Physical exercise improves plasmatic levels of IL-10, left ventricular end-diastolic pressure, and muscle lipid peroxidation in chronic heart failure rats

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Nunes RB, Tonetto M, Machado N, Chazan M, Heck TG, Veiga AB, Dall’Ago P. Physical exercise improves plasmatic levels of IL-10, left ventricular end-diastolic pressure, and muscle lipid peroxidation in chronic heart failure rats. J Appl Physiol 104: 1641–1647, 2008. First published April 10, 2008; doi:10.1152/japphysio.00062.2008.—Chronic heart failure (CHF) is characterized by left ventricular dysfunction, resulting in hemodynamic changes, sustained inflammatory state, as well as increase in oxidative stress. Physical exercise has been described as an important nonpharmacological procedure in the treatment of CHF, contributing to the improvement of the clinical outcomes in this disease. This study evaluated the effects of physical training on hemodynamics, muscle lipid peroxidation, and plasmatic levels of IL-10 in CHF rats. The left coronary artery was ligated to induce CHF, or sham operation was performed in control groups. Rats were assigned to one of four groups: trained CHF (T-CHF, n = 10), sedentary CHF (S-CHF, n = 10), trained sham (T-Sham, n = 10), or sedentary sham (S-Sham, n = 10). Trained animals had carried out a swimming protocol, 60 min/day, 5 days/wk, during 8 wk, whereas sedentary animals remained without training. Eight weeks of physical training promoted an improvement of diastolic function represented by a reduction of the left ventricular end-diastolic pressure in the T-CHF group compared with the S-CHF group (P < 0.05). Lipid peroxidation evaluated in gastrocnemius muscle using thiobarbituric acid reactive substance assay was higher in the S-CHF group compared with all other groups (P < 0.05). However, there were no differences between T-CHF compared with S-Sham and T-Sham groups. The plasmatic levels of IL-10 were lower in the S-CHF group compared with all other groups (P < 0.05). These findings demonstrate that regular physical training using a swimming protocol, with duration of 8 wk, improves the cardiac function and the anti-inflammatory response and reduces muscle cellular damage.

FUNCTIONAL LIMITATION IN CHRONIC heart failure (CHF) is determined by a combination of factors that include cardiovascular (43), skeletal muscle (15, 32, 46), and ventilatory response abnormalities (13, 36) during exercise. In CHF, these alterations are multifactorial, complex, and correlated (13). Exercise intolerance is the most common symptom observed in CHF and is associated with early muscle fatigue and dyspnea, which may reflect a reduced functional capacity (32, 54). In previous studies, functional capacity was mainly associated with skeletal muscle alterations due to a low-perfusion syndrome, which is a result of the neurohumoral hyperactive state that could be exaggerated during exercise (23, 42, 53). However, more recently, studies have demonstrated that mainly intrinsic metabolic and inflammatory abnormalities of the skeletal muscle are related to increase in the muscle fatigability (14, 15, 32, 43, 46). Several earlier studies have demonstrated reduction of skeletal muscle oxidative capacity (14), muscular atrophy (17, 48), as well as changes in the distribution of the fiber type (14, 15, 54) in CHF. In fact, severe left ventricular dysfunction lowers the activity of oxidative enzymes independently of muscle fiber composition (15). Beyond morphological and biochemical changes, the presence of increased plasmatic and muscular levels of proinflammatory cytokines, as well as low levels of anti-inflammatory cytokines, mainly interleukin-10 (IL-10), is well documented in patients with CHF (20). In this regard, the sustained inflammatory pattern is observed with a notable imbalance between pro- and anti-inflammatory cytokines (3, 4, 37, 52). Moreover, CHF is characterized by high levels of oxidative stress (47). The substantial participation of the aforementioned factors on circulatory and skeletal muscle dysfunction during physical exercise has already been shown, which perpetuates the functional limitation observed in CHF.

Recent reports have demonstrated that physical exercise not only reverses the abnormalities of the skeletal muscle, but also improves CHF symptoms (5, 11, 44). The beneficial effects of exercise on the skeletal muscle system include muscular hypertrophy, improvement of caquexia (24), and increase in the oxidative metabolism (26). More recently, a possible anti-inflammatory pivotal role was suggested for regular physical exercise, both in healthy individuals (25, 40, 55) and in chronic inflammatory conditions (30, 37), as observed in CHF. In accordance with this hypothesis, enhanced production of the anti-inflammatory factors from muscular contraction has been observed during physical exercise. However, the effect of physical exercise on the inflammatory condition in CHF remains poorly clarified. Furthermore, to the best of our knowledge, there are no published studies describing the effects of physical exercise on IL-10 levels and muscle oxidative stress in the CHF experimental model. Because of its potent anti-inflammatory effects, IL-10 has been implicated in a number of inflammatory conditions as an important regulator of the immune and inflammatory systems (34). Moreover, in vitro and in vivo studies suggest that IL-10 could be a potentially useful therapeutic agent for the treatment of acute and chronic, systemic, and localized inflammatory reactions.

The animal model of CHF resembles the chronic syndrome observed during heart failure in humans (18), allowing an

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important alternative to the study and understanding of CHF state. In addition, the precise mechanism of the functional limitation observed in CHF is still poorly understood.

On the basis of these observations, we hypothesized that physical exercise could be associated with improvements in hemodynamics, oxidative stress, and inflammatory condition in rats with CHF. Therefore, the aim of the present study was to evaluate the effects of physical exercise on hemodynamic function, skeletal muscle oxidative stress, and plasmatic levels of IL-10 in an animal model of CHF subsequent to myocardial infarction (MI).

METHODS

Animals

Experiments were performed on 40 male Wistar rats weighing between 200 and 250 g (~90 days of age), obtained from the Animal Breeding Unit of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). They were housed three per cage, receiving food and water ad libitum in an animal room under 12:12-h light-dark cycle, at 22°C. The investigation followed the ethical rules established by the Guide for Care and Use of Experimental Animals published by the National Institutes of Health (NIH publication no. 85-23, revised in 1996). All procedures outlined in this study were approved by the Ethics Committee Research of the UFCSPA (protocol 009/04).

Surgery to Induce MI

Rats were anesthetized with xilazine (12 mg/kg ip) and ketamine (90 mg/kg ip), intubated, and artificially ventilated (SamWay VR 15) with a respiratory frequency of 60 breaths/min and an oxygen inspired fraction of 100%. MI was induced as previously described by Pfeffer et al. (42). The heart was exposed through left thoracotomy between the fourth and fifth ribs. For the animals in which MI was induced, a mononylon suture 6-0 was passed in the main left descending coronary artery, in a point between 1 and 2 mm distal to the edge of the left atrium, and the left coronary artery was ligated. Sham-operated animals underwent the same procedure without tying the suture and served as control rats. The thorax was closed, the skin was sutured, and the pneumothorax was drained by a continuous aspiration system.

Experimental Design

After MI, rats were allowed a minimum of 4 wk of recovery (the necessary time to achieve the development of the CHF state) (42). Then they were assigned to one of four experimental groups: trained CHF rats (T-CHF, n = 10), sedentary CHF rats (S-CHF, n = 10), trained sham rats (T-Sham, n = 10), and sedentary sham rats (S-Sham, n = 10).

Physical exercise program. Four weeks after the MI or sham surgery, rats were adapted to a flat tank with tepid water (30–32°C) during 60 min, 5 days/wk, throughout 1 wk. In the following week, the animals started the protocol of physical exercise (60 min/day, 5 days/wk, during 5 wk). In the first 7 days, the rats swam for 20 min; on the subsequent days of training, the swimming time was extended to 30 min in the second week, 40 min in the third week, and finally 50 min in the fourth week. At the end of the fourth week until the end of the program (with 8 full wk), the animals had exercised continuously during 60 min. The tanks in which physical training was carried out presented capacity of 50 liters, and the water temperature was kept between 30 and 32°C. The swimming program followed the recommendations of the American Physiological Society (29).

Surgical preparation for hemodynamic evaluation. After 8 wk of physical exercise, animals were anesthetized with xilazine (12 mg/kg ip) and ketamine (90 mg/kg ip). A small incision in the anterior cervical region was performed for the insertion of a polyethylene catheter (PE-50) into the right carotid artery. The arterial pressure was first recorded during a 5-min period through a connection of the arterial cannula to a pressure transducer (strain-gauge, Narco Biosystem Miniature Pulse Transducer RP-155, Houston, TX), coupled to a pressure amplifier (Stemtec). Then the catheter was positioned into the left ventricle, and pulse wave was monitored by the typical graphic registration of ventricular pressure and was also recorded for 5 min.

Pressure analogical signals were digitalized by a data-acquisition system (CODAS-Data Acquisition System) with sampling rate of 2,000 Hz. These data were used to determine mean arterial pressure, heart rate, left ventricular systolic pressure, left ventricular maximum change over time, left ventricular minimum change in pressure over time, and left ventricular end-diastolic pressure (LVEDP). This last parameter was determined manually by the detection of the point of inflection to the end of diastoles from the analysis of the wave of ventricular pressure.

Blood samples and muscle collection. Blood samples were drawn from the catheter positioned in the right carotid artery, collected into a 1.5-ml tube containing sodium citrate 3.2% (1.9 vol/vol), centrifuged at 500 g and 4°C, and stored at ~80°C. Animals were killed after blood draw, and the gastrocnemius muscle of the right leg of each rat was removed, frozen in liquid nitrogen, and stored at ~80°C.

Lung and hepatic congestion. Lungs and liver of each animal were removed, weighed, and dehydrated (80°C) for 48 h and then weighed again to evaluate the water percentage.

Infarct size. Hearts were removed and weighed. Left ventricles were filled with an insufflating latex balloon and placed in formaldehyde for 24 h for subsequent analysis of the size of the infarction area, determined by planimetry (31).

Plasmatic levels of IL-10. IL-10 levels present in the plasma of each animal were determined by ELISA using the BD OptEIA ELISA Kit (BD Biosciences Pharmingen) following the manufacturer’s instructions. Measurements were performed in duplicates for calculation of results.

Muscle sample preparation. Gastrocnemius muscle samples were homogenized (7% wt/vol) in 120 mM KCi-0.30 mM phosphate-buffered saline, pH 7.4, containing 0.5 mM phenylmethanesulfonyl fluoride (0–4°C). The suspension was centrifuged at 600 g for 10 min, at 4°C, to remove cell debris, and the supernatant was used as sample. Protein concentration of muscle samples was measured by the Bradford method (8), using bovine serum albumin (1 mg/ml) as standard. Results are expressed in milligram of protein per milliliter of sample.

Oxidative Stress

Chemiluminescence. Chemiluminescence was measured in a liquid scintillation counter in the out-of coincidence mode (LKB Rack Beta Liquid Scintillation Spectrometer 1215, LKB Produkter). Gastrocnemius homogenates were placed in low-potassium vials at a protein concentration of 0.5–1.0 mg/ml in a reaction medium consisting of 120 mM KCi and 30 mM phosphate-buffered saline (pH 7.4). Measurement was started by the addition of tert-butyl hydroperoxide, and data were expressed as counts per second per milligram of protein.

Thiobarbituric acid reactive substance. To measure lipid peroxidation, homogenates were precipitated with 10% TCA, centrifuged, and incubated with thiobarbituric acid (Sigma, Chem.) for 60 min at 100°C. Thiobarbituric acid reactive substance (TBARS) was extracted using butanol (1:1 vol/vol). After centrifugation, the absorbance of the butanol layer was measured at 535 nm (9). The amount of TBARS formed was expressed in nanomoles of malondialdehyde per milligram of protein. Malondialdehyde standards were prepared from 1,1,3,3-tetramethoxypropane.

Catalase activity. Catalase activity was determined by following the decrease at 240-nm absorbance in a reaction medium containing 50 mM phosphate-buffered saline (pH 7.2) and 10 mM hydrogen peroxide (H2O2). It was expressed as microliters of H2O2 reduced per second per milligram of protein (1).
Statistical Analysis

Mean value and SD was calculated for each variable and group. Data were compared among groups with one-way ANOVA followed by Student-Newman-Keuls post hoc test. A \( P \) value of <0.05 was considered statistically significant. The GraphPad Prism 4 program (GraphPad Software, San Diego, CA) for Windows was used as computational tool for the data analysis.

RESULTS

Mortality in MI-induced CHF rats, during or immediately after surgery, was ~35%. There were no behaviors associated with stress or adverse effects in rats that participated in the swimming protocol.

Body Weight, Infarct Size, Pulmonary and Hepatic Congestion

MI was induced in 20 animals: 10 in the S-CHF group and 10 in the T-CHF group. Similarly, sham surgery was performed in 20 animals: 10 in the S-Sham group and 10 in the T-Sham group. All animals were weighed at the end of the experiment. No significant mean weight differences were detected among the four groups at any moment of the study (Table 1). A large area of scarring was observed in the anterior wall of the left ventricle of infarcted rats. The infarct size was determined in a percentage of the left ventricle total area, as an indication of ventricular dysfunction. For the groups S-CHF and T-CHF, the infarct size was 34.08 ± 1.4 vs. 34.71 ± 1.45% (Table 1), and there were no statistical differences between trained and sedentary groups. No detectable infarcts were found among the sedentary and the trained sham-operated rats.

Lung and liver wet-to-dry weight ratios were used to determine the percentage of water in these tissues, as an indicative of congestion. Table 1 shows that the groups of rats with CHF demonstrated an increase in wet-to-dry weight ratios in the lungs, as well as in the liver, compared with the sham groups, suggesting that these rats had significant lung and liver congestion. However, there were no differences in the liver wet-to-dry weight ratio between T-CHF and sham groups (Table 1).

Hemodynamic Variables

All CHF animals, either trained or sedentary, presented values of LVEDP above 20 mmHg, characterizing the presence of important ventricular dysfunction (35), compared with the sham groups (\( P < 0.001 \), Fig. 1A). However, as shown in Fig. 1A, when the T-CHF group was compared with the S-CHF group, there was an improvement of the diastolic function, represented by a reduction, although modest, of the LVEDP in T-CHF group (22.9 ± 3.2 vs. 26.2 ± 4.5 mmHg, \( P < 0.05 \)). In
addition, the LVEDP was lower in the T-Sham group compared with the S-Sham group (Fig. 1A).

Left ventricular systolic pressure was higher in the T-Sham group compared with T-CHF and S-CHF groups, without a difference between T-Sham and S-Sham groups (Fig. 1B). CHF rats exhibited depressed left ventricular maximum change in pressure over time and minimum change in pressure over time (Fig. 1, C and D, respectively). The mean arterial pressure measured during anesthesia was lower in the CHF groups compared with sham groups (S-Sham: 105.6 ± 15; T-Sham: 102.9 ± 11.6; S-CHF: 87.9 ± 12.1; and T-CHF: 90.2 ± 8.8 mmHg, \( P < 0.05 \)). In contrast, there were no differences in heart rate measured during anesthesia (data not shown).

**IL-10 Plasmatic Levels and Muscle Oxidative Stress**

The IL-10 plasmatic concentration, analyzed by ELISA, was lower in the S-CHF group compared with all other groups \( (P < 0.05) \). The T-CHF group demonstrated an improvement of IL-10 plasmatic levels as a consequence of physical exercise (Fig. 2A). Muscular oxidative stress was evaluated in the gastrocnemius of four experimental groups. Lipid peroxidation measured by TBARS was increased in the S-CHF group compared with all other groups \( (P < 0.05, \text{Fig. 2B}) \). In contrast, when we compared the T-CHF group with the sham groups, there were no differences in lipid peroxidation, suggesting that physical exercise decreases muscle lipid peroxidation in CHF rats. However, there were no significant differences in oxidative damage measured by chemiluminescence and by catalase enzyme activity (data not shown). In addition, IL-10 plasma levels did not correlated with TBARS \( (r = 0.49; P = 0.09) \).

**DISCUSSION**

The present study sought to evaluate the impact of an 8-wk protocol of physical exercise on hemodynamic variables, oxidative stress, and IL-10 plasmatic levels in Wistar rats with CHF subsequent to MI. A large number of investigations have been done in recent years to study the effects of physical exercise in the CHF state. However, investigations analyzing improvement of the LVEDP and the anti-inflammatory and antioxidant effects of a regular physical training are poor, especially with animal models of CHF. Therefore, we conducted this study to test the hypothesis that an 8-wk swimming program could be associated with an improvement in LVEDP, skeletal muscle oxidative stress, and plasmatic concentration of IL-10 in rats with CHF. The present report demonstrates, for the first time, that physical exercise based on a swimming protocol is able to reduce LVEDP, increase IL-10 plasmatic levels, and improve the levels of muscular lipid peroxidation.

The left coronary artery ligature produces marked left ventricular dysfunction that is directly related to the size of the infarcted area (10, 42) and simulates the most common cause of CHF. In our study, the average size of the infarcted area was around 34% of the total left ventricle area in CHF groups, which produced a significant increase in the end-diastolic pressure values \( (>20 \text{ mmHg}) \) of the left ventricle. This standard value has been assumed to characterize the development of the severe CHF (35). The hemodynamic dysfunction observed induces the development of structural and functional muscle changes, which, in turn, are related to impaired functional capacity (32, 46), muscular oxidative stress (6, 23, 49), increase in inflammatory cytokines, as well as reduction in the activity of anti-inflammatory cytokines (51).

Clinical studies have clearly demonstrated that the improvement of systemic (peripheral) function during CHF is associated with increased survival. However, pharmacological therapies, which increase left ventricular inotropic function, are found to be associated with an increase in mortality (12, 39, 49). On the other hand, many studies have demonstrated that physical exercise is a powerful factor to improve peripheral function in CHF, both in humans (5, 22, 44) and in animal models (53), without increases in mortality. These data support the notion that survival and prognosis were associated with the ability of the subject to adapt to a state of pump dysfunction rather than to the magnitude of change in pump function.

The results of our study have pointed out a positive effect of an 8-wk physical exercise program on cardiac function. The observed reduction on the LVEDP was 12.6% in the T-CHF group, compared with the S-CHF group. Similarly, the T-Sham group presented lower LVEDP values (17.1%) compared with S-Sham group, suggesting a relationship between the reduction in LVEDP and physical exercise, since it is present in both infarcted and noninfarcted trained rats. Accordingly, a recent meta-analysis about the effects of exercise in heart failure patients has demonstrated that programs of aerobic exercise can modify the cardiac remodeling and increase the functional capacity (22).

Among many subcellular changes, an increase in oxidative stress has been implicated in the development and progression...
of heart failure (27, 28, 50). The functional improvement resulting from physical exercise could be explained, at least in part, by a reduction of oxidative stress, an improvement of endothelial function, and an increase in anti-inflammatory cytokine levels, together with a reduction of proinflammatory cytokine levels (5, 11, 25, 55). Tissue levels of oxidative stress are determined by a balance between the production of reactive oxygen species and the enzymatic and nonenzymatic antioxidant defense production (23). The increase in oxidative stress may cause a reactive increase in the expression of antioxidant defense (23). In CHF, markers of oxidative damage, such as TBARS, are raised in plasma and tissue, and its levels are associated with the disease severity (6). In the present study, we could observe that physical exercise reduces TBARS activity in skeletal muscle of T-CHF rats in relation to the S-CHF group, showing a reduction of lipid peroxidation as a consequence of physical exercise. According to Moller et al. (33), physical exercise is related to oxidative stress in two ways: on one hand, acute physical exercise accelerates the oxidative metabolism, generating higher formation of free radicals, and, on the other hand, a protective antioxidant effect may be generated through regular sessions of physical exercise. In the present report, we selected the swimming protocol as the method of physical training, which was showed to be enough to reduce the lipid peroxidation levels in gastrocnemius of T-CHF rats. Surprisingly, the increase in lipid peroxidation of the CHF rats was not accompanied by an increase in catalase activity. There are several possibilities that may account for this disparity in our results, such as differences in the techniques used to determine catalase activity (using chemiluminescence) or differences in the effects of physical training between groups and the muscle capacity of adapting to chronic oxidative stress. In addition, regulation of antioxidant enzymes as a response to acute or chronic physical exercise stress is determined not only by the levels of oxidative stress but also by the availability of trace elements, such as Cu, Zn, Mn, Fe, and Se, which are reduced after exercise (2). Alternatively, IL-10 could be an important antioxidant agent, reducing free-radical generation (16).

Regular physical training has promoted an antioxidant enzymatic adaptation that reduces the free-radical-induced cellular damage (45). In healthy animals, the vasoconstriction induced by sympathetic nervous system is attenuated by nitric oxide (NO) production. Nevertheless, during CHF, NO production, release, and activity may be changed by oxidative stress. Physical training seems to improve the muscular metabolism by modifying the cellular redox state and inducing NO production (19, 50). In this regard, rats with CHF have a reduction of the endothelium-dependent vasodilatation in the skeletal muscle (53). On the other hand, physical exercise may restore, at least in part, the normal vasodilatation response in skeletal muscle. Therefore, the improvement in oxidative damage may be associated with better endothelium vasodilatation response, contributing to an improvement of the systemic function during CHF.

A large number of studies have suggested an increase in the activation of the immunological system with a marked rise in the circulating concentrations of proinflammatory cytokines during CHF, which can be observed in both humans and animals (20, 21, 41, 51). Physical exercise has been suggested as an important precursor in the activation of mechanisms of anti-inflammatory factors (25, 38, 40). In the present report, we demonstrated that CHF can reduce IL-10 levels in sedentary animals, while an 8-wk physical training can raise the IL-10 plasmatic concentration in T-CHF animals. IL-10 is a powerful anti-inflammatory cytokine that inhibits the release of TNF-α by peripheral blood mononuclear cells in CHF. This effect appears to be independent of disease severity, plasma cytokine levels, and endotoxin activity (7). Our results are consistent with previous investigations in which physical exercise induced an increase in anti-inflammatory cytokines in normal subjects (40). The authors suggested that the process of muscular contraction during exercise could stimulate the transcription of IL-6 mRNA, responsible for activating the cascade of anti-inflammatory cytokines, such as IL-10. This anti-inflammatory effect appears to be related to the regular physical training and the level of physical conditioning, because higher plasmatic levels of IL-10 are observed in physically active subjects, but not in sedentary ones (25). On the other hand, when the exercise is acutely performed, an increase of proinflammatory cytokines could be observed (55).

In conclusion, a regular 8-wk swimming training protocol for Wistar rats with CHF induced by MI was able to improve LVEDP and the anti-inflammatory response, with a significant increase in IL-10 plasmatic concentrations. In addition, our findings show that exercise decreases muscle cellular damage evaluated by TBARS. As it is fully applicable to humans, the present report provides an important contribution for the understanding of the benefits of regular physical exercise upon systemic alterations of CHF. Taking into account that CHF is a syndrome with systemic impacts, the use of simple, safe, and inexpensive strategies, such as physical exercise, can improve the patient functional capacity with a nonpharmacological therapeutic procedure that can be well accepted.

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DISCLOSURES
The authors do not have a financial relationship with a commercial entity that has an interest in the subject of this paper.

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