± SD for 10 Rats. Abbreviation; N(ω)-nitro-L-arginine methyl ester (L-NAME), ¥ significant different from saline group ($P < 0.001$), * significant different from L-NAME group ($P < 0.001$), # significant different from baseline group ($P < 0.001$).

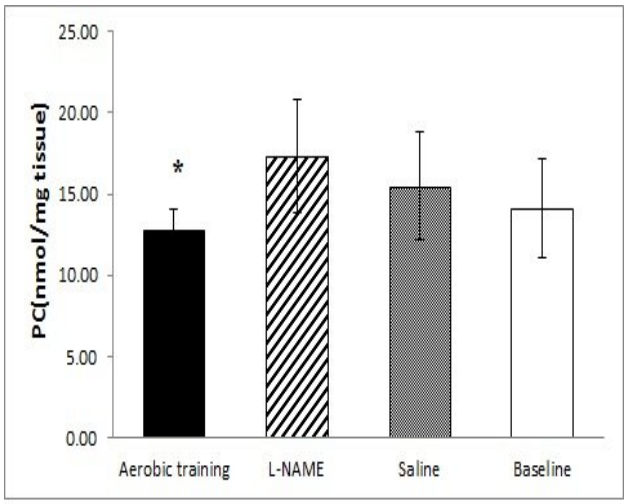

Figure 2: Changes of protein carbonyl (PC) levels following 8 weeks of aerobic training in rats during chronic exposure to L-NAME. Data are presented as the mean ± SD for 10 Rats. Abbreviation; N(ω)-nitro-L-arginine methyl ester (L-NAME), * significant different from L-NAME group ($P < 0.001$).

Figure 3 shows changes of ininterlukin-6 (IL-6) levels in the rats exposed to N(ω)-nitro-L-arginine methyl ester (L-NAME)-induced hypertension and rats in the control group. The administration of L-NAME (10 mg/kg) for 8 weeks resulted in a significant increase (74%) in IL-6 level, as compared to the saline group (figure 3). No significant differences were observed in IL-6 levels between rats in the control group and those in the saline group, however, 8 weeks of regular aerobic exercise significantly decreased IL-6 level in the aerobic group, comparing to the L-NAME group (42%)(figure 3).

Figures 4 and 5 show the changes in the biomarkers related to vascular function including Nitric oxide (NO) and angiotensin-converting enzyme (ACE) in the rats exposed to N(ω)-nitro-L-arginine methyl ester (L-NAME)-induced hypertension and rats in the control group. 8 weeks of intra-peritoneal administration of L-NAME (10 mg/kg) led to a significant increase in ACE level (28%) and a significant decrease in NO level (31%), as compared to the saline group. Despite a significant increase in NO level following 8 weeks

of aerobic exercise, as compared to saline and baseline groups (39%, 38%, respectively), an insignificant decrease(17%) were detected in the ACE levels between rats in the aerobic exercise and rats in the L-NAME group (figure 4,5). On the other hand, no significant differences were detected in NO and ACE levels between the baseline group and the saline group.

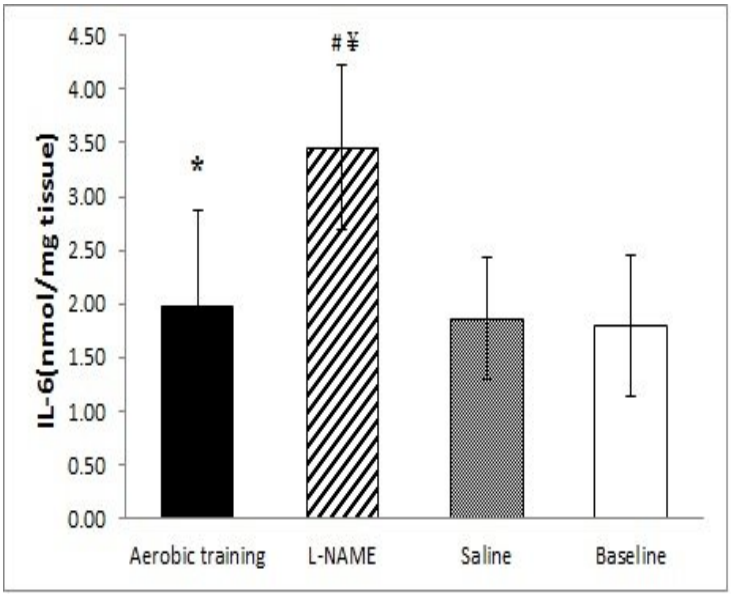

Figure 3: Changes of interleukin 6 (IL-6) levels after 8 weeks of aerobic training in rats during chronic exposure to L-NAME. Data are presented as the mean ± SD for 10 Rats. Abbreviation; N (ω)-nitro-L-arginine methyl ester (L-NAME), ¥ significant different from saline group ($P < 0.001$), * significant different from L-NAME group ($P < 0.001$), # significant different from baseline group ($P < 0.001$).

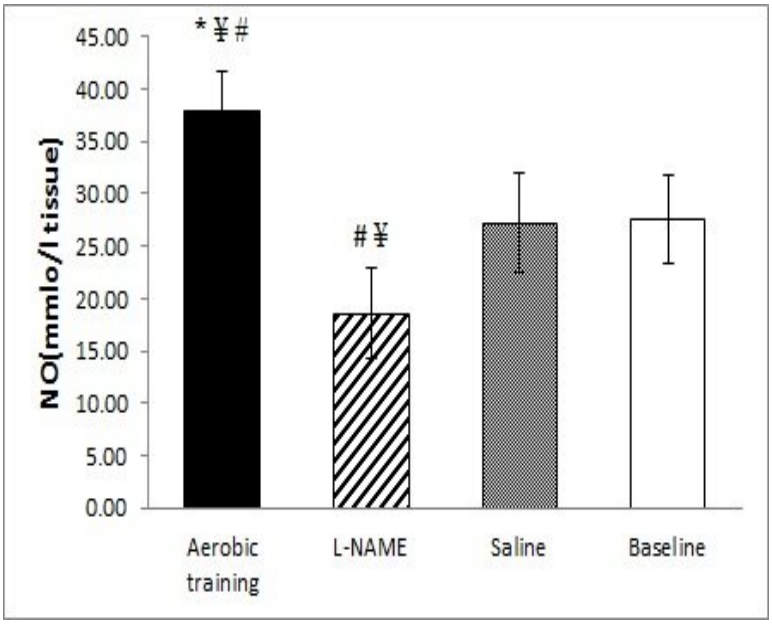

Figure 4: Changes of nitric oxide (NO) levels following 8 weeks of aerobic training in rats during chronic exposure to L-NAME. Data are presented as the mean ± SD for 10 Rats. Abbreviation; N(ω)-nitro-L-arginine methyl ester (L-NAME), ¥ significant different from saline group ($P < 0.001$), * significant different from L-NAME group ($P < 0.001$), # significant different from baseline group ($P < 0.001$).

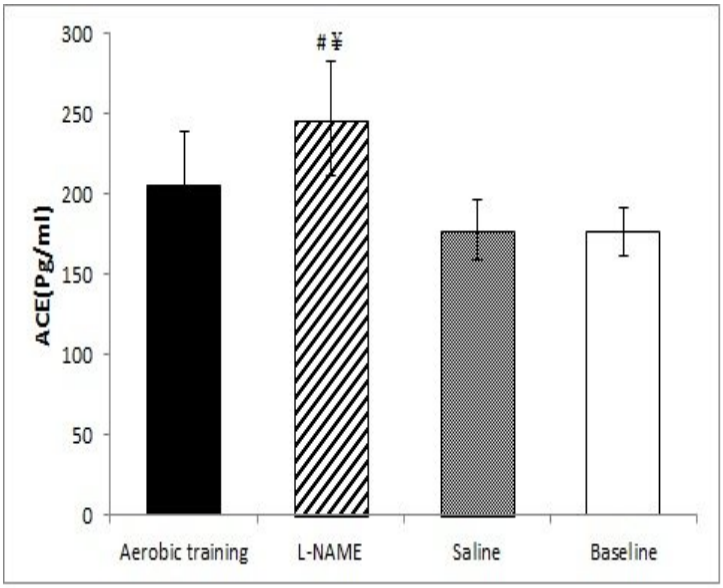

Figure 5: Changes of angiotensin-converting enzyme (ACE) levels following 8 weeks of aerobic training in rats during chronic exposure to L-NAME. Data are presented as the mean ± SD for 10 Rats. Abbreviation; N (ω)-nitro-L-arginine methyl ester (L-NAME), ¥ significant different from saline group ($P < 0.001$), # significant different from baseline group ($P < 0.001$).

Discussion and Conclusion

The present study investigated the effects of an 8-week aerobic training on interleukin 6 (IL-6), nitric oxide (NO), angiotensin-converting enzyme (ACE), superoxide dismutase (SOD) and protein carbonyl (PC) parameters of right ventricle tissue in male rats with chronic exposure to N(ω)-nitro-L-arginine methyl ester (L-NAME)-induced hypertension. The results of the present study indicate that intra-peritoneal administration of L-NAME (10 mg/kg) caused a significant increase in ACE, IL-6, an insignificant increase in PC levels and a significant decrease in NO and SOD levels, as compared to the saline and baseline groups. In contrast, the primary finding of the present study indicated that after 8 weeks of aerobic training a balance was detected in oxidants/antioxidants levels (PC and SOD) and vascular dysfunction markers (NO and ACE), as compared to the saline and L-NAME groups.

Recent studies reported pulmonary hypertension (PH) due to increased pulmonary vascular resistance putting an overload pressure on the right ventricle (RV), which, in turn, leads to RV hypertrophy [17]. A previous study on the PH in animal models has suggested a role for ROS in pathological RV remodeling [17]. In the present study, we found that intra-peritoneal administration of L-NAME (10 mg/kg) caused a significant increase in IL-6, an insignificant increase in PC levels and a significant decrease in NO and SOD levels, as compared to saline and baseline groups. Moreover, in this respect, we have previously shown that chronic administration of L-NAME resulted in an increased oxidative stress and Interlukine-6(IL-6) level in lung tissue. In addition, it reduced the nitric oxide (NO) levels, as compared to the baseline group [6]. These data suggest that the increased oxidative stress resulting from L-NAME could be blocked by a regular treadmill exercise, which improves antioxidants and vascular dysfunction.

Data from the current study provided additional understanding on how regular physical exercise, particularly treadmill run, could contribute to augmentation of heart resistance against oxidative stress-based toxicity induced by L-NAME administration. Hypertension is a ubiquitous and serious disease with a multifactorial etiology; therefore, several mechanisms may be involved in the hypotensive effects of aerobic training [18]. Regular exercise has been recommended as a strategy for the prevention and treatment of hypertension because of its effects on reducing clinical blood pressure [18]. Recent data implicated oxidative stress as a mediator of pulmonary hypertension (PH) and the pathological changes associated with the pulmonary vasculature and right

ventricle [5]. Increases in reactive oxygen species (ROS), altered redox state, and elevated oxidant stress have been demonstrated in the lungs and RV of several animal models with PH [5]. The imbalance in vasoactive mediators (e.g. NO) may lead to endothelial cell dysfunction and when accompanied with VSMC proliferation, could result in the chronic obstruction of small pulmonary arteries, leading to PH [5]. Regular physical exercise, which is known to promote a favorable cardiovascular state, may improve endothelial function via several mechanisms. Indeed, it augments blood flow and laminar shear stress, resulting in increased nitric oxide production and bioavailability [19]. In this regard, the beneficial effects of training on endothelial function can be mediated in an oxidant/antioxidant balance [19]. In our study, regular treadmill training induced a significant increase in NO levels in male rats during chronic exposures to L-NAME.

Inflammation is a feature of PH, and increased circulating levels of cytokines are reported in patients with PH [20].Pulmonary hypertension is characterized by a remodeling of pulmonary arteries with endothelial cell, fibroblast, and vascular smooth muscle cell activation and proliferation [21]. Also, in parallel with the recruitment of immune cells, pro inflammatory cytokines play complex roles in the development of heart failure [22]. A universal feature of cardiovascular disease is the dysfunction of the vascular endothelium, disrupting control of vasodilation, tissue perfusion, homeostasis and thrombosis [23]. Data from animal models also support the role of inflammatory cytokines in the initiation and progression of PH. For example, IL-6 levels consistently increased in animal models with experimental PH [21]. The implication of these pathophysiological mechanisms is that essential hypertension is primarily an inflammatory response to the vascular endothelium damage [6]. In contrast, regular moderate physical activity can elicit systemic molecular pathways connected with angiogenesis and chronic anti-inflammatory reaction with consequent modification of the endothelial function [19]. These results demonstrate that minimal increases in physical activity may decrease blood pressure, making adjustment to adding beneficial amounts of exercise more feasible for the sedentary hypertensive patients [6]. In the present study, the observations showed that aerobic exercise resulted in a significant decrease in IL-6, as compared to saline and baseline groups.

In summary, these findings suggest that aerobic training could have a protective effect against the toxicity and inflammation caused by N (ω)-nitro-L-arginine methyl ester (L-NAME)-induced hypertension. In addition, this study, for the first time, showed that treatment with regular aerobic exercise provided significant protection against L-NAME-induced toxicity in the right ventricle tissue through up-regulating antioxidant systems and down-regulating the inflammatory and vasoconstrictor factors in hypertensive rats. Overall, these results suggest that regular aerobic exercise during administration of L-NAME may be considered as a potentially useful strategy to limit toxicity in the right ventricle tissue.

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