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Parasite control and skeletal myositis in *Trypanosoma cruzi*-infected and exercised rats

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ABSTRACT

Non-pharmacological strategies have been rarely described in the treatment of infectious diseases. Although exercise training has been recently incorporated in the clinical management of Chagas disease, the rationale basis that supports this indication is poorly understood. Thus, we investigated the effect of an aerobic exercise on the parasitism, inflammation and oxidative tissue damage in a murine model of *Trypanosoma cruzi*-induced skeletal myositis. Wistar rats were randomized into four groups: trained not infected (TNI) and infected (TI), sedentary not infected (SNI) and infected (SI). A running training program was administered 5 days/week for 9 weeks. Then, infected animals were inoculated with *T. cruzi* and followed up for another 9 weeks. Exercise training induced beneficial adaptations by increasing time to fatigue and lactate threshold in TNI and TI animals. SI animals presented higher parasitemia, skeletal muscle parasitism, cell necrosis, leukocyte infiltration, cytokines levels, reactive oxygen species and nitric oxide production, thiobarbituric acid reactive substances, carbonyl proteins, myosin heavy chain I depletion, and increased catalase (CAT) and superoxide dismutase (SOD) activities. Beyond attenuation in all these variables, TI animals showed reduced TNF-α, CCL-2/MCP-1 and CX3CL1, and increased IL-10 muscle levels. Furthermore, these animals presented higher CAT and SOD activities and reduced lipid and protein oxidation. Taken together, our findings indicated that exercise training induced a protective phenotype in *T. cruzi*-infected mice, enhancing host defenses against the parasite and attenuating the pathological remodeling associated with skeletal myositis, aspects potentially associated to an improved immunological and redox balance in infected animals.

1. Introduction

Chagas disease is a neglected infectious disease caused by the intracellular protozoan *Trypanosoma cruzi* (Santos et al., 2015a; WHO, 2015). Due to high tropism by muscle tissues, skeletal muscles are intensely parasitized by *T. cruzi*, generating myositis, atrophy and necrosis in animals (Maldonado et al., 2004; Vizcaíno-Castillo et al., 2014; Báez et al., 2015) and humans (Montes de Oca et al., 2004; Torres et al., 2004). Despite the scarce evidence, skeletal muscle damage has been associated with direct cell parasitism (i.e. myocytolysis) and unspecific immune-mediated mechanisms, in which exacerbated Th1 inflammatory response (Zhang and Tarleton, 1999; Vizcaíno-Castillo et al., 2014) and regional overproduction of reactive metabolites (i.e. free radicals) have been implicated as pivotal mechanisms involved in the pathogenesis of Chagasic myopathy (Paiva et al., 2012; Báez et al., 2015).

As exacerbated immunological and pro-oxidant responses triggered by *T. cruzi* are coupled mechanisms directly involved in the worsening of Chagas disease, it has been proposed that anti-inflammatory and antioxidant agents could exert protective effects against tissue damage (Maçao et al., 2007; Novaes et al., 2016a). Furthermore, there is evidence that exercise training induces intense modulation of the immunological system and enhances endogenous antioxidant defenses in cardiac and skeletal muscle

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in animals (Niess et al., 1999; Powers et al., 2002; Malm, 2004) and humans (Niess et al., 1999; Gleeson 2007). In response to infections, physical training proved to be effective in inducing a Th1 immunological phenotype (Lowder et al., 2005) essential to promoting cell-mediated immunity and resistance against *T. cruzi* (Teixeira et al., 2002; Santos et al., 2015a). Although exercise training is effective in attenuating the severity of myopathies with different etiologies (Bacurau et al., 2016; Hedermann et al., 2016), the applicability of exercise in the treatment of infectious myopathies remains unknown.

Recently, exercise training has emerged as a non-pharmacological strategy for Chagas disease treatment (Lima et al., 2010, 2013; Novaes et al., 2016b). Although investigations in this area are scarce, a recent study indicated that a 12-week aerobic exercise training improves maximum oxygen consumption, peak heart rate, chronotropic response and exercise tolerance in Chagasic patients (Lima et al., 2010, 2013). Furthermore, an 8-week pre-infection treadmill training program proved to be effective in reducing parasitemia and weight loss induced by the infection in mice (Schebeleski-Soares et al., 2009). While all efforts have been directed to the cardiovascular system, the influence of exercise training on infected skeletal muscles remains unexplored. This study was designed to investigate the effect of an aerobic running training on parasitism, inflammation and oxidative tissue damage in a murine model of *T. cruzi*-induced skeletal myositis.

2. Materials and methods

2.1. Experimental design

Forty male Wistar rats (16-weeks old) were randomized into four groups containing 10 animals each. Two groups were subjected to treadmill running training, and the others remained sedentary. After the training period, animals from one trained and one sedentary group were infected with *T. cruzi*. The four groups were: trained not infected (TNI), trained infected (TI), sedentary not infected (SNI), and sedentary infected (SI). The animals were maintained in a controlled environment (temperature 22 ± 2 °C, humidity 60–70%, and 12/12 h dark/light inverted cycles) with free access to chow and water. The study was approved by the Animal Research Ethics Commission of the Federal University of Viçosa, Brazil (protocol 30/2009).

2.2. Running training and metabolic adaptation induced by exercise

All animals in the TNI and TI groups were subjected to an incremental running training program on a motor-driven treadmill (Insight Instruments®, Ribeirão Preto, Brazil), 5 days/week, for 9 weeks as previously described (Novaes et al., 2016b). Exercise training was initiated 24 h after determining the basal indexes of physical performance. To evaluate the effect of exercise training and inactivity on physical performance, all animals were subjected to a treadmill progressive running protocol until fatigue (Novaes et al., 2011). The fatigue was established as the moment at which the animals were unable to maintain the running cadence and interrupted the race by over 10 s. Every 3 min, peripheral blood (5 μl) was collected by tail puncture to measure lactate levels (Accutrend Lactate®, Roche, Basel, Switzerland). The transition point between the aerobic metabolism and anaerobic metabolism was determined by assessing the lactate threshold (LT). The protocol was repeated three times on alternating days before and after the exercise program. The results were considered as the average values obtained from three independent physical performance tests (Novaes et al., 2011).

2.3. Infection and parasitemia

Infected animals were intraperitoneally inoculated with *T. cruzi* (150,000 trypomastigotes [Y strain]/100 g body weight) obtained from the blood of infected mice (Novaes et al., 2011). The animals were inoculated 72 h after the last physical performance evaluation. Confirmation of infection, prepatent period, patent period, peak and mean parasitemia were determined using peripheral blood according to Brener's protocol (1962). Nine weeks after inoculation, the animals were euthanized under anesthesia with xylazine (2 mg/kg, i.p.) and ketamine (10 mg/kg, i.p.) and the tibialis anterior skeletal muscles were dissected and analyzed.

2.4. Cytokines immunoassay

The cytokine levels in muscle tissue were measured by enzyme-linked immunosorbent assay (ELISA). Briefly, muscle fragments (100 mg) were homogenized in phosphate buffer (pH 7.2) and centrifuged at 10,000*g* at 4 °C for 10 min. The cytokines TNF-α, IFN-γ, IL-6, IL-10 and the chemokines CCL-2/MCP-1 and CX3CL1 were assayed in the homogenate following the manufacturer's instructions (Promega, Madison, WI, USA). The reaction was revealed using peroxidase-conjugated streptavidin (Vector Lab., CA, USA) and the substrate containing 3,3′,5,5"-tetramethylbenzidine (Promega, WI, USA). The reactions were read in spectrophotometer at 450 nm, and the levels of cytokines were calculated by extrapolating the optical densities obtained from a standard curve using recombinant cytokines (Novaes et al., 2016b).

2.5. Histopathology and stereology

Skeletal muscle fragments were fixed for 48 h (10% w/v formaldehyde in 0.1 M phosphate buffer, pH 7.2). The fragments were embedded in paraffin, vertically sectioned at 4-μm thickness and stained by hematoxylin-eosin (H&E). Histological sections were analyzed by bright field microscopy (Olympus BX-60, Tokyo, Japan). Fifty histological fields (×400 magnification) were randomly sampled and the muscle (total area = 1.12×10^6 μm^2) was microscopically analyzed in each group (Novaes et al., 2011). The stereological method was used to assess the distribution of skeletal myocytes, tissue necrosis, amastigote nests and intensity of the inflammatory infiltrate in infected animals. A test area (A_T) of 3.15×10^3 μm^2 was used, containing 42 test points (Pt) applied in histological images obtained at × 1000 magnification across five random microscopic fields of each animal in a total of 160×10^3 μm^2 of muscle tissue for each group. The volume density of skeletal myocytes (Vv_{MY}) and necrotic areas (Vv_{NA}) were estimated as: $Vv_{MY/NA} = {}_{MY/NA}/P_T$, where $P_{MY/NA}$ is the number of test points hitting on skeletal myocytes or necrotic areas, and P_T is the total number of test points. The number density of inflammatory cells (Q_{AIC}) and amastigote nests of *T. cruzi* (QA_{TC}) was estimated as: $QA_{IC/TC} = (\Sigma_{IC/TC})/A_T$; where $_{IC/TC}$ is the total number of inflammatory cells or *T. cruzi* nests counted in the test area (A_T) (Novaes et al., 2013).

2.6. Oxidative/nitrosative stress markers

In situ production of reactive oxygen species (ROS) was evaluated in unfixed 5 μm thick muscle cryosections incubated at room temperature with dihydroethidium (DHE, 10 μmol/l in 0.01% dimethyl sulfoxide) for 30 min. Sections were examined by fluorescence microscopy (Olympus BX-60, Olympus, Tokyo, Japan) and the image was captured at 400× magnification. Five random microscopic fields were analyzed for each animal. Red fluorescence produced by DHE oxidation to hydroxyethidium was evaluated

by using Image Pro-Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA) (Santos et al., 2015a,b).

The same muscle homogenate used in the cytokine immunoassays were used for assessing oxidative and nitrosative markers. Thiobarbituric acid-reactive substances (TBARS) were quantified to determine lipid peroxidation in muscle tissue (Gutteridge and Halliwel, 1990). Protein oxidation was verified by the quantification of protein carbonyls (PCN) using the 2,4-dinitrophenylhydrazine (DNPH) procedure (Levine et al., 1990). Nitric oxide (NO) was indirectly determined by the quantification of nitrite/nitrate levels using the Griess method (Ricart-Jané et al., 2002).

2.7. Endogenous antioxidant enzymes

Catalase (CAT) activity was evaluated by measuring the rate of decomposition of hydrogen peroxide (H_2O_2) (Aebi, 1984). Superoxide dismutase (SOD) activity was estimated by a xanthine oxidase method (Sarban et al., 2005). Glutathione-s-transferase (GST) activity was estimated spectrophotometrically at 340 nm, as described by Habig et al. (1974), and calculated from the rate of nicotinamide adenine dinucleotide phosphate (NADPH) oxidation.

2.8. Myosin heavy chain analysis

Biochemical analysis of myosin heavy chain I (MyHC I) was performed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Bär and Pette, 1988). Muscle samples were placed in an extraction solution (10% glycerol, 5% 2-mercaptoethanol, and 2.3% sodium dodecyl sulfate in a 0.9% Tris-HCl buffer [pH 6.8]). For electrophoretic separation, muscle extracts were loaded onto a 7%–10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gradient gel over a period of 26 h at 185 V. The gels were stained with coomassie blue to identify the MyHC isoforms according to their molecular weights. The relative MyHC I content was identified according to the molecular mass and quantified by densitometry using Image J Software (US National Institutes of Health, USA).

2.9. Statistical analysis

Data were reported as mean and standard deviation (mean ± S.D.). Data distribution was verified by D'Agostino-Pearson test. Parameters of exercise tolerance, biochemical and immunological data were compared using one-way analysis of variance ANOVA followed by Tukey's post hoc test. Parasitological parameters were compared using the Mann–Whitney–Wilcoxon test. Morphological data were compared using the Kruskal–Wallis test. A probability of $p < 0.05$ was considered statistically significant.

3. Results

As indicated in Fig. 1, trained animals presented increased exercise tolerance, which was evidenced by prolongation in lactate threshold and time to fatigue compared to sedentary animals ($p < 0.05$).

Infected and trained (TI) animals presented increased prepatent period, as well as reduced patent period, peak and mean parasitemia compared to sedentary infected animals (SI) ($p < 0.05$), Table 1.

Tissue levels of all cytokines investigated (INF-γ, TNF-α, IL-6, IL-10, and CCL2/MCP-1) were similar in the TNI and SNI groups ($p > 0.05$), except CX3CL1, which presented low levels in the SNI group ($p < 0.05$). In general, both infected groups (SI and TI) presented increased levels of all cytokines investigated compared to uninfected animals (SNI and TNI) ($p < 0.05$). While IL-10 was increased, TNF-α, CCL2/MCP-1 and CX3CL1 levels were reduced in the TI group compared to the SI group ($p < 0.05$), Fig. 2.

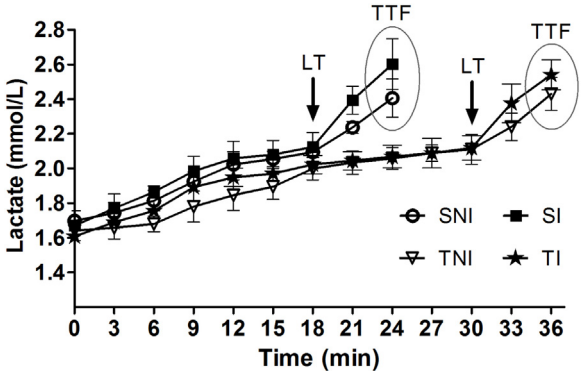

Fig. 1. Lactate threshold (LT) and total time to fatigue (TTF) in sedentary and trained mice previous to *T. cruzi* inoculation. TNI, trained not infected; TI, trained infected; SNI, sedentary not infected; SI, sedentary infected. Data are expressed as mean and standard deviation.

Skeletal muscle from animals in the SNI and TNI groups presented a normal microscopic structure, which presented myocytes with well-defined cross-striation, surrounded by scarce connective tissue presenting low interstitial cellularity. Both SI and TI animals presented evident skeletal myositis, tissue necrosis, connective expansion and diffuse distribution of amastigote nests of *T. cruzi* (Fig. 3).

Stereological analysis indicated that both SI and TI animals presented marked tissue necrosis, reduced myocyte distribution and intense inflammatory infiltration compared to the SNI and TNI groups ($p < 0.05$). All these parameters were reduced in the TI group compared to the SI group ($p < 0.05$), Table 2.

Animals in the SI and TI groups showed intense muscle production of ROS and reduced MyHC I levels compared to the SNI and TNI groups ($p < 0.05$). Skeletal muscle from TI animals presented reduced ROS and increased MyHC I levels compared to the SI animals ($p < 0.05$), Fig. 4.

TBARS, PCny and NO muscle levels were drastically increased in both SI and TI groups compared to the SNI and TNI groups ($p < 0.05$). These parameters were markedly attenuated in TI compared to SI animals ($p < 0.05$), Fig. 5.

The antioxidant enzymes CAT, SOD and GST showed increased activities in skeletal muscle from both SI and TI compared to the SNI and TNI groups ($p < 0.05$). CAT and SOD, but not GST activities, were increased in TI compared to SI animals ($p < 0.05$), Fig. 6.

4. Discussion

While studies with humans (Lima et al., 2010, 2013; Fialho et al., 2012) and animals (Preto et al., 2015; Novaes et al., 2016b) are mainly focused on the cardiovascular system, the impact of exercise therapy on skeletal muscle during *T. cruzi*-infection remains neglected. Corroborating the assumption that skeletal muscles are primary targets of exercise therapy (Goldspink, 2003; Hedermann et al., 2016), our results indicated that running training induced metabolic adaptations, improved parasitological control and reduced skeletal myositis in infected animals, aspects potentially related to modulation of cytokine production, reduced ROS production, improved redox balance, attenuation of skeletal muscle pathological remodeling and reduction of MyHC I depletion.

Considering that the parasitic load is closely correlated to tissue parasitism and damage, parasitological control plays a pivotal role in the treatment of Chagas disease (Zhang and Tarleton, 1999; Veloso et al., 2008). Parasitological control observed in our study has been potentially linked to the immunomodulatory effect of exercise training (Novaes et al., 2016b). As Th1 cytokines (especially IFN-γ and TNF-α) were markedly increased in infected animals,

Table 1
Effect of exercise training on parasitological parameters of *Trypanosoma cruzi*-infected mice.

	Prepatent period (days)	Patent period (days)	Peak of parasitemia (parasites/0.1 mL × 10^3)	Mean parasitemia (parasites/0.1 mL × 10^3)
SI	4.2 ± 0.42	9.7 ± 0.95	21.59 ± 2.84 × 10^3	3.82 ± 1.48
TI	5.5 ± 0.53*	7.4 ± 1.16*	20.58 ± 2.63 × 10^3	1.92 ± 0.63*

SI, sedentary infected; TI, trained infected. Data are expressed as mean and standard deviation.

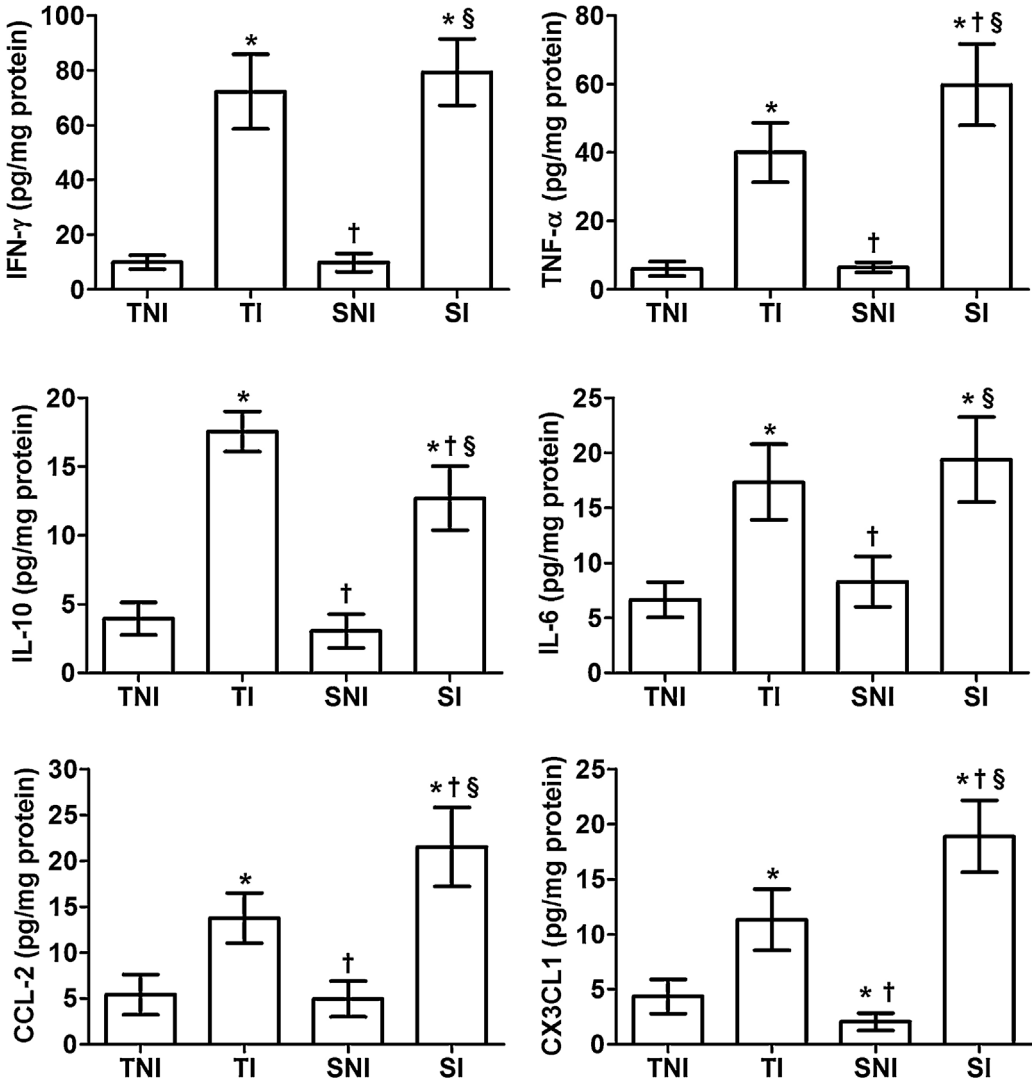

Fig. 2. Cytokine and chemokine levels in the skeletal muscle from *T. cruzi*-infected mice. TNI, trained not infected; TI, trained infected; SNI, sedentary not infected; SI, sedentary infected. Data are expressed as mean and standard deviation. *†§ Statistical differences among the groups ($p < 0.05$); *vs.* TNI, †*vs.* TI, § *vs.* SNI.

Table 2
Effect of exercise training on skeletal muscle structure in *T. cruzi*-infected mice.

	Vv myocytes (%)	Vv necrosis (%)	QA_{IC} (cells/mm^2)	QA_{TC} (nests/mm^2)
TNI	92.17 ± 3.68	ND	824.39 ± 236.41	ND
TI	72.26 ± 6.19*	4.28 ± 1.05	1808.11 ± 415.74*	11.38 ± 5.08
SNI	90.52 ± 4.41†	*ND*	958.41 ± 253.60†	*ND*
SI	55.70 ± 7.11*†§	9.65 ± 3.21§	2951.80 ± 617.25*†§	20.25 ± 7.41

Vv, volume density; QA, number density; $_{IC}$, interstitial/inflammatory cells; $_{TC}$, *T. cruzi* nests; ND, not detected. TNI, trained not infected; TI, trained infected; SNI, sedentary not infected; SI, sedentary infected. Data are expressed as mean and standard deviation. *†§Statistical differences among the groups ($p < 0.05$); *vs.* TNI, †*vs.* TI, § *vs.* SNI.

exercise training does not impair the polarization of the immunological response, an aspect that could attenuate the parasitological control and amplify muscle damage. This immunomodulatory effect was also clearly observed in infected trained animals, which presented similar IFN-γ and IL-6 levels, reduction in TNF-α, CCL-2/MCP-1 and CX3CL1, as well as increased IL-10 tissue levels compared to infected sedentary animals. As regulatory molecules, the Th1/Th2 cytokine balance is crucial to the establishment of an adjusted inflammatory process, which is able to ensure parasite control without determining intense secondary immune-mediated

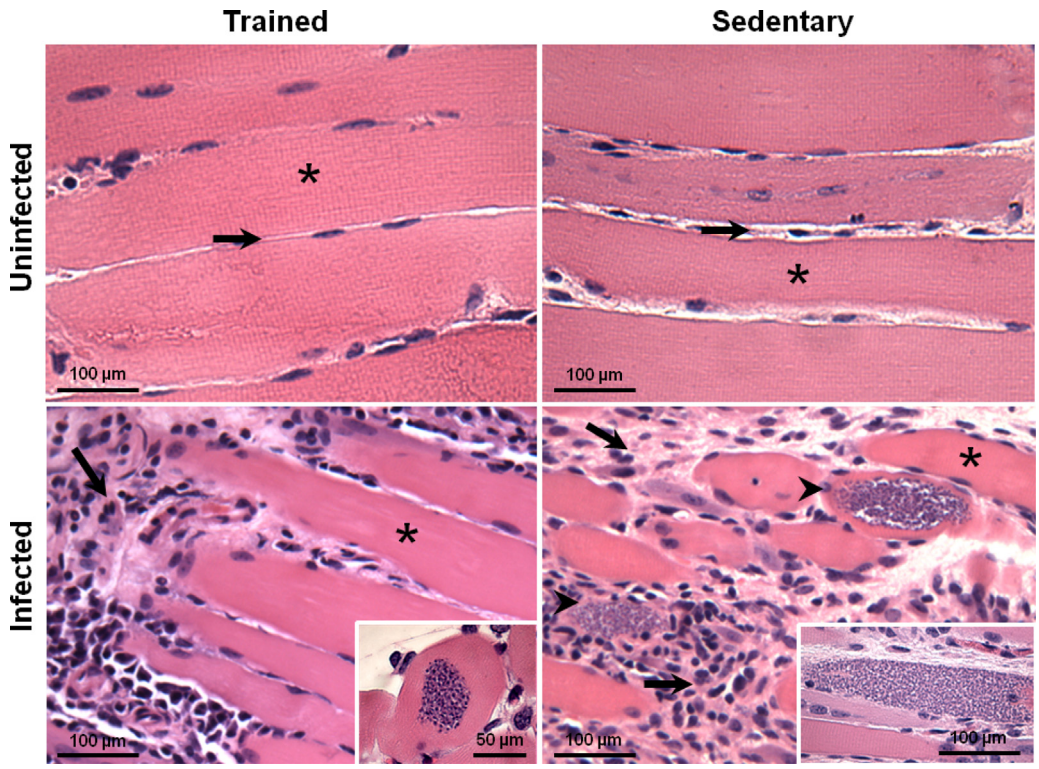

Fig. 3. Representative photomicrographs of the skeletal muscle from uninfected control and *Trypanosoma cruzi*-infected mice (H&E staining, bright field microscopy). Trained and sedentary uninfected animals presented scarce connective stroma (arrow), low tissue cellularity and evident myocytes (asterisk) cross-striation. Trained and sedentary infected mice showed intense skeletal myositis, connective tissue expansion (arrow) and intracellular *T. cruzi* amastigote nests (arrowheads).

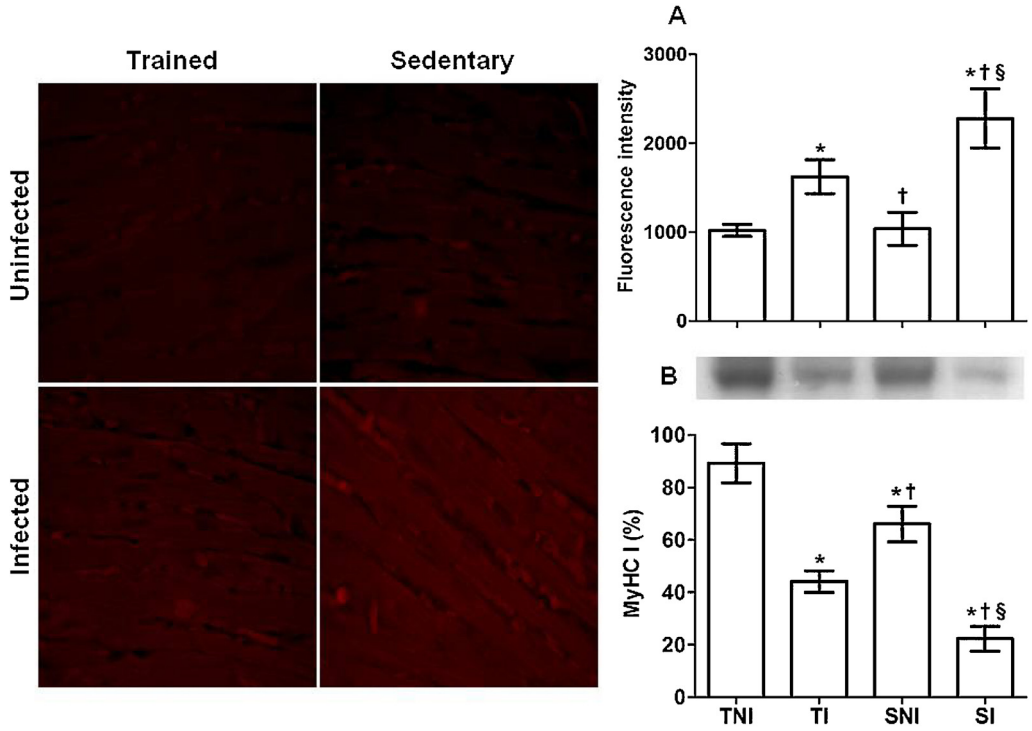

Fig. 4. Reactive oxygen species (ROS) and myosin heavy chain I (MyHC I) levels in the skeletal muscle from *T. cruzi*-infected mice. TNI, trained not infected; TI, trained infected; SNI, sedentary not infected; SI, sedentary infected. The images were obtained by fluorescence microscopy. Brighter images indicate high ROS production, which was indirectly quantified in A. MyHC I expression was determined optical densitometry from polyacrylamide gel electrophoresis. (A and B) Data are expressed as mean and standard deviation. *†§ Statistical differences among the groups ($p < 0.05$); *vs.* TNI, †*vs.* TI, § *vs.* SNI.

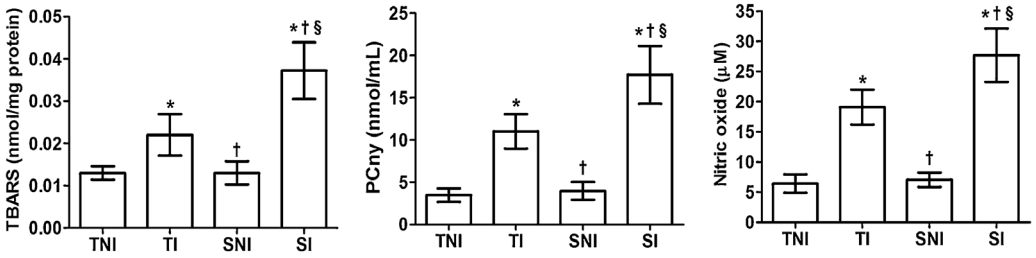

Fig. 5. Markers of reactive stress in the skeletal muscle from *T. cruzi*-infected mice. TBARS, Thiobarbituric acid reactive substances; PCN, protein carbonyl. Nitric oxide was expressed as nitrite/nitrate (NO_2^-/NO_3^-). TNI, trained not infected; TI, trained infected; SNI, sedentary not infected; SI, sedentary infected. Data are expressed as mean and standard deviation. *†§ Statistical differences among the groups ($p < 0.05$); **vs.* TNI, †*vs.* TI, § *vs.* SNI.

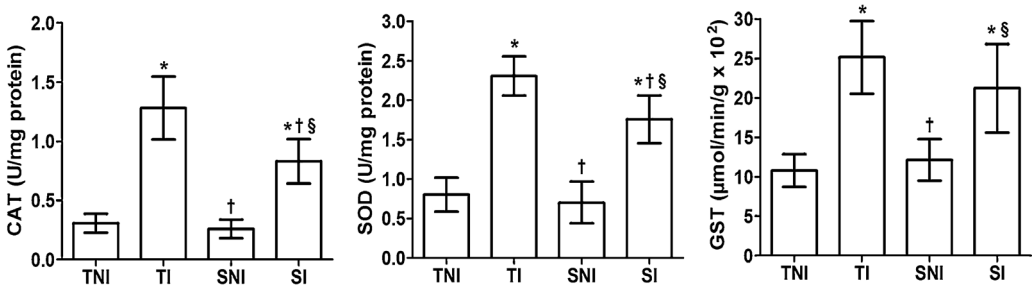

Fig. 6. Activity of endogenous antioxidant enzymes in the skeletal muscle from *T. cruzi*-infected mice. CAT, catalase; GST, glutathione-s-transferase; SOD, superoxide dismutase. TNI, trained not infected; TI, trained infected; SNI, sedentary not infected; SI, sedentary infected. Data are expressed as mean and standard deviation. *†§ Statistical differences among the groups ($p < 0.05$); **vs.* TNI, †*vs.* TI, § *vs.* SNI.

tissue damage caused by an exacerbated Th1 response (Gomes et al., 2003; Novaes et al., 2016a,b). Taken together with the parasitological, histopathological and stereological results, our findings indicated that reduction in typical Th1 molecules (i.e. TNF-α, CCL-2/MCP-1) do not determine increased host susceptibility to *T. cruzi* infection. Conversely, this reduction was potentially achieved from the improved immunological control and attenuation of skeletal muscle inflammation. This proposition is coherent with CCL-2/MCP-1 reduction, a molecule directly involved in leukocyte chemoattraction to infected organs (Teixeira et al., 2002; Yamauchi et al., 2007). Furthermore, as a potent anti-inflammatory cytokine, increased IL-10 levels also reinforced the inflammatory control and reduction in skeletal myositis severity.

Because of the improved immunological control, attenuation in the skeletal muscle pathological remodeling was expected. Beyond direct myocytolysis and necrosis induced by direct intracellular parasite proliferation, tissue damage is secondarily determined by exacerbated regional inflammatory process directed to *T. cruzi* (Gomes et al., 2003; Novaes et al., 2016a). As cytotoxins, including radical (i.e. $O_2^{\bullet -}$, OH^-, $ONOO^-$) and non-radical (i.e. NO, H_2O_2, HClO, HNO_2) reactive molecules, produced by inflammatory cells recruited to the infected tissues act indiscriminately on parasites and host cells, the inflammation intensity is directly related to tissue damage severity in Chagas disease (Paiva et al., 2012; Novaes et al., 2015). Thus, reduced cell necrosis, inflammatory infiltration and higher distribution of intact myocytes are consistent with reduced tissue parasitism (i.e. tissue amastigote nests) observed in trained animals. Taking into account that skeletal myocytes present limited regenerative potential (Maldonado et al., 2004; Souza et al., 2014), this effect seems to be relevant in protecting the host against the progressive immunomediated tissue damage typically observed in Chagas disease (Zhang and Tarleton, 1999; Torres et al., 2004).

Exercise training also presented beneficial effects against MyHC I depletion in infected skeletal muscle, a finding potentially related to the higher preservation of intact myocytes. Conversely, sedentary animals presented increased parenchyma damage (i.e. necrotic myocytes) and connective expansion, which could be partially responsible for the reduced MyHC I levels when adjusted by muscle mass or total muscle protein. However, increased MyHC I levels could also be related to the ability of exercise training to induce MyHC I gene expression by mechanic-transduction mechanisms (Bär and Pette, 1988; Goldspink, 2003). Although this mechanism is more relevant in explaining higher MyHC I levels in uninfected and trained animals, further studies are required to confirm if these propositions is coherent in infected animals. Protection against MyHC I depletion represents a remarkable finding in skeletal myopathies, since this molecule is directly associated with the production of energy, force and velocity of contraction by myocytes, aspects with marked influence on muscle function (Bär and Pette, 1988; Goldspink, 2003).

The improved redox balance also indicated the protective role of exercise training in infected animals compared to sedentary ones. This effect was mediated by reduced ROS and NO production, which was related to reduced lipid (i.e. TBARS) and protein (PCny) oxidation in skeletal muscle from trained animals. Although reactive stress has been directly implicated in heart morphofunctional damage and Chagas cardiomyopathy progression (Maçao et al., 2007; Gupta et al., 2009), the impact of this process on skeletal myositis is poorly understood. Apparently, mitochondrial dysfunction in parasitized skeletal myocytes is an intrinsic source of ROS and RNS (Báez et al., 2015). However, the inflammatory process and "respiratory burst" of recruited leucocytes has been proposed as the main source of reactive mediators, especially NO, H_2O_2, $O_2^{\bullet -}$, and OH^- (Paiva et al., 2012; Santos et al., 2015b). This proposition is supported given that anti-inflammatory strategies are accompanied by a marked reduction in ROS and RNS production in Chagas disease (Novaes et al., 2016a,b). Thus, by reducing leucocyte migration and inflammation severity, exercise training regulates a pivotal source of reactive metabolites, reducing ROS and RNS tissue levels and secondary reactive tissue damage. Although this mechanism seems to be similar to those observed in Chagas cardiomyopathy (Novaes et al., 2016b), the ROS and RNS production pathways and

the role of these molecules to the development of skeletal myositis was not completely elucidated, requiring further studies.

In the present study, reduced lipid and protein oxidation cannot be exclusively attributed to the attenuation in muscle inflammation and reduced reactive metabolite production. Thus, increased antioxidant enzyme activity (i.e. CAT and SOD) observed in infected and trained animals is also a remarkable adaptation that contributes to counteract ROS and RNS, restricting reactive tissue damage. Improvements in redox metabolism have been systematically described in animals and humans, an adaptation that together with immunological modulation has provided the rationale basis that supports the indication of exercise training to treat several health conditions, including metabolic (Bacurau et al., 2016; Hedermann et al., 2016) and infectious diseases (Lowder et al., 2005; Schebeleski-Soares et al., 2009).

Taken together, our findings indicated that exercise training induced a protective phenotype in *T. cruzi*-infected mice, enhancing the host defenses against the parasite and attenuating the extent of pathological remodeling associated with skeletal myositis. Taking into account that the reduction of skeletal muscle damage was accompanied by an attenuation of the inflammatory process, ROS and NO production and reactive tissue damage, as well as the upregulation of antioxidant enzyme activity, the beneficial adaptations induced by exercise were potentially associated with an improved immunological and redox balance in infected animals.

Conflict of interest statements

There are no conflicts of interest. All authors contributed to data collection and analysis, article preparation and have approved the final manuscript.

Acknowledgments

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