Swimming Exercise Modifies Oxidative Stress in Skeletal and Cardiac Muscles of Diabetic Rats

Talita Fernanda Pio
Centro Universitario Euripedes de Marilia
Lucas Eduard Orzari
Instituto Brasileiro de Medicina de Reabilitacao
Armindo Antônio Alves
Centro Universitario da Fundação Hermínio Ometto
Paulo Cesar Lock Silveira
Centro Universitario da Fundação Hermínio Ometto
Marcelo Augusto Marretto Esquisatto (marcelosquisatto@fho.edu.br)
Centro Universitário da Fundação Hermínio Ometto https://orcid.org/0000-0002-2588-619X
Thiago Antônio Moretti de Andrade
Centro Universitário da Fundação Herminio Ometto
Maira Felonato Mendes
Centro Universitario da Fundação Herminio Ometto
Rodrigo Augusto Dalia
Centro Universitario da Fundação Herminio Ometto

Research

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

Posted Date: July 9th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-38287/v1

Abstract

Background: Oxidative stress is a key factor in the worsening of diabetes, requiring an increase in the activity of the antioxidant system, culminating in a balance in the body. The aim of the present study was to evaluate the redox state in the skeletal and cardiac muscles in rats induced to diabetes model and submitted to swimming exercise training for 4 weeks.

Methods: Wistar rats were separated into the following four groups: untrained control (C); trained control (T); untrained alloxan-induced diabetes (D); trained alloxan-induced diabetes (TD). The animals were submitted to swimming exercise training for 4 weeks. The redox state of skeletal and cardiac muscles was analyzed by TBARS, -SH groups and, H_2O_2 production as well as by the SOD and Catalase activity. Besides, a histomorphometry analysis was performed in the cardiac muscle in which the total number of cardiomyocytes and the total area of collagen fibers were measured.

Results: On the Soleus muscles, the TD group showed increased of H_2O_2 and Catalase compared to T group; and SOD compared to D group. About the red gastrocnemius, TD group presented higher SOD and lower Catalase relation to D group. Regarding to cardiac muscle, TD group presented lower TBARS than D group, while it was higher in -SH groups and Catalase compared to D group. Moreover, the swimming exercise led to a decrease in hyperglycemia, benefits in pathogenesis and physio-pathogenesis, evidenced by a reduced number of cardiomyocytes and area of collagen fibers.

Conclusions: The swimming protocol exercise in diabetic rats was able to control the hyperglycemia, control of oxidative damage and minimize the fibrosis on cardiac muscle in diabetic rats.

Background

Type 1 diabetes is characterized by the auto-immune destruction of β cells, resulting in hyperglycemia [1, 2]. World Health Organization (WHO) data show that diabetes has increased in recent years and now constitutes one of the main public health problems [3–5].

The problems associated with diabetes can be progressive, especially when the production of free radicals exceeds the body's capacity to neutralize them, leading to oxidative stress [6, 7]. Damage to the cell membrane includes lipid peroxidation, enzyme problems, DNA damage, and impaired carbohydrate metabolism [8, 9]. There is consequent atrophy in the skeletal muscles [10] and proteolysis in the cardiac muscle [11].

However, swimming exercises training with can be used to improve exercise capacity and muscle function in patients with a combination of chronic heart failure and diabetes [12]. Swimming exercises training has been shown to provide satisfactory results in terms of better levels of antioxidants, including reduced glutathione (GSH), superoxide dismutase (SOD), and catalase [13, 14]. These effects are associated with the control of redox signaling in several pathways, involving the nuclear factor kappa B (NF-κB), the mitogen-activated protein kinase (MAPK), and the gamma co-activator of the 1-alpha

receptor activated by the proliferating peroxisome (PGC it) [15, 16]. There is also a decrease in hyperglycemia, activating the AMP-activated protein kinase (AMPK) pathway, which reduces the inflammatory process due to decreased hyperglycemia in diabetes [17]. Another effect occurs when the entry of high levels of glucose into the cell leads to the activation of the pentose pathway, which increases GSH [18]. A third effect is an activation of xanthine oxidase (XO), characterized by the production of reactive species sufficient to activate antioxidants in the pathways without causing damage [19, 20]. A fourth effect involves coupled mitochondria, which at maximum speed (known as state 3) result in the production of fewer reactive species in the electron transport chain [21].

Therefore, it is important to evaluate the redox state in the skeletal and cardiac muscles of rats induced to hyperglycemia, after four weeks of aquatic exercise training using model protocol with increasing loads.

Material And Methods

Animals and ethical considerations

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

All the experimental procedures were performed according to the protocols of the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health. The study also complied with the standards of the Brazilian College of Animal Experimentation (COBEA), Brazilian legislation on the scientific use of animals [22], and the Ethics Committee on Animal Use (CEUA) of Herminio Ometto Foundation - FHO, protocol 083/2014.

Induction of diabetes and experimental groups

The male Wistar rats were administered alloxan monohydrate (Sigma-Aldrich) dissolved in citrate buffer, at a dose of 32 mg/Kg, intravenously (in the penile dorsal vein), after 12 h of fasting. After this procedure, the animals were placed in cages and were administered a solution of glucose (15%) for 24 h, in order to avoid complications of alloxanic hypoglycemia [23]. One week after induction, blood samples were collected from the tail veins of the animals and the levels of glycemia were determined using a portable glucose meter (Accu-Chek Active). Rats with glycemia between 200 and 600 mg/dL were included in the subsequent experiments.

Thirty days after confirming hyperglycemia, the animals were randomly separated into the following groups: untrained control (C); trained control protocol (T); untrained diabetic (D); trained diabetic protocol

(TD) (n = 8 rats/group). About the animals not induced to diabetes, it were made the same protocol, but without aloxane in solution injected (Fig. 1).

Physical exercise protocol

The physical exercise protocol was performed in a swimming tank with controlled water temperature of 31 ± 1 °C. First, the animals were adapted 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 × 80 × 80 cm) with a maximum 50 cm height of water. The periods used were 5 min on 1st day, 10 min on 2nd day, and 15 min on 3th day, using a low water level [16]. From 4th day onwards, the water level was raised, requiring the animals to swim for 10 min on 4th day and for 15 min on 5th day. On 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 [24].

Afterwards, a minimum lactate (ML) test was used to determine the aerobic/anaerobic metabolic transition, with an initial load of 13% of the body weight (for induction of hyperlactacidemia) and training for 30 sec. After resting for 30 sec, the rats were submitted to swimming with a 13% load until exhaustion. After resting for 9 min, a blood sample (25 μL) was collected from the distal tail vein for determination the lactate concentration, and the animals started exercise with progressively higher loads [24].The load was initially 2.0% of the body mass 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. The ML was determined using a second order polynomial fit to the data of lactate plotted against the workload [24].

After the ML test, the exercise groups were submitted 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 has been associated with increasing muscular endurance in humans [25]. Besides, it has been demonstrated a mean gain of 15% in the performance of rodents that exercise for 4 weeks at ML intensity [26]. Therefore, the initial training load was equivalent to 115% of the individual ML and the time spent on exercise totalized 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 at the end of each training week (n = 8 animals per group). Blood samples were collected from the tail and it was determined using reagent strips and a glucose meter (Abbott, Chicago, USA). The glucose response during glycemia the end of each training week was calculated by estimating the total area under the curve using the trapezoidal method [27].

Harvesting of biological tissues

The animals were euthanized intraperitoneally with 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 the most work in the swimming training were the red gastrocnemius (RG; 35–62% type I, 30–51% type IIA, 1–8% type IIB), containing a predominance of mixed fibers (oxidative/glycolytic), the white gastrocnemius (WG; 0% type I, 0% type IIA, 92% type IIB), with predominantly type IIB (glycolytic) fibers, and the soleus muscle (84% type I, 7% type IIA, 0% type IIB), with predominantly oxidative fibers. These muscles were removed and separated for biochemical (skeletal and cardiac muscles) [28] and histomorphometrical analysis (cardiac muscle).

Biochemical analyses of redox status (TBARS, H_2O_2, –SH groups, SOD and Catalase)

The white and red portions of the gastrocnemius muscle, the soleus muscle, and part of the left ventricle were homogenized in specific buffer using a Polytron Model PT 10/35 homogenizer (Brinkmann Instruments, Westbury, NY, USA) [29].

As a biomarker of oxidative stress, lipid peroxidation was detected by determination of MDA production. Briefly, samples of the RG, WG, soleus, and cardiac muscles were homogenized (using the Polytron device) in phosphate buffer and centrifuged at 5,000 rpm for 10 min at 18 °C. TBARS quantification was performed by colorimetric MDA analysis, using a spectrophotometer at 535 nm [4, 29].

H_2O_2 was determined by fluorescence (Amplex UltraRed Reagent kit, Life Technologies Corporation, Grand Island, New York, USA) [28].

As a biomarker of antioxidants, the quantification of -SH groups employed a colorimetric method involving reaction of the sulfhydryl group with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), followed by spectrophotometric measurement at 412 nm [29].

SOD was measured by the inhibition of adrenaline oxidation, which was adapted from a previous study. Plasma samples were homogenized in glycine buffer. Volumes of 5, 10 and 15 µl of sample were separated after homogenization and 5,0 ml of catalase (0.0024 mg/mL of distilled water), 175–185 mL of glycine buffer (0.75 g in 200 mL of distilled water at 32 ºC, 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 performed for 180 sec at 10 sec intervals and measured on ELISA reader at 480 nm. Values were expressed as unit of SOD per

milligram of protein (U/mg protein) [30]. Catalase activity was determined based on the rate of H_2O_2 decomposition by the enzyme present in the sample using 10 mM H_2O_2 in potassium phosphate buffer, pH 7.0. The maximum H_2O_2 decomposition rate was measured in a spectrophotometer at 240 nm, and values were expressed as catalase units per milligram of protein [31].

Histomorphometry

A portion of the heart was fixed in 10% paraformaldehyde buffered at pH 7.4 for 24 h, followed by dehydration, embedding in paraffin, and cutting into 5,0 µm sections. The sections were stained with Ferric Hematoxylin (to determine the number of cardiomyocytes - n/10^4 µm^2) and Picro Sirius Red (to determine the percentage area of collagen fibers - % in 10^4 µm^2) in order to evaluate the effects of the diabetic condition and the training protocols. Histological sections were photographed in the LEICA®DM-2000 optical microscope with LEICA®DFC-300 FX camera connected to the computer with LAS®software - Leica Application Suite for image capture Measurements were made using the Sigma Scan Pro 5.0 software [32] in triplicate for each animal, with determination of the group means from the sections from each animal.

Statistical analysis

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

Results

Glycemia

Evaluation of the effect of physical the training showed that the training method was effective in decreasing hyperglycemic effects in diabetic animals (Fig. 2A, B).

Analysis of pro and antioxidant activity

Soleus muscle

The soleus muscle showed a higher value of TBARS for the D group, compared to the C and TD groups (Fig. 3A).

In analyze of H_2O_2, the TD group showed greater, compared to T (Fig. 3B), while the production of –SH was higher in D, compared to C group (Fig. 3C).

SOD, showed higher expression in TD group, compared to the D group (Fig. 3D). Catalase was higher in TD, compared to T group (Fig. 3E).

Red gastrocnemius

In the red gastrocnemic muscle, there was not difference between the levels of TBARS, H_2O_2 and –SH groups in the different groups (Fig. 4A-C).

The analysis of SOD, it was greater in TD group, compared to D group (Fig. 4D) and D group was shown decreased compared to the C (Fig. 4D). Catalase was reduced in TD when compared to D (Fig. 4E).

White gastrocnemius

No differences between the experimental groups were observed for the pro and antioxidants white portion of the gastrocnemius muscle (Fig. 5A-E).

Cardiac muscle

The D group showed greater TBARS compared to C and TD groups (Fig. 6A). H_2O_2, Catalase, SOD showed no difference between the experimental groups (Fig. 6B-D).

About Catalase, the TD group showed an increase when compared to D (Fig. 6E).

Cardiomyocytes and collagen fibers

The numbers of cardiomyocytes in D group was lower than others groups (Fig. 7A,C). About area of collagen fiber, TD group showed lower than D (Fig. 7B,D).

Discussion

The swimming protocol exercise used in the present study was effective in decrease the glycemia in alloxan-induced diabetic rats (TD group vs D group) in agreement with previous findings [33]. Blood hyperglycemia leads to advanced glycation end products (AGEs). Studies have found dysfunction induced in endothelial progenitor cells (EPCs), sirtuin-3 (SIRT3) impairment [21], and dysfunction via AMPK/p38/NF-kB causing high OS, such as increased production of ON, which is believed to be a mediator of pancreatic islet damage [21, 34, 35]. Analysis of the pancreas of diabetes-induced rats submitted to eight weeks of moderate- to high-intensity training resulted in a 33% increase in βcells, due to improved glycemic and inflammatory control [34, 36, 37]. Diabetes-induced rats submitted to eight weeks of exercise showed a 21% decrease in hyperglycemia, compared to a group without exercise [38]. The application of exercise resulted in glycemic control and no occurrence of diabetic ketoacidosis, with

greater expression of PGC-1α, compared to control (resting) animals. IL-6, an anti-apoptotic factor that is also involved in redox regulation, has been suggested to act against pro-inflammatory cytokines, providing better control of BCL-2 and BCL-xL [32, 35, 39].

Diabetes is characterized by an increase in ROS, which together with inflammatory cytokines contributes to muscle atrophy, generating a vicious circle of decreased glucose uptake and increased ROS [37, 40]. In agreement with these data, the alloxan-induced diabetes group (D) showed increased production of TBARS and -SH groups in the soleus muscle. On the other hand, the TD group in the same muscle showed improvement of TBARS and -SH groups, and has shown increased SOD, and catalase activity. SOD activity was also increased in the red gastrocnemius muscle. Therefore, our data suggest that the swimming protocol exercise used in the present study was effective in to modulate the oxidative stress associated with alloxan-induced diabetes model.

The results of lipid peroxidation in skeletal muscles showed a balance in pro and antioxidant activity after training. The exercise in DM is associated with improvements in physical ability, endothelial function, and signal transduction, as well as a favorable lipid profile and avoidance of cancer due to lipid peroxidation [41]. Exercise training-induced a decrease of TBARS content (81%) in the soleus muscle from rats and presents an antioxidant effect on skeletal muscle [42]. The TBARS decrease observed with exercise in the diabetics animals in the soleus muscle was comparable to those reported previously using animal and humans models, drug treatments, diet control, and high-intensity intermittent exercise [20].

No elevation of H_2O_2 in gastrocnemius was found for the experimental groups, indicating that predominantly glycolytic musculature (type IIB) was not associated with exacerbation of H_2O_2, as observed previously in a study comparing glycolytic and oxidative fibers [20]. In contrast, the soleus muscle of groups TD showed increases of H_2O_2. It has recently been suggested that at controlled levels, H_2O_2 could stimulate SIRT1, with deacetylation of FOXO-3, hence reducing oxidation and stimulating the expression of SOD, Catalase, and GPx in skeletal and cardiac muscle [20]. High-intensity training has shown good results in terms of redox control, since H_2O_2 does not cause damage directly, but in critical situations can lead to the development of other pathologies such as hyperthyroidism due to elevated OH^- [20].

The antioxidant enzymes SOD, Catalase, and GPx are the main defenses against ROS, especially during physical training [40]. The results obtained in the present work for the soleus musculature showed higher levels of SOD, and Catalase in group TD. Similar elevation of Catalase in the soleus muscle was found in a study using healthy animals exercised at 60% of maximum speed, with a single molecule of Catalase acting to convert millions of molecules of water and oxygen [43, 44]. Deficiency of Catalase has been shown to increase the effects of DM1 and increase the risk of type II diabetes in healthy individuals [45].

Considering the results for the soleus muscle, the aquatic exercise training in this experimental model was effective antioxidants and could have been associated with the increasing time training [36]. The time factor is important in physical training, since the exercise can be adapted, allowing evolution and

better adjustment of the entire organism to the increasing load [7]. Similar results with progressive increase of time were obtained in a study with DM individuals who performed training (above 80% bpm) for 30 min initially and for 40 min in the last week, resulting in increases of SOD and GPX [6].

Studies report that in oxidative part of the gastrocnemius muscle, the SOD enzyme was increased in the hyperglycemic state, as well as with the type 1 high-intensity physical training. In the case of SOD (with lower mitochondrial number), similar results have been reported previously for training at 60% of the maximum velocity in animals without the pathology [33, 46, 47]. It was also found that NOX was higher in the soleus and RG muscles, compared to the WG muscle, which could have been due to lower vascular development in the WG muscle [47].

The cardiac muscle showed an increased expression of catalase enzymes in TD group, corroborating with literature [17], and a decrease in TBARS. In a study with rats submitted to anaerobic training (75% VO_2max 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, together with an increase of reduced glutathione [48]. Physical training has been shown to reverse the IkB/NF-kB pathway in DM1 and DM2 [49]. This could be explained by the activation of PGC-1α, which in addition to increasing antioxidant levels also regulates the expression of VEGF and angiogenesis in skeletal and cardiac muscle cells [49]. In addition to SOD, and CAT produced by the cardiac muscle can lead to vascular improvements [46, 50].

The morphometric analyses evidenced that the cardiac cells were damaged due to hyperglycemia and ROS. A smaller number of cardiomyocytes were found for the D group, which was related to increased fibrosis, representing a loss of cardiac function characteristic of diabetic cardiomyopathy [20]. 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 [20].

In the diabetic individual, endothelial dysfunction is manifested as problems associated with prostaglandins and ON, with non-esterified fatty acids altering the basal membranes of blood vessels and affecting blood flow and the quantities of cardiomyocytes [51]. Moderate-intensity long-term exercise (over 15 weeks) leads to physiological cardiac growth, and in previous work, no significant cardiomyocyte apoptosis or myocardial fibrosis was found in trained C57BL/6 wild-type rats [20, 52].

In terms of the amount of collagen, there was evidence of cellular and interstitial fibrosis in this experimental model, as found elsewhere for animals with diabetes [53]. Reduced myocyte hypertrophy and decreased collagen deposition (fibrosis), evidencing cardiac damage, was observed in Sprague-Dawley rats trained at 80% of their maximum capacity for four weeks, showing that the high-intensity exercise was able to reverse cardiac remodeling in the diabetic heart [52]. In previous work in which diabetes was induced using alloxan and training was performed at above 80% of maximum intensity, positive impacts on cardiac remodeling were found, as evidenced by a reduction in myocyte hypertrophy

and decreased collagen deposition (fibrosis) [52, 54], normalization of the increased levels of collagen type III, and reduced apoptosis and myocardial fibrosis [20].

Swimming exercise results in a significant increase in antioxidant capacity, enabling the use of shorter exercise time in order to encourage adherence to the treatment regime [37, 45]. Antioxidant enzymes should be increased naturally, otherwise, as in animals with over expression (knockout) of SOD, and CAT, a variety of cellular and signaling problems can occur [37, 46].

Conclusion

It could be concluded that there were decreases of the hyperglycemia condition in the groups submitted to exercise. The training protocol involving increasing the volume and decreasing the intensity for four weeks was able to induce oxidative stress balance as well as minimize the fibrosis. These findings characterize the protocol with therapeutic potential for patients with Diabetes.

Declarations

Ethics approval and consent to participate

Animal experimental procedures were in accordance with standards of the Brazilian College of Animal Experimentation (COBEA), Brazilian legislation on the scientific use of animals and approved by the Ethics Committee on Animal Use (CEUA) of Herminio Ometto Foundation – FHO, Araras/SP, Brazil.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

Funding for this project was provided by Hermínio Ometto Foundation – FHO.

Authors' contributions

T.F.P. designed the study, acquisition, and interpretation of data, technical procedures and drafted the manuscript. L.E.O. acquisition and interpretation of data, technical procedures and drafted the manuscript. A.A.A. designed the study, revised the manuscript for important intellectual content. M.A.M.E. performed histological analyses, technical procedures, drafted the manuscript and final approval. T.A.M.A acquisition and interpretation of data, revised the manuscript for important intellectual content, provided the strain analysis program and drafted the manuscript. M.F. acquisition and interpretation of data, revised the manuscript for important intellectual content, provided the strain analysis program and drafted the manuscript. R.A.D. conception and design of the study, manuscript preparation, and critical revision.

Acknowledgements

We thank Renata Barbieri for technical assistance. We thank Jéssica Roberta Mendonça, Rodrigo Martins Pereira and Felipe Christofoletti Hellmeister for technical procedures. We thank also Danielle Bernardes and Gláucia Maria Tech dos Santos for manuscript final revision

References

1. Coleman SK, Rebalka IA, D'Souza DM, Hawke TJ. Skeletal muscle as a therapeutic target for delaying type 1 diabetic complications. World J Diabetes. 2015;17:1323-36.
2. Manna TD, Setian N, Savoldelli RD, Guedes DR, Kuperman H, Menezes HC, Filho L, Steinmetz V, Cominato L, Dichtchekenian , Damiani D . Diabetes mellitus in childhood: an emerging condition in the 21st Rev Assoc Med Bras. 2016;62:594-601.
3. Katsarou A, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, Jacobsen LM, Schatz DA, Lernmark Å. Type 1 diabetes mellitus. Nat Rev Dis Primers. 2017;3:17016.
4. Camiletti-Moirón D, Aparicio VA, Nebot E, Medina G, Martínez R, Kapravelou G, Andrade A, Porres JM, López-Jurado M, Aranda P . High-protein diet induces oxidative stress in rat brain: protective action of high-intensity exercise against lipid peroxidation. Nutr Hosp. 2015;31:866-74.
5. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp L, Guariguata L, Cho NH, Cavan D, Shaw JE, Makaroff LE. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract. 2017;128:40-50.
6. Aro CE, Russell JA, Soto Muñoz ME, Villegas González BE. Effects of high intensity interval training versus moderate intensity continuous training on the reduction of oxidative stress in type 2 diabetic adult patients: CAT. Medwave. 2015;15:e6212.
7. Powers SK, Hogan MC. Exercise and oxidative stress. J Physiol. 2016;594:5079-80.
8. Accattato F, Greco M, Pullano SA, Fiorillo AS, Pujia U, Montalcini T, Foti DP, Brunetti UM, Gulletta E. Effects of acute physical exercise on oxidative stress and inflammatory status in young, sedentary

obese subjects. PLoS One. 2017;12:e0178900.

9. Kaul K, Apostolopolou M, Roden M. Insulin resistance in type 1 diabetes mellitus. Metabolism. 2015;64:1629-39.
10. Hirata Y, Nomura K, Senga Y, Okada Y, Kobayashi K, Okamoto S, Minokoshi Y, Imamura M, Takeda S, Hosooka, Ogawa W. Hyperglycemia induces skeletal muscle atrophy via a WWP1/KLF15 axis. JCI insight. 2019; 4:e124952.
11. Hu J, Klein JD, Du J, Wang XH. Cardiac muscle protein catabolism in diabetes mellitus: activation of the ubiquitin-proteasome system by insulin deficiency. Endocrinology. 2008;149:5384-90.
12. Asa C, Maria S, Katharina SS, Bert A. Aquatic exercise is effective in improving exercise performance in patients with heart failure and type 2 diabetes mellitus. Evid Based Complement Alternat Med. 2012;2012:349209.
13. Lubkowska A, Bryczkowska I, Gutowska I, Rotter I, Marczuk N, Baranowska-Bosiacka I, Banfi G. The effects of swimming training in cold water on antioxidant enzyme activity and lipid peroxidation in erythrocytes of male and female aged rats. Int J Environ Res Public Health. 2019;16:647.
14. Yardley JE, Sigal RJ. Exercise strategies for hypoglycemia prevention in individuals with type 1 diabetes. Diabetes Spectr. 2015;28:32-8.
15. Ji LL, Zhang Y. Antioxidant and anti-inflammatory effects of exercise: role of redox signaling. Free Radic Res. 2014;48:3-11.
16. Hall KE, McDonald MW, Grisé KN, Campos OA, Noble EG, Melling CW. The role of resistance and aerobic exercise training on insulin sensitivity measures in STZ-induced type 1 diabetic rodents. Metabolism. 2013;62:1485-94.
17. Anaruma CP, Ferreira M Jr, Sponton CH, Delbin MA, Zanesco A. Heart rate variability and plasma biomarkers in patients with type 1 diabetes mellitus: Effect of a bout of aerobic exercise. Diabetes Res Clin Pract. 2016;111:19-27.
18. Turksoy K, Paulino TM, Zaharieva DP, Yavelberg L, Jamnik V, Riddell MC, Cinar A. Classification of physical activity: information to artificial pancreas control systems in real time. J Diabetes Sci Technol. 2015,9:1200-7.
19. Ji DK, Zhang Y, Zang Y, J Li, Chen GR, Ele XP, Tian H. Targeted intracellular production of reactive oxygen species by a 2d molybdenum disulfide glycosheet. Adv Mater. 2016;28:9356-63.
20. Wang H, Bei Y, Lu Y, Sun W, Liu Q, Wang Y, Cao Y, Chen P, Xiao J, Kong X. Exercise prevents cardiac injury and improves mitochondrial biogenesis in advanced diabetic cardiomyopathy with PGC-1α and Akt activation. Cell Physiol Biochem. 2015;35:2159-68.
21. He X, Zeng H, Chen JX. Ablation of SIRT3 causes coronary microvascular dysfunction and impairs cardiac recovery post myocardial ischemia. Int J Cardiol. 2016;215:349-57.
22. Amaral MEC, Ribeiro RA, Vanzela CE, Barbosa-Sampaio HC. Reduced AMPKα2 protein expression restores glucose-induced insulin secretion in islets from calorie-restricted rats. J. Exp. Pathol. 2016;97:50-5.

23. Lenzen S. Alloxan and streptozotocin diabetes, time structures of endocrine systems project framework. Endocrinology. 2007;8:119-38.
24. Araújo MB, Vieira Junior RC, Moura LP, Costa Junior M, Dalia RA, Sponton ACS, Ribeiro C, Mello MAR. Influence of creatine supplementation on indicators of glucose metabolism in skeletal muscle of exercised rats. Motriz: rev. educ. fis. 2013;19:709-16.
25. Rhea MR, Phillips WT, Burkett LN, Stone WJ, Ball SD, Alvar BA, Thomas AB. A comparison of linear and daily undulating periodized programs with equated volume and intensity for local muscular endurance. J Strength Cond Res. 2003;17:82-7.
26. Cunha VNC, Cunha RR, Russo Segundo P, Moreira SR, Simões HG. Treinamento de natação na intensidade do limiar anaeróbio melhora a aptidão funcional de ratos idosos. Rev Bras Med Esporte. 2008;14:533-38.
27. Le Floch JP, Escuyer P, Baudin E, Baudon D, Perlemuter L. Blood glucose area under the curve: methodological aspects. Diabetes Care. 1990;13:172-5.
28. Ramos-Filho D, Chicaybam G, de-Souza-Ferreira E, Guerra Martinez C, Kurtenbach E, Casimiro-Lopes G, Galina A. High Intensity Interval Training (HIIT) induces specific changes in respiration and electron leakage in the mitochondria of different rat skeletal muscles. PLoS One. 2015;10:e0131766.
29. Mataix J, Quiles JL, Huertas JR, Battino H, Mañas M. Tissue specific interactions of exercise, dietary fatty acids, and vitamin E in lipid peroxidation. Free Radic Biol Med. 1998;24:511–21.
30. Bannister JV, Calabrese L. Assays for superoxide dismutase. Methods Biochem Anal. 1987;32:279-312
31. Aebi H. Catalase *in vitro*. Methods Enzymol. 1984;105:121-6.
32. Relive M, Mukhopadhyay P, Bátkai S, Hasko L, Liaudet G, Huffman JW, Csiszar U , Ungvari Z , Mackie K , Chatterjee S , Pacher P .CB2-receptor stimulation attenuates TNF-alpha-induced human endothelial cell activation, transendothelial migration of monocytes, and monocyte-endothelial adhesion. Am J Physiol Heart Circ Physiol. 2007;293:2210-8.
33. Lee Y, Kim JH, Hong Y, Lee SR, Chang KT, Hong Y. Prophylactic effects of swimming exercise on autophagy-induced muscle atrophy in diabetic rats. Lab Anim Res. 2012;28:171-9.
34. Lascar N, Kennedy A, Jackson N, et al. Exercise to preserve beta cell function in recent-onset type 1 diabetes mellitus (EXTOD)--a study protocol for a pilot randomized controlled trial. Trials. 2013;14:180.
35. Paula FM, Leite NC, Vanzela EC, Kurauti MA, Freitas-Dias R, Carneiro EM, Boschero AC, Zoppi CC. Exercise increases pancreatic β-cell viability in a model of type 1 diabetes through IL-6 signaling. FASEB J. 2015;29:1805-16.
36. Golbidi S, Badran M, Laher I. Antioxidant and anti-inflammatory effects of exercise in diabetic patients. Exp Diabetes Res. 2012;2012:941868.
37. Moura LP, Bertolini NO, Ghezzi AC, Bertucci DR, Bonfim MR, Serafim THS, Pereira AS, Garuffi M, Mello MAR, Luciano E. Glucose homeostasis in type 1 diabetic rats after acute physical activity. JEPonline. 2011;14:8-19.

38. Kim JS, Lee YH, Kim JC, Ko YH, Yoon CS, Yi HK. Effect of exercise training of different intensities on anti-inflammatory reaction in streptozotocin-induced diabetic rats. Biol Sport. 2014;31:73-9.
39. Colberg SR, Laan R, Dassau E, Kerr D. Physical activity and type 1 diabetes: time for a rewire? J Diabetes Sci Technol. 2015;9:609-18.
40. Marcinko K, Steimberg GR. The role of AMPK in controlling metabolism and mitochondrial biogenesis during exercise. Exp Physiol. 2014;12:1581-5.
41. Fatima N, Faisal SM, Zubair S, Ajmal M, Siddiqui SS, Moin S, Owais M. Role of pro-inflammatory cytokines and biochemical markers in the pathogenesis of type 1 diabetes: correlation with age and glycemic condition in diabetic human subjects. PLoS One. 2016;11:e0161548.
42. Lambertucci RH, Levada-Pires AC, Rossoni LV, Curi R, Pithon-Curi TC. Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats. Mech Ageing Dev. 2007;128(3):267-75.
43. de Araujo GG, Papoti M, Dos Reis IG, de Mello MA, Gobatto CA. Short and long term effects of high-intensity interval training on hormones, metabolites, antioxidant system, glycogen concentration, and aerobic performance adaptations in rats. Front Physiol. 2016;7:505.
44. Gomes EC, Silva AN, de Oliveira MR. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. Oxid Med Cell Longev. 2012;2012:756132.
45. Lawler JM, Rodriguez DA, Hord JM. Mitochondria in the middle: exercise preconditioning protection of striated muscle. J Physiol. 2016;594:5161-83.
46. Lei XG, Zhu JH, Cheng WH, Bao Y, Ho YS, Reddi AR, Holmgren A, Arnér ES. Paradoxical roles of antioxidant enzymes: basic mechanisms and health implications. Physiol Rev. 2016;96:307-64.
47. Loureiro AC, do Rêgo-Monteiro IC, Louzada RA, Ortenzi VH, de Aguiar AP, de Abreu ES, Cavalcanti-de-Albuquerque JP, Hecht F, de Oliveira AC, Ceccatto VM, Fortunato RS, Carvalho DP. Differential expression of NADPH oxidases depends on skeletal muscle fiber type in rats. Oxid Med Cell Longev. 2016;2016:6738701.
48. Gonchar O. Effect of intermittent hypoxia on pro- and antioxidant balance in rat heart during high-intensity chronic exercise. Acta Physiol Hung. 2005;92:211-20.
49. He F, Li J, Liu Z, Chuang CC, Yang W, Zuo L. Redox Mechanism of Reactive Oxygen Species in Exercise. Front Physiol. 2016;7:486.
50. Naderi R, Mohaddes G, Mohammadi M, Ghaznavi R, Ghyasi R, Vatankhah AM. Voluntary exercise protects heart from oxidative stress in diabetic rats. Adv Pharm Bull. 2015;5:231-36.
51. Konior A, Schramm A, Czesnikiewicz-Guzik M, Guzik TJ. NADPH oxidases in vascular pathology. Antioxid Redox Signal. 2014;20:2794-814.
52. Novoa U, Arauna D, Moran M, Nuñez M, Zagmutt S, Saldivia S, Valdes C, Villaseñor J, Zambrano CG, Gonzalez DR. High-intensity exercise reduces cardiac fibrosis and hypertrophy but does not restore the nitroso-redox imbalance in diabetic cardiomyopathy. Oxid Med Cell Longev. 2017;2017:7921363.

53. Chu PY, Walder K, Horlock D, Williams D, Nelson E, Byrne M, Jandeleit-Dahm K, Zimmet P, Kaye DM. CXCR4 antagonism attenuates the development of diabetic cardiac fibrosis. PLoS One. 2015;10:e0133616.
54. Haghani K, Bakhtiyari S, Doost Mohammadpour J. Alterations in plasma glucose and cardiac antioxidant enzymes activity in streptozotocin-induced diabetic rats: effects of trigonella foenum-graecum extract and swimming training. Can J Diabetes. 2016;40:135-42.

Figures

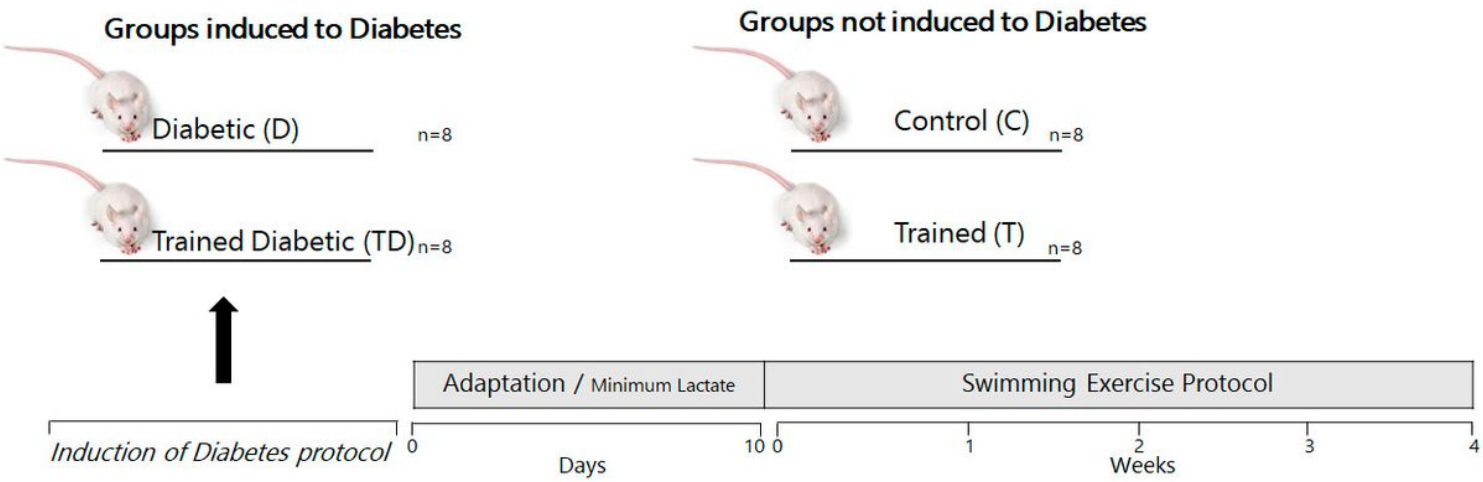

Figure 1

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

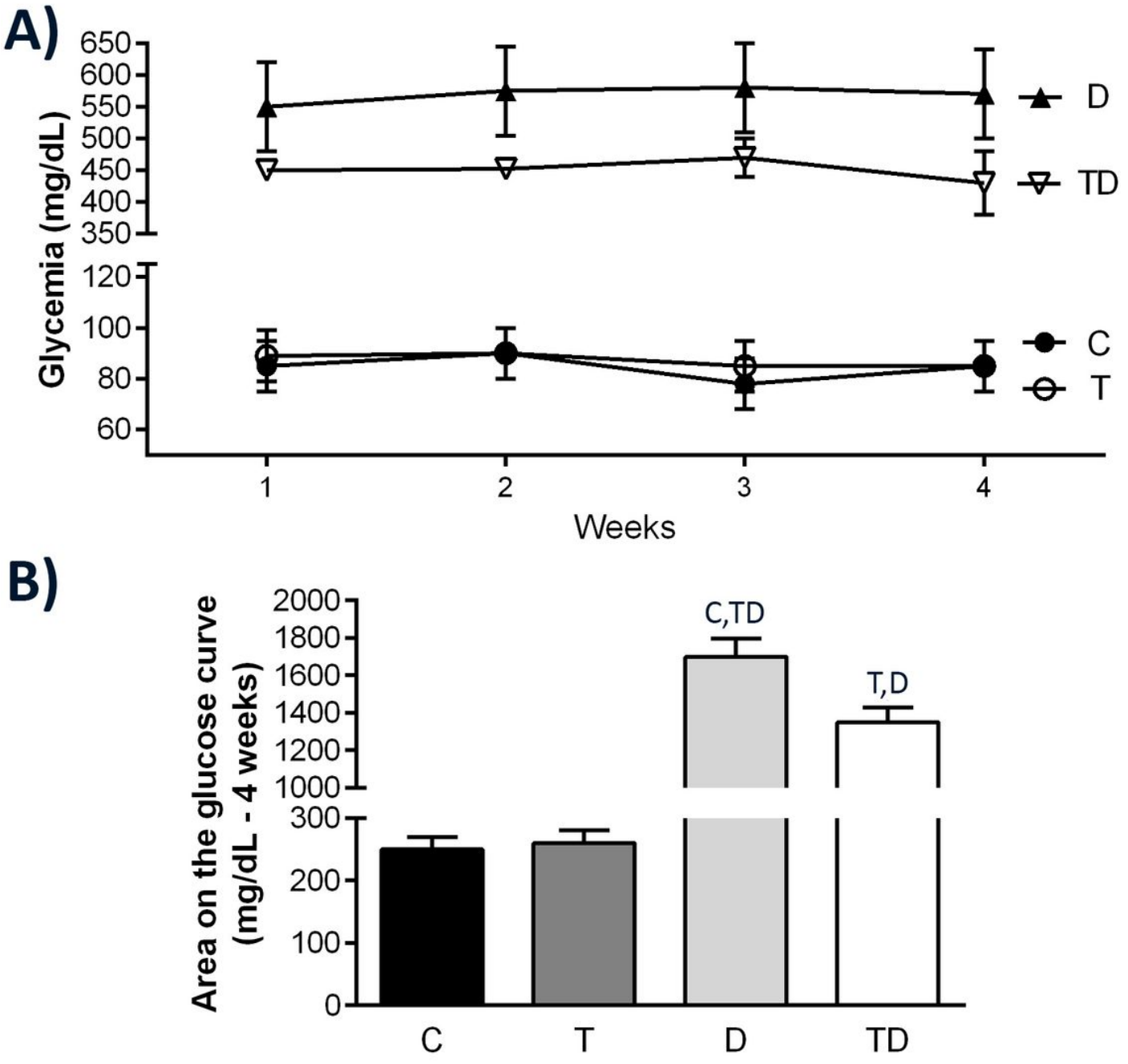

Figure 2

Glycemia at the end of each training week. (A) representative graph of the weekly glycemia of the animals throughout the experimental period. (B) Area over the weekly glycemia curve of the animals over the experimental period. Results expressed as mean ± standard deviation of the mean. Untrained control (C); trained control protocol (T); untrained diabetic (D); trained diabetic protocol (TD) (n=8 rats/group). a: $p<0.05$ in relation to C group. b: $p<0.05$ in relation to T group. c: $p<0.05$ in relation to D group. d: $p<0.05$ in relation to TD groups (ANOVA Two-way/Tukey post-test).

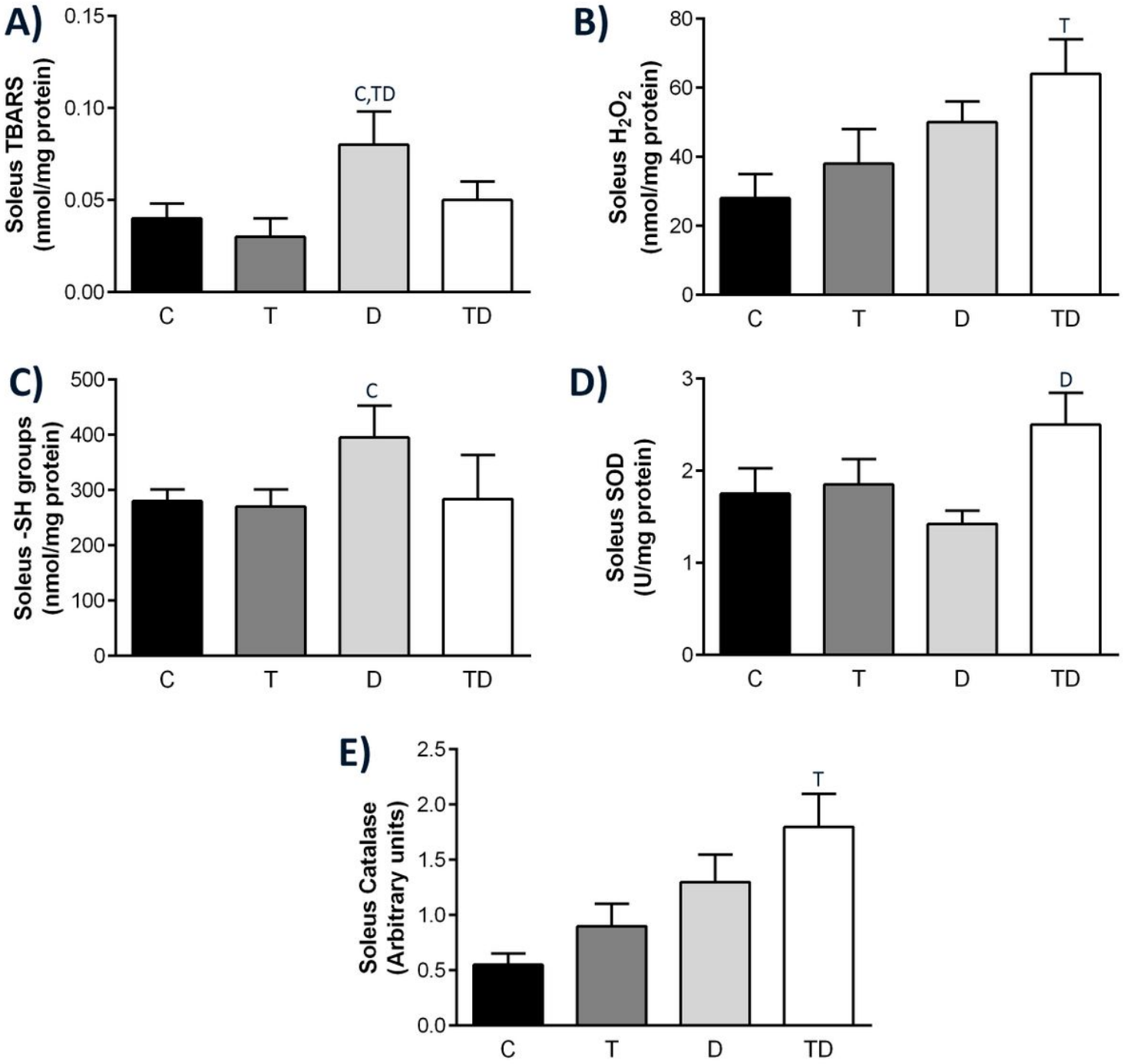

Figure 3

Soleus muscle analysis of (A) Thiobarbituric Acid (TBARS), Hydrogen Peroxide (H2O2), (C) Sulfhydryl Groups (-SH) (D) Superoxide Dismutase (SOD) and Catalase . Untrained control (C); trained control protocol (T); untrained diabetic (D); trained diabetic protocol (TD) (n=8 rats/group). a: $p<0.05$ in relation to C group. b: $p<0.05$ in relation to T group. c: $p<0.05$ in relation to D group. d: $p<0.05$ in relation to TD groups (ANOVA Two-way/Tukey post-test).

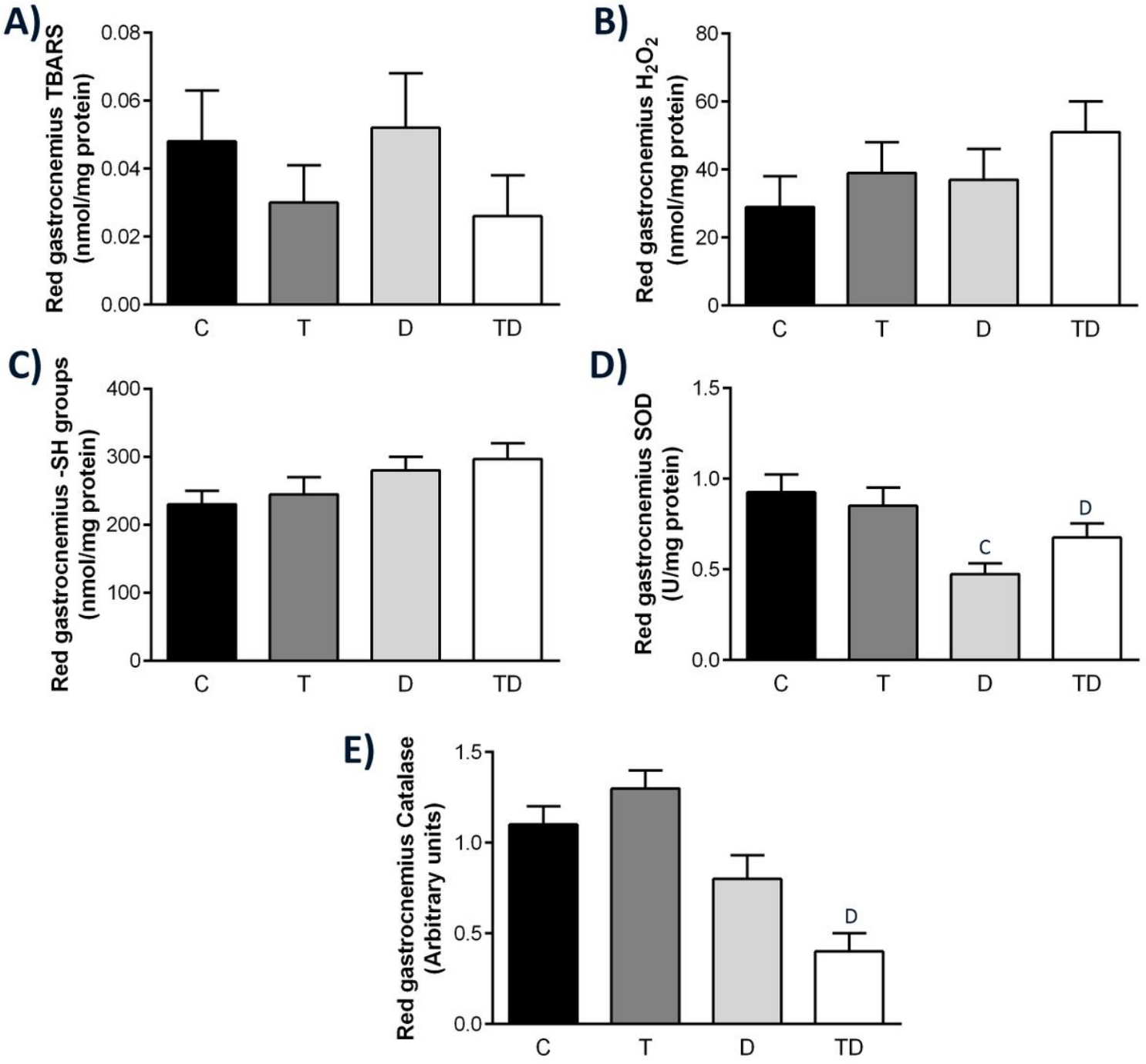

Figure 4

Red gastrocnemius analysis of (A) Thiobarbituric Acid (TBARS), Hydrogen Peroxide (H2O2), (C) Sulfhydryl Groups (-SH) (D) Superoxide Dismutase (SOD) and Catalase. Untrained control (C); trained control protocol (T); untrained diabetic (D); trained diabetic protocol (TD) (n=8 rats/group). a: $p<0.05$ in relation to C group. b: $p<0.05$ in relation to T group. c: $p<0.05$ in relation to D group. d: $p<0.05$ in relation to TD groups (ANOVA Two-way/Tukey post-test).

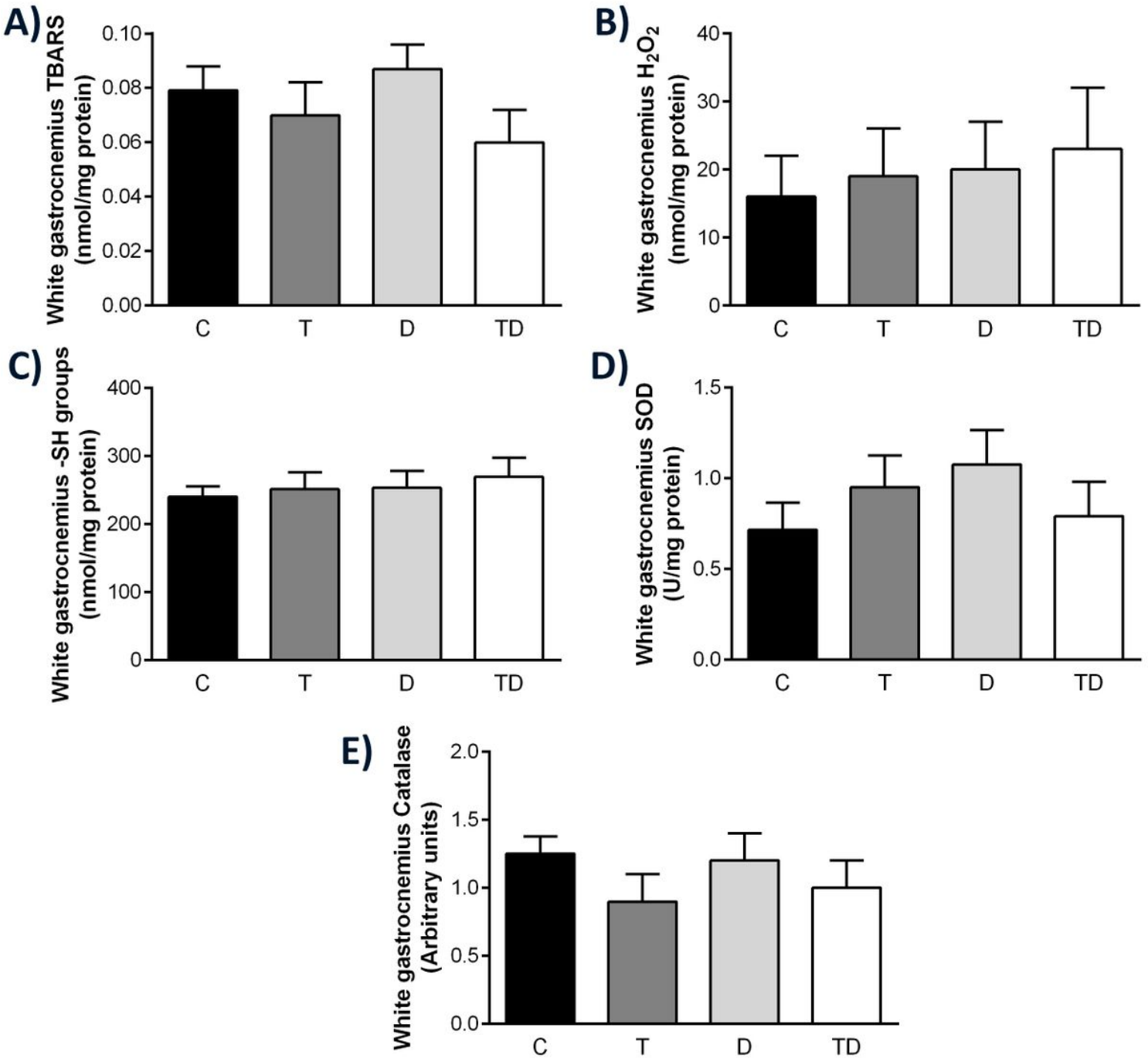

Figure 5

White gastrocnemius analysis of (A) Thiobarbituric Acid (TBARS), Hydrogen Peroxide (H2O2), (C) Sulfhydryl Groups (-SH) (D) Superoxide Dismutase (SOD) and Catalase. Untrained control (C); trained control protocol (T); untrained diabetic (D); trained diabetic protocol (TD) (n=8 rats/group). a: $p<0.05$ in relation to C group. b: $p<0.05$ in relation to T group. c: $p<0.05$ in relation to D group. d: $p<0.05$ in relation to TD groups (ANOVA Two-way/Tukey post-test).

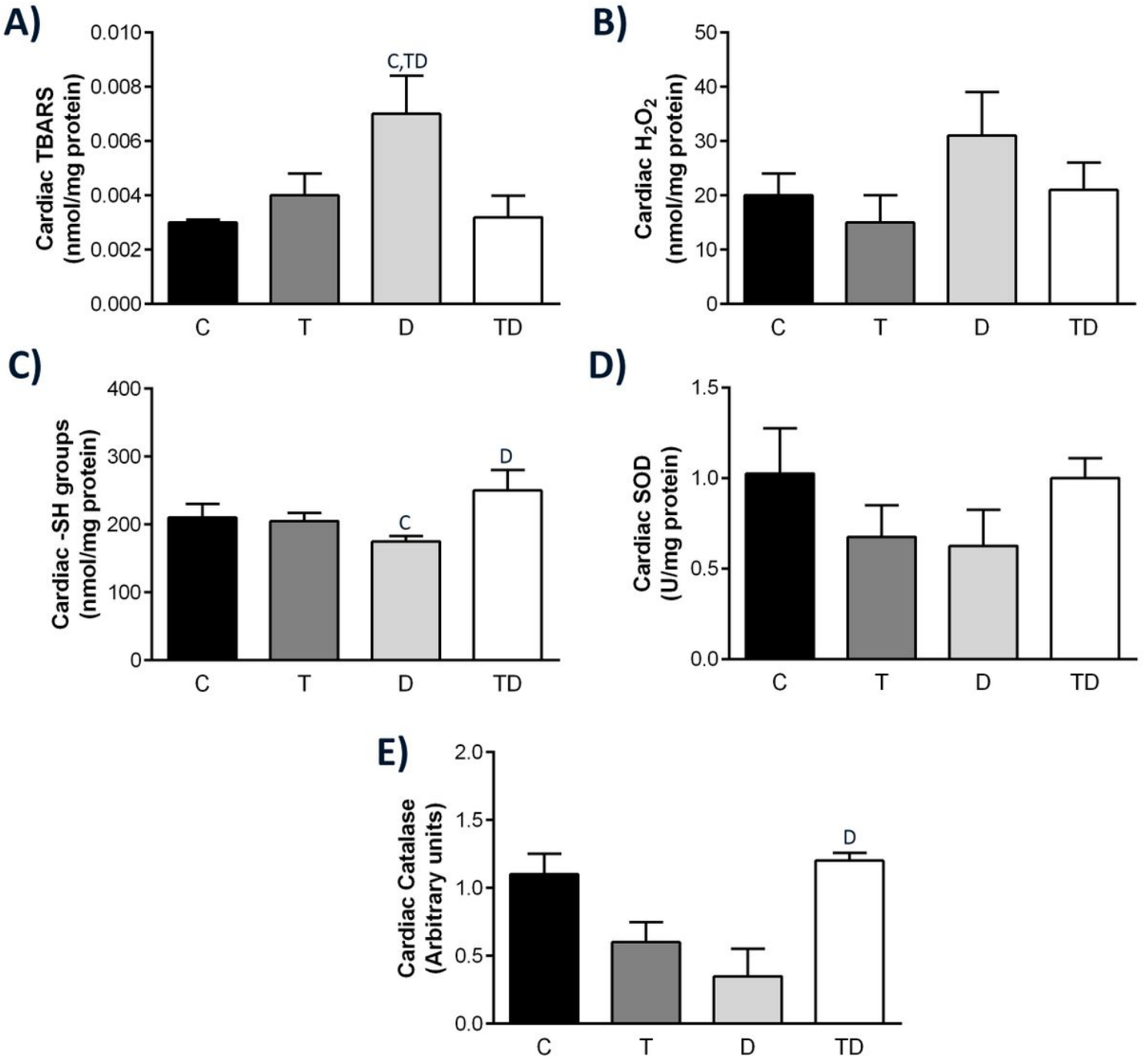

Figure 6

Cardiac analysis of (A) Thiobarbituric Acid (TBARS), Hydrogen Peroxide (H2O2), (C) Sulfhydryl Groups (-SH) (D) Superoxide Dismutase (SOD) and Catalase. Untrained control (C); trained control protocol (T); untrained diabetic (D); trained diabetic protocol (TD) (n=8 rats/group). a: $p<0.05$ in relation to C group. b: $p<0.05$ in relation to T group. c: $p<0.05$ in relation to D group. d: $p<0.05$ in relation to TD groups (ANOVA Two-way/Tukey post-test).

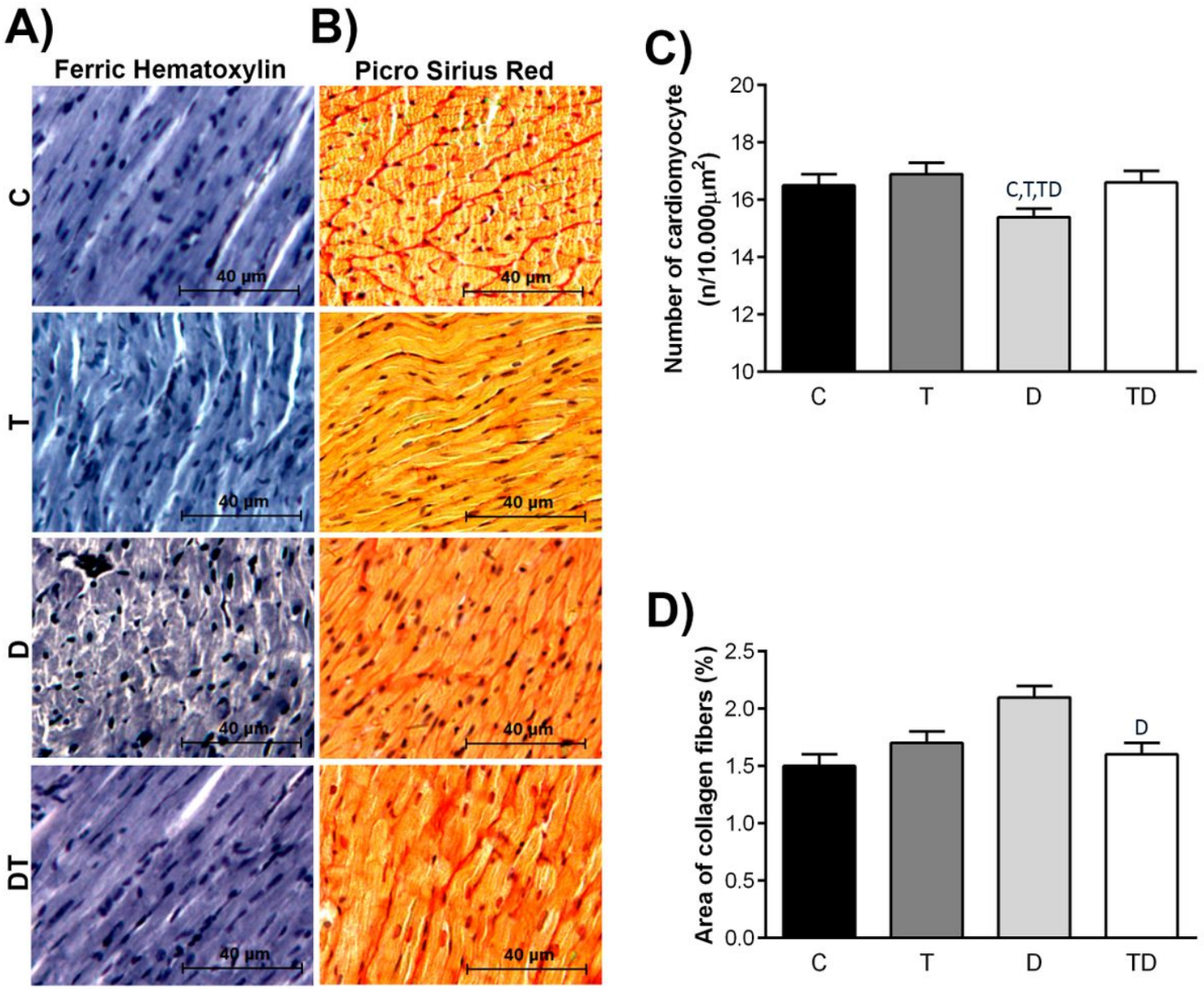

Figure 7

Histomorphometry of cardiac muscle to evaluate (A,C) the number of cardiomyocyte from Ferric hematoxylin stained (B,D) total percentage of the Collagen Fiber area from Picro Sirus Red stained. Results expressed as mean ± standard deviation of the mean, n = 3 animals per group. Untrained control (C); trained control protocol (T); untrained diabetic (D); trained diabetic protocol (TD) (n=8 rats/group). a: $p<0.05$ in relation to C group. b: $p<0.05$ in relation to T group. c: $p<0.05$ in relation to D group. d: $p<0.05$ in relation to TD groups (ANOVA Two-way/Tukey post-test).