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Swimming exercise modifies oxidative stress in skeletal and cardiac muscles of diabetic rats

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ABSTRACT

Introduction: Oxidative stress is a key factor leading to the deterioration of diabetes. Oxidative stress exacerbates diabetes and induction of the activity of the antioxidant system may be required to prevent this effect. **Objective:** The aim of the present study was to evaluate the redox state in the skeletal and cardiac muscles in a diabetes rat model subjected to swimming exercise for 4 weeks. **Methods:** Wistar rats were divided into four groups: untrained control (C), trained control (T), untrained alloxan-induced diabetes (D), and trained alloxan-induced diabetes (TD). The redox state of the skeletal and cardiac muscles was assessed by analyzing TBARS, -SH groups, H2O2 production, and SOD and catalase activity. The total number of cardiomyocytes and the total area of collagen fibers in the cardiac muscle were measured by histomorphometry. **Results:** In the Soleus muscles, the TD group showed increased H2O2 levels and catalase activity compared to the T group, and SOD activity compared to the D group. Regarding the red gastrocnemius, the TD group presented higher SOD and lower catalase activities than the D group. Regarding the cardiac muscle, the TD group presented lower TBARS and higher levels of -SH groups and catalase activity than the D group. Swimming exercise decreased hyperglycemia and reduced pathology, as evidenced by the reduced number of cardiomyocytes and the area of collagen fibers. **Conclusion:** Swimming exercise in diabetic rats controlled hyperglycemia and oxidative damage, and the reduced fibrosis in the cardiac muscle of diabetic rats.

Keywords: Swimming exercise, diabetes, oxidative damage, skeletal muscle, cardiac muscle, fibrosis.

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1. INTRODUCTION

Type 1 diabetes is characterized by the autoimmune destruction of β cells, resulting in hyperglycemia (COLEMAN, *et al*., 2015, KATSAROU, *et al*., 2017). The World Health Organization (WHO) data show that diabetes has increased in recent years and now constitutes one of the main public health problems (OGURTSOVA *et al*., 2017).

Diabetes-associated pathology can be progressive, especially when the production of free radicals exceeds the body's capacity to neutralize them, leading to oxidative stress (ARO, *et al*., 2015). Oxidative stress may result in lipid peroxidation, reduction in enzyme activity, DNA damage, and impaired carbohydrate metabolism (KAUL *et al*., 2015, ACCATTATO *et al*., 2017). There is also consequent atrophy of skeletal muscles (HIRATA, *et al*., 2019) and proteolysis in the cardiac muscle (HU *et al*., 2008).

However, swimming exercise improves exercise capacity and muscle function in patients with a combination of chronic heart failure and diabetes (ASA, *et al*., 2012). In rats, swimming exercise provides satisfactory results in terms of higher levels of antioxidants, including glutathione (GSH), superoxide dismutase (SOD), and catalase (LUBKOWSKA, *et al*., 2019). These are general mechanisms of exercise and are associated with the control of redox signaling through several pathways, including nuclear factor kappa B (NF-κB), mitogen-activated protein kinase (MAPK), and peroxisome-activated 1-alpha receptor gamma coactivator proliferating (PGC-1α) (HALL, *et al*., 2013, JI & ZHANG, 2014).

In this study we evaluated the redox state of the skeletal and cardiac muscles of hyperglycemic rats, after four weeks of aquatic exercise using a model protocol with increasing loads.

2. MATERIALS AND METHODOS

Animals and ethical considerations

Male Wistar rats (Rattus norvegicus albinus) were obtained from the Center for Animal Experimentation (CEA) of the University Center of Herminio Ometto Foundation (Araras, Sao Paulo, Brazil). The rats, weighing between 200 and 250 g, were placed in cages (four animals per cage) and kept under controlled conditions of temperature (23 ± 1ºC), humidity, and illumination (12 h light/dark cycles), with free access to water and rodent feed during the entire experiment.

All surgical and experimental procedures used in this study were conducted in

accordance with the experimental requirements and biodiversity rights of the Guide for the

Care and Use of Laboratory Animals, National Institutes of Health. The study also complied with the standards of the Brazilian College of Animal Experimentation (COBEA), conducted according to the rules established by the Arouca Law, Brazilian legislation on the scientific use of animals, conformed to the ethical principles in animal research adapted by the Conselho Nacional de Controle de Experimentação Animal (CONCEA), and approved by the Animal Use Ethics Committee of the University Center of Hermínio Ometto Foundation (FHO) protocol 083/2014.

Induction of diabetes and experimental groups

Male Wistar rats were intravenously (in the penile dorsal vein) administered 32 mg/kg alloxan monohydrate (Sigma-Aldrich, San Luis, Missouri, EUA) dissolved in citrate buffer, 12 h after fasting. Then, the animals were placed in cages and administered a 15% glucose solution for 24 h to avoid complications of alloxanic hypoglycemia (LENZEN, 2007). One week after induction, blood samples were collected from the tail veins of the animals, and glycemia levels were determined using a portable glucose meter (Abbott, Chicago, IL, USA). Rats with glycemia levels between 200 and 600 mg/dL were included in the subsequent experiments.

Thirty days after confirming hyperglycemia, the animals were randomly divided into the following groups: untrained control (C), trained control protocol (T), untrained diabetic (D), and trained diabetic protocol (TD) (n=8 rats/group). Animals in which diabetes was not induced (C and T groups) were treated with the same protocol, but without alloxan (Figure 1).

Physical exercise protocol

The physical exercise protocol was performed in a swimming tank with a controlled water temperature of 31 ± 1°C. First, the animals were acclimated to the water environment for 10 days. For that, the animals were placed in individual bays (1 rat per bay, 12 bays per tank), separated by transparent acrylic divisions, in tanks (100 x 80 x 80 cm) with a maximum depth of 50 cm. The animals were acclimatized and trained as follows: 5 min on 1st day, 10 min on 2nd day, and 15 min on 3th day, using a low water level. From the day onwards, the water level was raised, requiring the animals to swim for 10 min on the 4th day and for 15 min on the 5th day. On the 6th and 7th days, a load of 3% of the body weight was added to each rat for 10 and 15 min, respectively. The purpose of the adaptation was to reduce animals stress, without causing physiological adaptations (ARAUJO *et al*., 2013).

Afterwards, a minimum lactate (ML) test was performed to determine the aerobic/anaerobic metabolic transition, with an initial load of 13% of their body weight (for induction of hyperlactacidemia) and training for 30 s. After resting for 30 s, the rats were subjected to swimming with the 13% load until exhaustion. After resting for 9 min, a blood sample (25 µL) was collected from the distal tail vein to determine the lactate concentration, and the animals started exercise with progressively higher loads (ARAUJO *et al*., 2013). The load was initially 2.0% of their body weight and was increased by 0.5% every 5 min until exhaustion. At each load change, a blood sample (25 µL) was collected for lactate determination. At the end of each exercise, the rats were dried with a towel and returned to their cages. ML was determined using a second order polynomial fit to the data of lactate plotted against the workload (ARAUJO *et al*., 2013).

After the ML test, the exercise groups were subjected to the swimming exercise in individual tanks containing water at 31 ± 1 ºC, on six days per week, during four consecutive weeks, with lead weights attached to the thorax. The exercise training protocol used in the present study was based in a reverse periodization model in which intensity is initially at its highest and volume at its lowest. This protocol was chosen because it is associated with increased muscular endurance in humans (RHEA *et al*., 2003). Besides, a mean gain of 15% in the performance of rodents that exercise for 4 weeks at ML intensity has been demonstrated (CUNHA

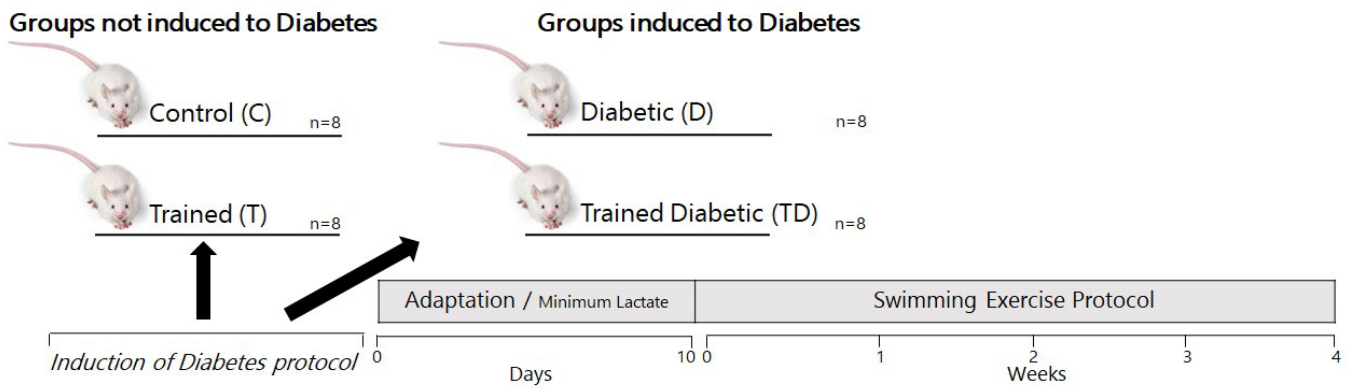

Figure 1. Experimental groups and experimental design: Untrained control (C); trained control protocol (T); untrained diabetic (D); trained diabetic protocol (TD) (n=8 rats/group)

et al., 2008). Therefore, the initial training load was equivalent to 115% of the individual ML, and the time spent on exercise totaled 25 min/day. Thereafter, an overload decrease of 5% was applied each week up to the 4th week (110% of ML in the 2nd week, 105% of ML in the 3th week, and 100% of the ML in the 4th week), with an increase of 5 min in the total period of activity (30 min in the 2nd week, 35 min in the 3th week, and 40 min in the 4th week).

Glycemia

The glycemia test was performed at the end of each training week (n=8 animals per group). Blood samples were collected from the tail vein and glycemia was determined using reagent strips and a glucose meter (Abbott, Chicago, IL, USA). The glucose levels at the end of each training week were calculated by estimating the total area under the curve using the trapezoidal method (LE FLOCH, *et al*., 1990).

Harvesting of biological tissues

The animals were euthanized by intraperitoneal injection of a solution of ketamine hydrochloride (75 mg/kg) and xylazine hydrochloride (25 mg/kg) 24 h after the final exercise session. The heart was removed and immersed in 14 mM KCl solution, followed by washing in ice-cold 0.9% NaCl and sectioning. The muscles that performed most of the work during the swimming training were the red gastrocnemius (RG; 35-62% type I, 30-51% type IIA, 1-8% type IIB), containing predominantly mixed fibers (oxidative/glycolytic), the white gastrocnemius (WG; 0% type I, 0% type IIA, 92% type IIB), comprised predominantly type IIB (glycolytic) fibers, and the soleus muscle (84% type I, 7% type IIA, 0% type IIB), consisting predominantly of oxidative fibers. These muscles were harvested for biochemical (skeletal and cardiac muscles) (RAMOS-FILHO, *et al*., 2015) and histomorphometrical analysis (cardiac muscle).

Biochemical analyses of redox status

The white and red portions of the gastrocnemius muscle, the soleus muscle, and part of the left ventricle were homogenized in specific buffer (7.4 pH, 10 mmoL/L Tris-HCl, 1 mmoL/L EDTA and 250 mmol/L sucrose) using a Polytron Model PT 10/35 homogenizer (Brinkmann Instruments, Westbury, NY, USA) (MATAIX *et al*., 1998).

As a biomarker of oxidative stress, lipid peroxidation was assessed by determining MDA production. Briefly, samples of the RG, WG, soleus, and cardiac muscles were homogenized (using the Polytron device) in phosphate buffer and centrifuged at 2795 g for 10 min at 18°C. TBARS (Thiobarbituric acid reactive substances) quantification was performed by colorimetric MDA analysis (Lipid Peroxidation (MDA) Assay Kit (Colorimetric/Fluorometric)-ab118970, Abcam- United Kingdom) using a spectrophotometer at 535 nm (MATAIX *et al*., 1998).

The levels of H2O2 were determined by fluorescence, by using the Amplex UltraRed Reagent kit (Life Technologies Corporation, Grand Island, New York, USA) according to the manufacturer's instructions (RAMOS-FILHO, *et al*., 2015).

As a biomarker of antioxidants, the levels of -SH groups were assayed using a colorimetric method that involved the reaction of the sulfhydryl group with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), followed by spectrophotometric measurement at 412 nm (MATAIX *et al*., 1998).

SOD activity was measured by using the inhibition of adrenaline oxidation method. Plasma samples were homogenized in glycine buffer. A total of 5, 10, and 15 µL of sample were aliquoted and 5.0 mL of catalase (0.0024 mg/mL in distilled water), 180 mL of glycine buffer (0.75 g in 200 mL of distilled water at 32ºC, pH 10.2), and 5.0 µL of adrenaline (60 mM in distilled water plus 15 mL/mL fuming HCl) were added. The readings were acquired for 180 s at 10 s intervals and measured on an ELISA reader (Fabricante: BioTek® Instruments, Inc., Winooski, Vermont, USA) at 480 nm. Values were expressed as units of SOD per milligram of protein (U/mg protein) (BANNISTER & CALABRESE, 1987). Catalase activity was determined based on the rate of H2O2 decomposition by the enzyme present in the sample using 10 mM H2O2 as a substrate in potassium phosphate buffer, pH 7.0. The maximum H2O2 decomposition rate was measured using a spectrophotometer (ThermoFischer Scientific, Madison, Wisconsin, USA) at 240 nm, and values were expressed as catalase units per milligram of protein (AEBI, 1984).

Histomorphometry

A portion of the heart was fixed for 24 h in 10% paraformaldehyde buffered at pH 7.4, dehydrated, embedded in paraffin, and cut into 5.0 µm sections. The sections were stained with ferric hematoxylin (to determine the number of cardiomyocytes - n/104 µm2) and picrosirius red (to determine the percentage area of collagen fibers - % in 104 µm2) to evaluate the effects of the diabetes and the training protocols. Histological sections were photographed using the LEICA®DM-2000 optical microscope enabled with LEICA®DFC-300 FX camera (Wetzlar, Germany) connected to a computer with LAS®software - Leica Application Suite for image capture. Measurements were made using the ImageJ in triplicate for each animal, by determining the group means from the sections of each animal (RELIVE, *et al*., 2007).

Statistical analysis

All the data were first analyzed by the Shapiro-Wilk normality test, followed by multifactorial analysis of variance (ANOVA two-way) and Tukey's post-hoc test. The results were expressed as mean ± standard error, and results with $p<0.05$ were considered statistically significant. Statistical analyses were performed using GraphPad Prism 6.0 software.

3. RESULTS

Glycemia

Evaluation of the effect of physical training showed that the training method was effective in decreasing hyperglycemia in diabetic animals (Figure 2a, b).

Analysis of pro- and antioxidant activity

Soleus muscle

The soleus muscle showed a higher value of TBARS in the D group than in the C and TD groups (Figure 3a).

In the analysis of H2O2, the TD group showed greater levels compared to the T group (Figure 3b), whereas the levels of –SH were higher in the D group than in the C

group (Figure 3c).

The activity of SOD was higher in the TD group compared to the D group (Figure 3d). The activity of catalase was higher in the TD group than in the T group (Figure 3e).

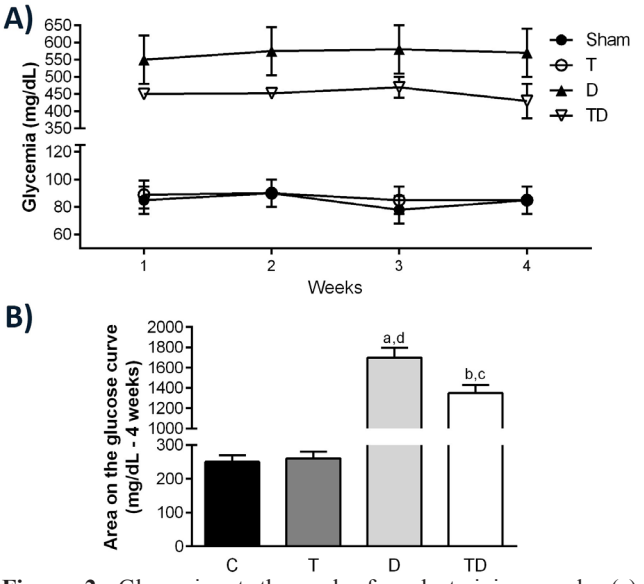

Figure 2. Glycemia at the end of each training week. (a) representative graph of the weekly glycemia of the animals throughout the experimental period. (b) area under the curve of the animals over the experimental period. Results are expressed as mean ± standard deviation of the mean. Untrained control (C); trained control (T); untrained diabetic (D); trained diabetic (TD) (n=8 rats/group). Letters in the bar graphs correspond to $p < 0.05$ relative to the corresponding group (ANOVA Two-way/Tukey post-test)

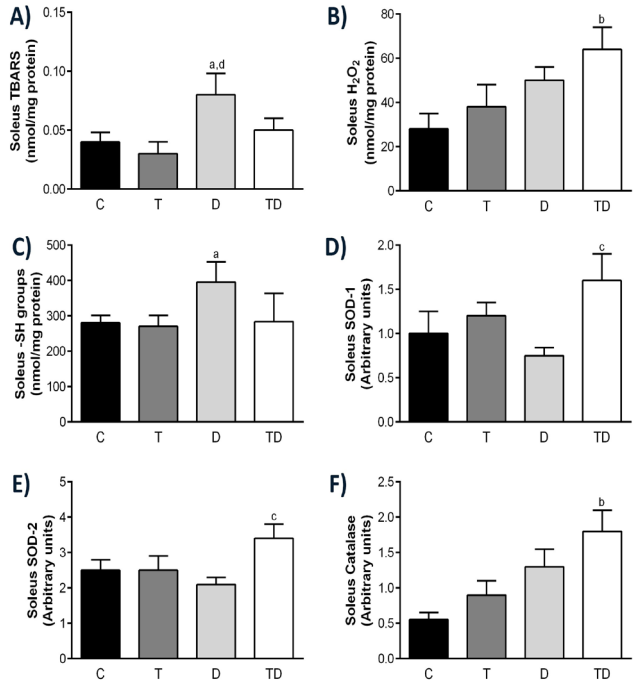

Figure 3. Soleus muscle analysis of (a) thiobarbituric acid (TBARS), (b) hydrogen peroxide (H2O2), and (c) sulfhydryl groups (-SH) levels, (d) superoxide dismutase (SOD) and (e) catalase activities. Untrained control (C); trained control (T); untrained diabetic (D); trained diabetic (TD) (n=8 rats/group). Letters in the bar graphs correspond to $p < 0.05$ relative to the corresponding group (ANOVA Two-way/Tukey post-test)

Red gastrocnemius

In the red gastrocnemius muscle, no difference was observed in the levels of TBARS, H2O2, and –SH groups between the different groups (Figure 4a-c).

The activity of SOD was greater in the TD group compared to D group, and the D group showed decreased activity compared to the C group (Figure 4d). The activity of catalase was reduced in the TD group compared to the D group (Figure 4e).

White gastrocnemius

No differences were observed between the experimental groups for the pro- and antioxidant markers regarding the white portions of the gastrocnemius muscle (Figure 5a-e).

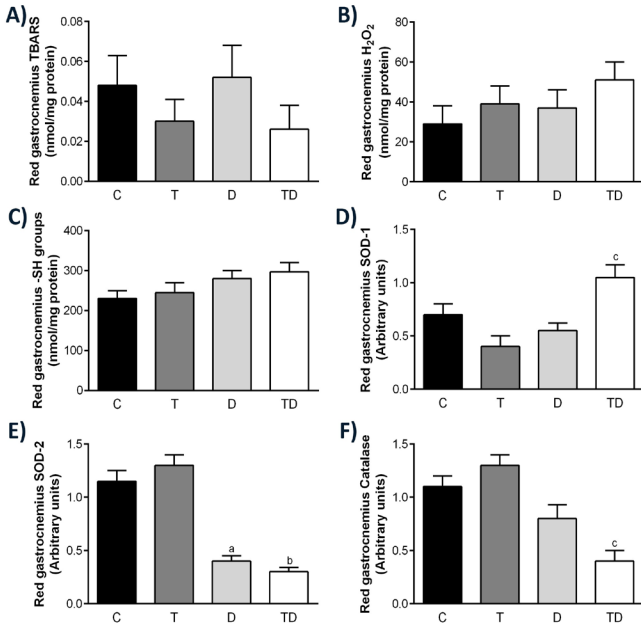

Figure 4. Red gastrocnemius analysis of (a) thiobarbituric acid (TBARS), (b) hydrogen peroxide (H2O2), and (c) sulfhydryl groups (-SH) levels, (d) superoxide dismutase (SOD) and (e) catalase activities. Untrained control (C); trained control (T); untrained diabetic (D); trained diabetic (TD) (n=8 rats/group). Letters in the bar graphs correspond to $p < 0.05$ relative to the corresponding group (ANOVA Two-way/Tukey post-test)

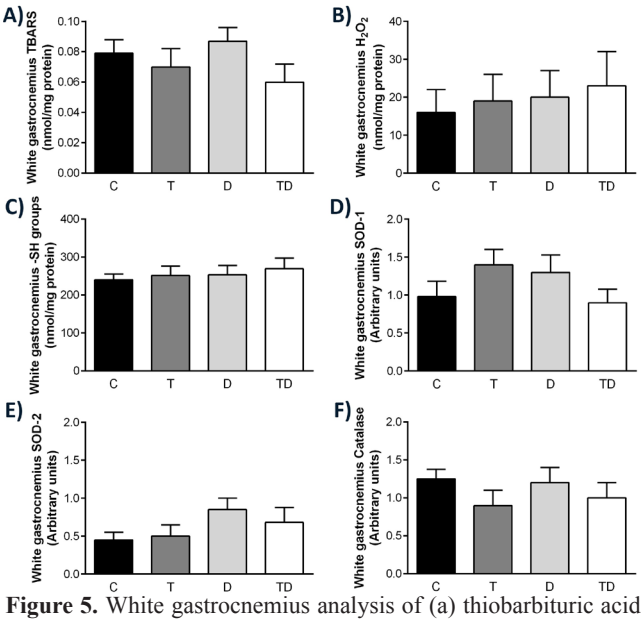

Figure 5. White gastrocnemius analysis of (a) thiobarbituric acid (TBARS), (b) hydrogen peroxide (H2O2), and (c) sulfhydryl groups (-SH) levels, (d) superoxide dismutase (SOD) and (e) catalase activities. Untrained control (C); trained control (T); untrained diabetic (D); trained diabetic (TD) (n=8 rats/group). Letters in the bar graphs correspond to $p < 0.05$ relative to the corresponding group (ANOVA Two-way/Tukey post-test)

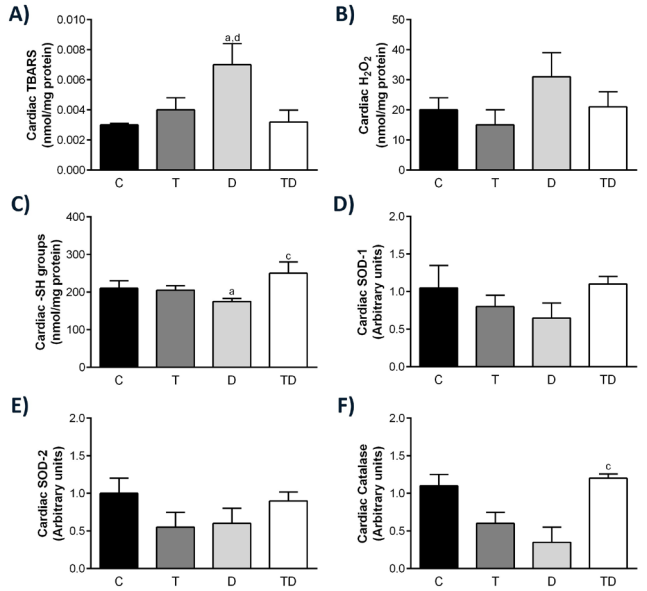

Figure 6. Cardiac analysis of (a) thiobarbituric acid (TBARS), (b) hydrogen peroxide (H2O2), and (c) sulfhydryl groups (-SH) levels, (d) superoxide dismutase (SOD) and (e) catalase activities. Untrained control (C); trained control (T); untrained diabetic (D); trained diabetic (TD) (n=8 rats/group). Letters in the bar graphs correspond to $p < 0.05$ relative to the corresponding group (ANOVA Two-way/Tukey post-test)

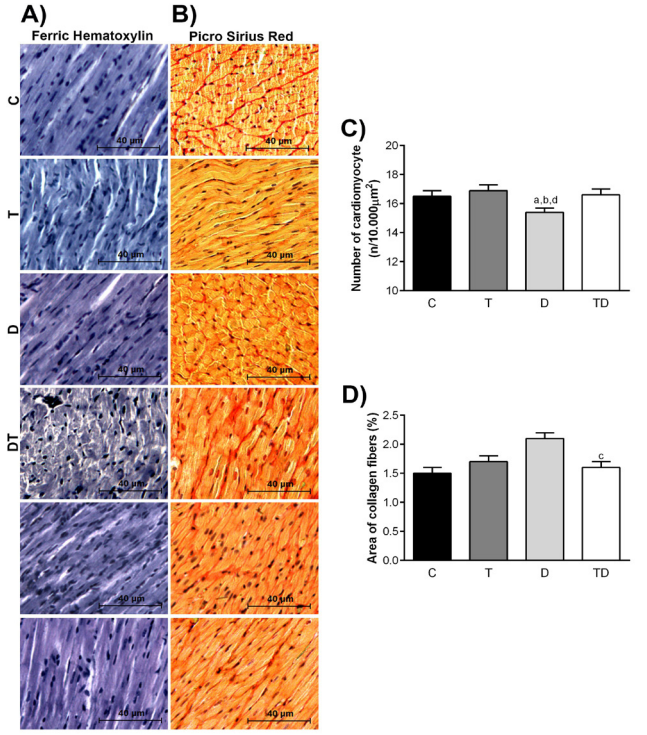

Figure 7. Histomorphometry of cardiac muscle to evaluate (a, c) the number of cardiomyocytes using Ferric hematoxylin staining (b, d) total percentage of the collagen fiber area using picrosirius red staining. Results are expressed as mean ± standard deviation of the mean, n=3 animals per group. Untrained control (C); trained control (T); untrained diabetic (D); trained diabetic (TD) (n=8 rats/group). Letters in the bar graphs correspond to $p < 0.05$ relative to the corresponding group (ANOVA Two-way/Tukey post-test)

Cardiac muscle

The D group showed greater levels of TBARS than the C and TD groups (Figure 6a). The levels of H2O2, and SOD showed no difference between the experimental groups (Figure 6b, d). The level of –SH groups showed an increase in the TD group compared to D group, while this one showed lower level compared to group C (Figure 6c). The TD group showed an increase catalase activity compared to D group (Figure 6e).

Cardiomyocytes and collagen fibers

The number of cardiomyocytes was lower in the D group than that in the other groups (Figure 7a, c). The area of collagen fiber in the TD group was lower than that in the D group (Figure 7b, d).

4. DISCUSION

The swimming exercise used in the present study was effective in decreasing glycemia in alloxan-induced diabetic rats (TD group vs. D group), which was in agreement with previous findings (LEE, *et al*., 2012). Blood hyperglycemia leads to advanced glycation end products (AGEs). Furthermore, it has been shown that it results in dysfunctions of endothelial progenitor cells (EPCs), sirtuin-3 (SIRT3) impairment, and dysfunction via AMPK/p38/NF-kB causing high oxidative stress, such as increased production of Nitric Oxide (LASCAR *et al*., 2013, PAULA *et al*., 2015, HE, *et al*., 2016). Analysis of the pancreas in the diabetic rats showed that eight weeks of moderate- to high-intensity training resulted in a 33% increase in the number of β-cells, due to improved glycemic and inflammatory control (MOURA, *et al*., 2011, GOLBIDI, *et al*., 2012, LASCAR *et al*., 2013). Diabetic rats subjected to eight weeks of exercise showed a 21% decrease in hyperglycemia, compared to the group without exercise (KIM, *et al*., 2014). Exercising resulted in glycemic control and absence of diabetes related ketoacidosis, with greater expression of PGC-1α in the pancreatic β-cell, compared to control (resting) animals (RELIVE, *et al*., 2007, COLBERG *et al*., 2015, PAULA, *et al*., 2015).

Diabetes is characterized by increased levels of ROS, which together with inflammatory cytokines contribute to muscle atrophy, generating a vicious circle of decreased glucose uptake and increased ROS (MOURA, *et al*., 2011, MARCINKO & STEIMBERG, 2014). In agreement with these data, the alloxan-induced diabetes group (D) showed increased levels of TBARS and -SH groups in the soleus muscle. However, the same muscle in the TD group showed improved levels of TBARS and -SH groups, and increased SOD and catalase activities. SOD activity also increased in the red gastrocnemius muscle. Therefore, our data suggest that the swimming exercise protocol used in the present study was effective in modulating the oxidative stress associated with the alloxan-induced diabetes model.

The results regarding the lipid peroxidation in skeletal muscles showed an enhanced oxidant activity after training. Exercise in diabetic condition has been associated with improvements in physical ability, endothelial function, and signal transduction as well as a favorable lipid profile and suppresses cancer caused by lipid peroxidation (FATIMA, *et al*., 2016). Exercise induced a decrease in TBARS content (81%) in the soleus muscle of rats and had an antioxidant effect on the skeletal muscle (LAMBERTUCCI, *et al*., 2007). In diabetic animals, the decrease in TBARS levels observed in the soleus muscle in response to exercise

is comparable to those reported earlier in animal and human models regarding drug treatments, diet control, and high-intensity intermittent exercise (WANG, *et al*., 2015).

H2O2 levels in the gastrocnemius did not increase in experimental groups, indicating that predominantly glycolytic muscles (type IIB) were not affected by the oxidative stress caused by H2O2, as previously observed in a study comparing glycolytic and oxidative fibers (WANG, *et al*., 2015). In contrast, the soleus muscle of the TD group showed an increase in H2O2. It has recently been suggested that at physiological levels, H2O2 could stimulate SIRT1 and deacetylate FOXO-3, thereby reducing oxidation and stimulating the expression of SOD, catalase, and GPx in skeletal and cardiac muscle (WANG, *et al*., 2015).

The antioxidant enzymes SOD, catalase, and GPx are the main agents of defenses against ROS, especially during physical training (MARCINKO & STEIMBERG, 2014). In the present study, the results obtained for the soleus musculature showed higher levels of SOD and catalase activity in the TD group. Similar elevation in catalase activity in the soleus muscle were reported in a study using healthy animals that exercised at 60% of maximum speed, with a single molecule of catalase converting millions of molecules of water and oxygen (GOMES, *et al*., 2012, DE ARAUJO, *et al*., 2016). A deficiency in catalase has been shown to increase the effects of type I diabetes and increase the risk of type II diabetes in healthy individuals (LAWLER, *et al*., 2016).

The results for the soleus muscle suggest that aquatic exercise in this experimental model was an effective inducer of antioxidant defense and may be associated with increasing training time (GOLBIDI, *et al*., 2012). The time factor is important in physical training allowing evolution and better adjustment of the organism to the increasing load (POWERS & HIGAN, 2016). Similar results showing progressive increase with time were reported in a study with diabetic individuals who initially trained (above 80% bpm) for 30 min and for 40 min in the last week, resulting in increases in the activity of SOD and GPX (ARO, *et al*., 2015).

Studies have reported that in the oxidative part of the gastrocnemius muscle, the SOD enzyme was increased in the hyperglycemic state, as well as in type 1 high-intensity physical training. In the case of SOD, similar results have been reported previously for training at 60% of the maximum velocity in animals without pathology (LEE, *et al*., 2012, LEI, *et al*., 2016, LOUREIRO, *et al*., 2016). It has also been found that Nitrogen Oxide is higher in the soleus and RG muscles than in the WG muscle, which could be due to the lower vascular development in the WG muscle (LOUREIRO, *et al*., 2016).

The cardiac muscle showed an increased expression of catalase in the TD group, corroborating the literature (ANARUMA *et al*., 2016), and a decrease in TBARS. In a study on rats subjected to anaerobic training (75% VO2max (maximum rate of oxygen) for 30 min, 5 days per week, for 4 weeks), the analysis of cardiac muscle revealed decreases in TBARS similar to those obtained in this study, along with an increase in reduced glutathione (GONCHAR, 2005). Physical training has been shown to reverse the IkB/NF-κB pathway in type I and II diabetes (HE, *et al*., 2016). This could be explained by the activation of PGC-1α, which in addition to increasing antioxidant levels, regulates the expression of VEGF and angiogenesis in skeletal and cardiac muscle (HE, *et al*., 2016). In addition to SOD, CAT produced by the cardiac muscle can lead to vascular improvements (LEI *et al*., 2016, NADERI *et al*., 2015).

The morphometric analyses showed that the cardiac cells were damaged by hyperglycemia and ROS. A smaller number of cardiomyocytes were found in the D group, which related to increased fibrosis, representing a loss of cardiac function characteristic of diabetic cardiomyopathy (WANG, *et al*., 2015). Interstitial fibrosis and the accumulation of glycoproteins are associated with the presence of collagen types I and III, resulting in phenotypic and functional differentiation. The literature suggests that functional alterations involve metabolic rather than structural characteristics (WANG, *et al*., 2015).

In individuals with diabetes, endothelial dysfunction manifests as problems associated with prostaglandins and Nitrogen Oxide. Furthermore, non-esterified fatty acids alter the basal membranes of blood vessels, affect blood flow, and the numbers of cardiomyocytes (KONIOR, *et al*., 2014). Moderate-intensity long-term exercise (over 15 weeks) leads to physiological cardiac growth. In a previous study, no significant cardiomyocyte apoptosis or myocardial fibrosis was observed in trained C57BL/6 wild-type rats (WANG, *et al*., 2015, NOVOA *et al*., 2017).

In terms of the amount of collagen, there was an evidence of cellular and interstitial fibrosis in our experimental model, as reported elsewhere in animals with diabetes (CHU *et al*., 2015). Reduced myocyte hypertrophy and decreased collagen deposition (fibrosis), suggesting cardiac damage, have been observed in Sprague-Dawley rats that trained at 80% of their maximum capacity for four weeks, showing that high-intensity exercise reversed cardiac remodeling in the diabetic heart (NOVOA *et al*., 2017). In a previous study in which diabetes was induced using alloxan and training was performed at > 80% of maximum intensity, positive effects on cardiac remodeling were observed, as evidenced by a reduction in myocyte hypertrophy and collagen deposition (fibrosis) (HAGHANI, *et al*., 2016, NOVOA *et al*., 2017), normalization of the levels of collagen type III, and reduced apoptosis and myocardial fibrosis (WANG *et al*., 2015).

Swimming exercise results in a significant increase in antioxidant capacity, and use of shorter exercise time encourages adherence to the treatment regimen (MOURA, *et al*., 2011, LAWLER, *et al*., 2016). Antioxidant enzymes should be increased naturally, otherwise, as in animals that overexpress SOD, and CAT, a variety of cellular and signaling processes can be dysregulated (MOURA *et al*., 2011, LEI, *et al*., 2016).

5.CONCLUSION

We conclude that swimming, as a physical exercise, was found to decrease hyperglycemia in the diabetic animals. The training protocol involved an increase in the volume and a decrease in the intensity for four weeks,

and reduced oxidative stress and minimized fibrosis in animal tissues. These findings suggest that a physical exercise protocol may hold therapeutic potential for patients with diabetes.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest regarding the publication of this paper.

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