ORIGINAL ARTICLE

Effect of Mild Aerobic Exercise in Atrial Granules of Mice with Chronic Chagas Disease

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Abstract

Background: Chagas disease presents in different clinical forms, ranging from asymptomatic to acute, with destruction of heart cells and a possibility of death. In the chronic phase, the parasites can cause serious injuries to different tissues.

Objectives: Our objective was to study the effects of physical exercise (swimming) in atrial granules and components of cardiomyocytes in mice with chronic Chagas disease.

Methods: In total, 20 male mice were divided into four different groups: untrained control (UC), trained control (TC), untrained infected (UI), and trained infected (TI). In the UI and TI groups, 1,000 forms of *Trypanosoma cruzi* (Y strain) were inoculated intraperitoneally. After 40 days of infection and proof of chronic phase, the exercise protocol began. The UC and UI groups performed exercise for 10 min/day, and the TC and TI groups followed a training protocol five times a week for 30 minutes during 8 weeks. Ultrathin sections were subjected to morphometric and stereological analyses using electron photomicrographs (x15000) obtained by transmission electron microscopy.

Results: The TI group showed the lowest percentage of small granules (58%), while the UI group presented 80% of these granules. The volume density of the Golgi complex and myofibrils in the TI group were reduced compared with those in the UI group, while the parameters of atrial granules and mitochondria increased.

Conclusion: Our results suggest that mild physical exercise changes the morphological and morphometric parameters of granules and organelles in the cardiac atrium of mice infected with *T. cruzi*, and produces moderate beneficial effects on the cardiovascular system. (Int J Cardiovasc Sci. 2018;31(6)585-593)

Keywords: Chagas Disease; Exercise; Atrial Natriuretic Factor; Mice.

Introduction

Cardiac manifestations of Chagas disease remain the leading cause of death in several countries in Latin America and have become a public health problem in nonendemic countries due to migration.[1] Chronic Chagas heart disease is considered a major cause of nonischemic cardiomyopathy worldwide.[2] With an annual incidence of 28,000 cases in the region of the Americas, Chagas disease affects approximately 6 to 8 million people and causes, on average, about 12,000 deaths per year.[3]

Chronic Chagas heart disease is characterized by cardiac dysfunction in varying degrees evolving to heart failure, bradycardia, biventricular cardiomyopathy or right ventricular dysfunction, severe arrhythmias, thromboembolism, syncope, and sudden death. It is a cardiomyopathy with a prognosis determined by systolic dysfunction and diastolic failure, especially among patients with heart failure.[2,4-6]

Both atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are synthesized and stored in the cytoplasm of atrial and ventricular cardiomyocytes

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in the shape of granules of varying size, and play an important role in the pathophysiology of heart failure, including that of Chagas etiology. ANP and BNP are very similar to each other with respect to amino acid sequence and pharmacological spectrum. Patients with congestive heart failure have high levels of ANP and BNP in the atrial and ventricular cardiomyocytes and increased secretion of their contents.[1,7-11] The pathophysiological role of ANP and BNP in cardiovascular diseases is related to their endogenous diuretic and vasodilator action, with both peptides working as protectors of the cardiovascular system in situations of volume and pressure overload.[12-14] In addition, ANP or BNP administration produces clinical improvement in patients with heart failure.[15,16]

According to Bianchi et al.,[17] the action of exercise on the normal heart provides benefits. The increase in blood volume caused by physical exercise raises the ANP and BNP levels, facilitating the metabolism of all organs involved.[17]

The effect of physical exercise on ANP levels in healthy subjects and in individuals with nonspecific heart failure is evident. In these cases, depending on the type and intensity, physical exercise increases ANP production and secretion in cardiomyocytes, greatly increasing the serum levels of this peptide.[18,19]

The aim of this study was to evaluate the effect of an exercise program on the cardiomyocytes of mice with chronic Chagas disease. The data showed an experimental basis for measuring the effects of regular exercise practice in patients with chronic Chagas disease.

Methods

Animals and procedures

Experimental animals

The experiment included 20 young, male, Swiss mice (20 - 25 g, 21 days old) from the Animal House of the Dante Pazzanese Institute of Cardiology, São Paulo, Brazil. The animals were housed in collective polycarbonate cages in a temperature-controlled room (21 - 24°C) with a 12 h dark-light cycle (light 7:00 am to 7:00 pm). Water and food were available *ad libitum*.

All procedures were approved by the Research Ethics Committee of the *Universidade São Judas Tadeu* (060/2007). This investigation was conducted in accordance with the Principles of Laboratory Animal Care formulated by the National Institutes of Health (Publication No. 96–23, Revised 1996).

The mice were randomly assigned to four groups: untrained control (UC, n = 5), trained control (TC, n = 5), untrained infected (UI, n = 5), and trained infected (TI, n = 5). The TC and TI groups were submitted to swimming exercise. The sample size was defined based on the parameters established by the *Conselho Nacional de Controle de Experimentação Animal* (CONCEA) concerning the use of animals in research. The number of animals used was sufficient to evaluate the hypothesis of this study.

Parasitemia and exercise training

Inoculum and strains of 20-day-old *Trypanosoma cruzi* were inoculated intraperitoneally in 10 Swiss mice (groups UI and TI) with 10^3 trypomastigotes of the Y strain of *T cruzi*.[19] The parasitemia curve and parasitemia peak were determined by collecting 5 μL blood samples from the animals' tails using the Brenner protocol.[19] Blood was collected daily from the second day of the infection until no parasites were observed (~40 days), characterizing the chronic phase of the infection.[20,21]

After 60 days of life, all animals were adapted to the liquid medium in collective tanks with a temperature of 30 ± 2ºC for a week during 15 minutes in order to reduce their stress during physical exercise in the water. The training protocol (swimming) adapted from Lancha et al.[22] was performed by the TC and TI groups for 8 weeks, 5 days a week, lasting 30 minutes per day. The training load was equal to 5% of the body weight of each animal. This protocol was characterized as low-intensity and long-term training.[22]

Tissue sample preparation

At the end of the experiment, when the animals were around 120 days old, they were sacrificed by decapitation. Subsequently, thoracotomy was performed, and the hearts in diastole were removed and weighed. After that, the hearts were perfused via the aorta at a constant pressure of 80 mmHg using 0.1 M cacodylate buffer (3 min), followed by 2.5% glutaraldehyde solution diluted in cacodylate buffer. Subsequently, in each animal, the atria were separated from the ventricles, and the right atrium (RA) was separated from the left atrium.

Right atrium

Fragments of the RA of approximately 3 mm wide and 5 mm length were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M buffer for 2 h at 4°C and postfixed in 1% osmium tetroxide in the same buffer for 2 h at 4°C. The

samples were dehydrated in ethanol series and embedded in Epon resin. Thin sections were double-stained with uranyl acetate and lead citrate. Two randomly chosen blocks from each RA in which the myocytes were cut in cross sections were used for quantitative analysis. The ultrathin sections were placed on a copper grid and, using a JEOL transmission electron microscope, 10 randomly chosen fields per block were selected for micrographs.

Ultrastructural morphometry and stereology

Twenty RA electron micrographs per animal, chosen by systematic random sampling of squares, were taken at a final magnification of x15000, and the numerical density of granules / field and the diameter of all granules present in the field were determined. For the volume density of granules, mitochondria, myofibrils, Golgi complex, and interstitium present in the field, the electron micrographs were analyzed by a stereological test system with 82 points using the Image J software (version 1.47, National Institutes of Health; Collins, 2007), and the values were expressed as percentages.[23]

Statistical analysis

The data were evaluated with the software Stata 7.0 and are expressed as mean ± standard error of mean (SEM). All continuous variables were normally distributed (Friedman test), and statistical differences between the groups were obtained by two-way analysis of variance (ANOVA) and *post hoc* Tukey test. P values below 5% were considered statistically significant.

Results

Body and heart mass

At the beginning of the protocol, all study groups presented similar body mass values (35.7 ± 1.9 g). At the end of the study, body weight was similar in the UC (40.6 ± 0.94 g), TC (43.4 ± 0.62 g), UI (42.52 ± 1.4 g), and TI (41.94 ± 1.02 g) groups. Heart mass and heart mass / body mass ratio showed no significant difference among the groups (Table 1).

Morphology of atrial cardiomyocytes

The electron photomicrographs in Figure 1 show the structural aspects of the atrial cardiomyocytes in the UC and TC groups, and those in Figure 2 show the structural aspects of the atrial cardiomyocytes in the UI and TI groups.

The frequency distribution histogram of the atrial granules showed that *T. cruzi* infection (UI and TI groups) promoted an increase of small granules (16.7 to 29.9 nm) and a reduction of large granules (50.0 to 75.0 nm) when compared with the UC group. However, a comparison between the UI and TI groups showed a decrease in the diameter of the small granules and an increase in the diameter of large granules promoted by training in both control and infected animals, reversing the process induced by infection. The average granules (30.0 to 49.9 nm) were unchanged in all studied groups (Figure 3).

Volume density of the organelles of atrial cardiomyocytes

Table 2 shows morphological and quantitative data of the organelles of atrial cardiomyocytes in the RA of animals in the experimental groups. The organelle density parameters observed in the TI group were similar to those in the TC group, except for the myofibril density, which was lower in the TI group. In addition, the TI group showed increased density of atrial granules and mitochondria, and reduced density of myofibrils and Golgi complex compared with the UI group, but these parameters were comparable to those in the TC.

Table 1 - Body and heart mass of the four groups of studied animals

Parameters/groups (n = 5)	UC	TC	UI	TI
Body mass, initial (g)	37.8 ± 0.58	37.7 ± 0.56	36.80 ± 1.07	33.20 ± 1.58
Body mass, final (g)	40.6 ± 0.95	43.5 ± 0.52	42.52 ± 1.4	41.94 ± 1.02
Heart mass (g)	0.19 ± 0.01	0.19 ± 0.0073	0.176 ± 0.015	0.19 ± 0.02
Heart / body mass, $x10^{-3}$	4.69 ± 0.043	4.56 ± 0.012	4.12 ± 0.026	4.65 ± 0.039

Values are presented as mean ± standard error of mean. Abbreviations: UC: untrained control; TC: trained control; UI: untrained infected; TI: trained infected.

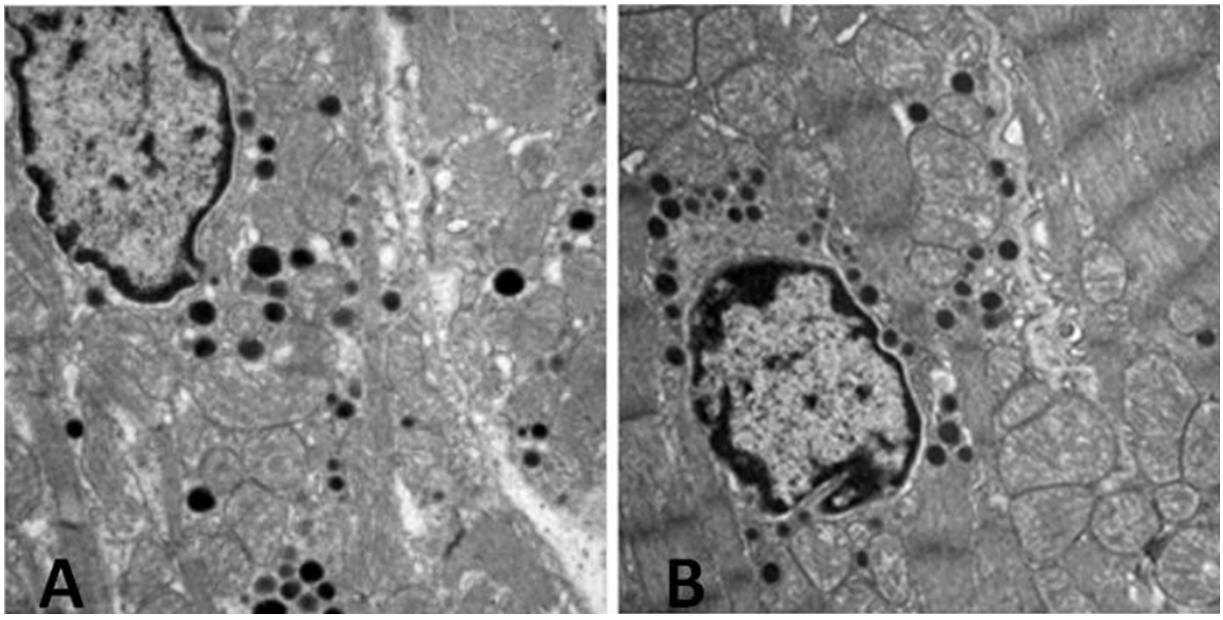

Figure 1 - Electron micrographs of atrial cardiomyocytes in the control groups. The image in (A) shows the untrained group (UC) with plenty of interstitium with regular architecture and presence of collagen fibers. Preserved mitochondria, Golgi complex, myofibrils, and Z line are observed in the cytoplasm. The nucleus shows nuclear chromatin with heterogeneous density, clear nuclear envelope, and irregular contour at the intersection with the cytoplasm. The atrial granules have different size and density and agglomerate closer to mitochondria, Golgi complex, and polar region of the nucleus. In (B), the trained control group (TC) shows numerous lined mitochondria, thickened myofibrils, and Z lines, reduced interstitium and, consequently, reduced collagen fibers. The nuclear envelope presents extremely irregular and electron-dense contour. Compared with the untrained control group (UC), in the TC, most atrial granules display variable size and electron density, are dispersed in the cytoplasm in less quantity around the nuclear envelope and closer to the mitochondria, Golgi complex, and the periphery of myocytes.

Together, the trained groups (TC and TI) showed a decrease in volume density of myofibrils and increase in volume density of mitochondria when compared with the untrained groups (UC and UI). The distribution of organelles of cardiomyocytes and interstitium in the RA (Figure 4) showed a greater presence of granules in the trained groups, especially in the TI.

Discussion

Our results show an influence of a nonpharmacologic treatment (physical exercise) on cardiovascular control in an experimental model of chronic Chagas disease. We showed an improvement promoted by moderate aerobic exercise in both endocrine activity and mechanical action of the heart in animals with chronic Chagas disease.

The histopathological analysis followed the Dallas criteria advocated by Aretz[24] and confirmed the occurrence of chagasic myocarditis in the studied animals. Moreover, the positive influence of light exercise on heart morphological and morphometric parameters in an experimental model of mice with Chagas disease has been reported with evaluation of the left and right ventricles of these animals.[25]

Corroborating previous data, our results indicated an increased density of atrial granules by area and number of mitochondria in cardiomyocytes promoted by exercise training, giving evidence of increased ANP plasma in animals submitted to physical exercise. Other studies have shown that physical activity modulates the increase in ANP gene expression in atrial receptors.[26,27] This effect is explained by increased pressure in atrial walls caused by blood volume during physical exercise, inducing an increase in ANP levels in the swimming animals, and regulating the cardiovascular response.[28]

During physical exercise, there is an increase in plasma levels of catecholamines and ANP, as well as physiological lipolysis with an increase in fatty acids, providing important nutrition to the heart.[29,30] Hu et al.[31] investigated the effects of physical exercise and ANP circulation in patients with and without ischemic

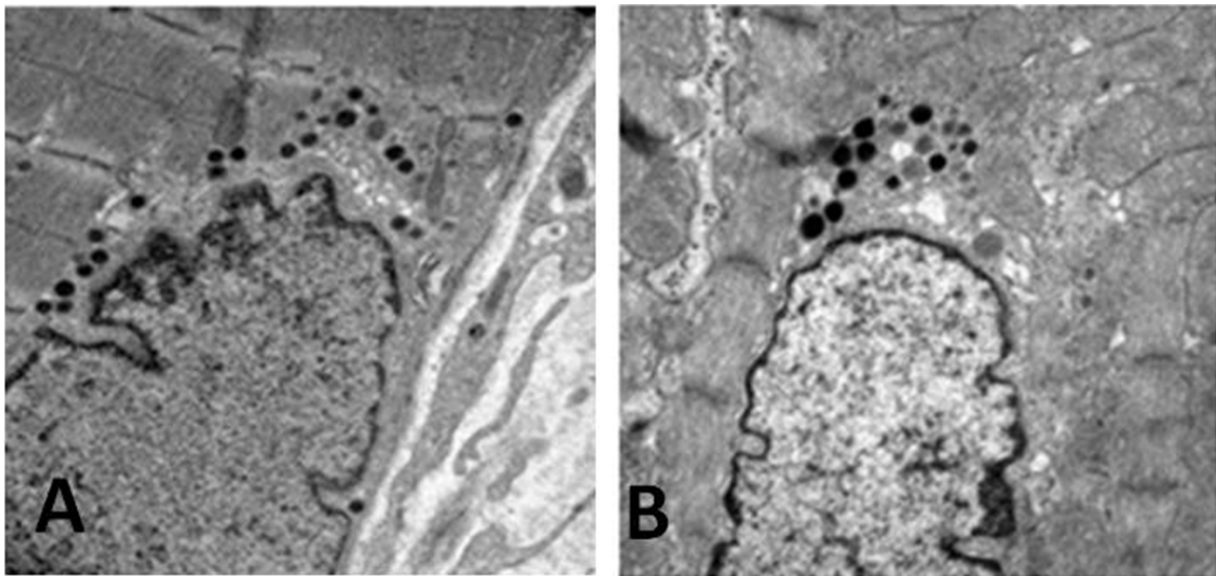

Figure 2 - Electron photomicrographs of atrial cardiomyocytes of infected groups. In (A), the untrained infected group (UI) presents a large amount of collagen fibers in the interstitium. Some nuclear envelopes have an irregular thickness and numerous invaginations, while others have a rectilinear thickness and precise contours with the cytoplasm. The nuclear chromatin shows heterogeneous electron density with intensely dense regions near the nuclear envelope, mitochondria without evident ridges, concentration of granules, mostly small, on the periphery of myocytes, and preserved structure of myofibrils and Z lines. In (B), the trained infected group (TI) presents irregular interstitium with plenty of collagen fibers. The cytoplasmic membrane in most myocytes has an irregular contour with numerous invaginations, and the cytoplasm has numerous scattered or grouped mitochondria and rare suggestion of degeneration. The nuclear chromatin is heterogeneous with electron-dense regions disseminated throughout the nucleus, and along the nuclear envelope. There is a predominance of small atrial granules scattered in the cytoplasm, in the nuclear poles, and the surface of the cytoplasmic membrane. Both myofibril and Z line are thickened.

Table 2 - Apparatus of atrial cardiomyocytes in mice in the untrained control (UC), trained control (TC), untrained infected (UI), and trained infected (TI) groups

Parameters /group	UC	TC	UI	TI
Vv [gr] (%)	4.43 ± 0.36	4.02 ± 0.66	3.18 ± 0.19*	6.16 ± 0.5+
Vv [mit] (%)	16.5 ± 1.05	26.91 ± 4.3*	15.56 ± 1.16*#	23.68 ± 2.08*+
Vv [miof] (%)	56.32 ± 1.67	46.01 ± 4.01*	55.43 ± 1.60#	44.45 ± 2.02*#+
Vv [golgi] (%)	4.74 ± 0.30	4.94 ± 0.60	7.15 ± 0.39*#	5.48 ± 0.38+
Vv[others] %	7.23 ± 1.63	11.82 ± 4.0	8.15 ± 1.6	9.64 ± 1.66
Vv [int] (%)	10.28 ± 1.17	7.55 ± 1.97	10.6 ± 0.94	9.02 ± 1.06

*Values are presented as mean ± standard error of mean. *p < 0.05 versus UC; #p < 0.05 versus TC and +p < 0.05 versus UI. Abbreviations: Vv[gr]: volumetric density of granules; Vv[mit]: mitochondria; Vv[myofib]: myofibrils; Vv[golgi]: Golgi complex; Vv[int]: interstitium; Vv[others]: others.*

heart disease, and found a disproportionate elevation of ANP after physical exercise in ischemia. Zhu et al.[32] analyzed postmortem pericardial ANP and showed that ANP levels correlated negatively with pericardial cardiac troponin levels, while Tanaka et al.[33] found that RA cardiomyocytes are prevalent in the production of ANP. Many studies have been performed analyzing the stimulation of ANP secretion by physical exercise.[34-38]

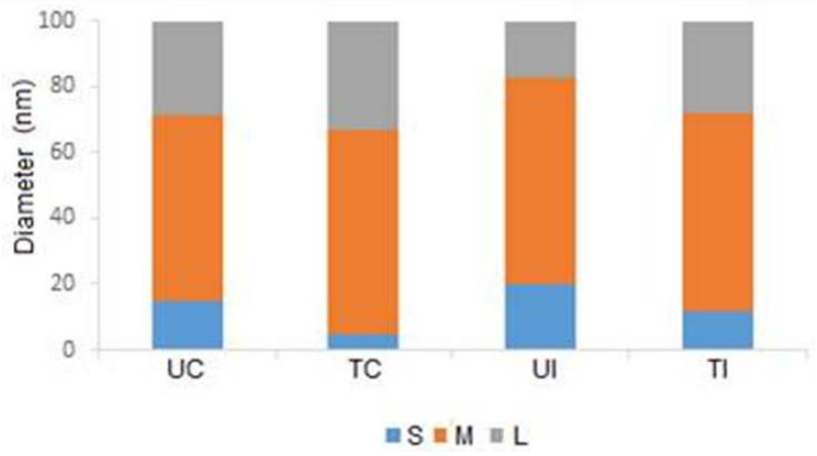

Figure 3 - Frequency distribution histogram of atrial granules in relation to their diameter (small [S], medium [M], and large [L]) in the studied groups.

In acute Chagas disease, there is intense myocarditis associated with changes in the secretory complex in atrial myoendocrine cells and consequent heart failure. Additionally, it is known that ANP and BNP levels also increase in patients with Chagas with echocardiographic changes.[1] Our data showed that the UI group had a significant reduction in the density of granules when compared with the UC group. However, this difference was not observed in the density per area, indicating no substantial difference in the amount of granules between the UI and UC groups.

Additionally, the TI group presented density parameters similar to those observed in the TC group and different from those in the UI group, indicating that exercise training can approximate the physiology of infected cardiac cells to that found in uninfected cells. As for the interstitium, there was no significant difference between the untrained groups compared with the swimming groups. The myofibril density was higher among groups that performed exercise (TC and TI) compared with those that did not exercise (UC and UI). This indicates that physical activity contributes to increasing the number of myofibrils in the heart muscle.[39,40]

When comparing the numerical density of atrial granules in all four groups of animals, we observed no statistically significant difference among the groups ($p > 0.05$). However, there were differences between large and small granules. Interestingly, physical training promoted a decrease in the diameter of small granules and an increase in the diameter of large granules in both controls and infected animals. Additionally, we also observed that the frequency distribution of granules in relation to the areas was significant in the UI group compared with the other groups, with 80% of small granules. The TI group showed the smallest percentage of large granules (0.9%) compared with the other groups. Activation of the renin-angiotensin-aldosterone system in congestive heart failure results in sodium and fluid retention, with increased blood volume and central venous pressure, and consequent stretching of the atrial wall,[41,42] as well as morphologic and morphometric changes in cytoplasmic organelles in atrial and ventricular cells.[43,44] The lowest concentration of large granules observed may represent a more effective elimination of the contents present in the granules, resulting in endocrine benefits to the cardiovascular and renal systems.

Structural differences were identified in untrained animals with Chagas disease when compared with those in the UC group. The granules of UI animals had a heterogeneous size and dispersed electron density in the cytoplasm permeating the sarcolemma, cytoplasmic condensation, and characteristic changes in cell chromatin and nuclear envelope with several indentations in the cytoplasm.

The chagasic swimming group (TI) presented reduced interstitium and plasma membrane of irregular contour with numerous invaginations containing electron-dense granules. Numerous mitochondria, some with signs of degeneration, were also observed. Nuclear chromatin appeared hazy and electron dense,

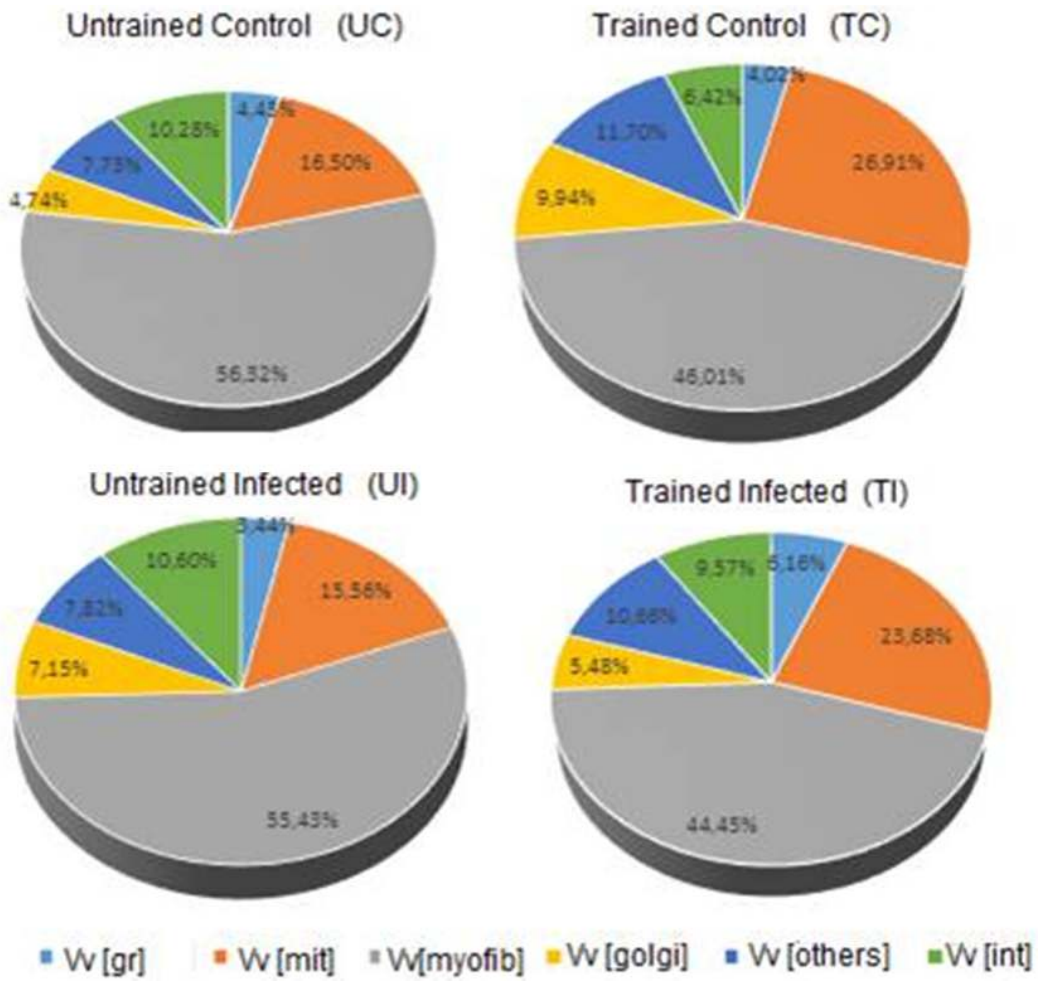

Figure 4 - Distribution of volume densities of cytoplasmic organelles and by interstitium between the UC, TC, UI, and TI groups. Volumetric density of granules (Vv[gr]), mitochondria (Vv[mit]), myofibrils (Vv[myofib]), Golgi complex (Vv[golgi]), interstitium (Vv[int]), and others (Vv[others]).

concentrated in the nuclear envelope and dispersed in the nucleus. A reduced number of granules was observed, with a predominance of small granules dispersed in the cytoplasm and around the Golgi apparatus and mitochondria, and in the peripheral region of the cytoplasmic membrane.

Conclusion

Our results suggest that physical exercise (light) performed by chagasic animals is beneficial to the heart, promoting rehabilitation of sequelae caused by myocyte injuries during parasitemia throughout the acute phase of the disease. This benefit was likely due to increased heart rate, which stimulates the production of electron-dense atrial granules and ANP in response, providing greater blood volume and pressure of the atrial walls, and consequently increased secretion of ANP.

Author contributions

Conception and design of the research: Ferraboli R, Ornelas EM, Fonseca FLA, Cardoso CG, Maifrino LBM. Acquisition of data: Ferraboli R, Ornelas EM, Fonseca FLA. Analysis and interpretation of the data: Ferraboli R, Ornelas EM, Fonseca FLA, Veiga GL, Marques MR, Maifrino LBM. Statistical analysis: Ferraboli R, Fonseca FLA. Obtaining financing: Maifrino LBM. Writing of the manuscript: Ferraboli R, Veiga GL, Cardoso CG, Maifrino LBM. Critical revision of the manuscript for intellectual

content: Ferraboli R, Ornelas EM, Fonseca FLA, Veiga GL, Cardoso CG, Marques MR, Maifrino LBM.

Potential Conflict of Interest

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

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Study Association

This article is part of the thesis of master submitted by Roberto Ferraboli, from *Universidade São Judas Tadeu*.

Ethics approval and consent to participate

This study was approved by the Ethics Committee on Animal Experiments of the *Universidade São Judas Tadeu* (060/2007) under the protocol number 060/2007 the National Institutes of Health (Publication No. 96–23, Revised 1996).

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