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Original Article

Swimming training attenuates the morphological reorganization of the myocardium and local inflammation in the left ventricle of growing rats with untreated experimental diabetes

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ABSTRACT

Diabetic cardiomyopathy is associated with cardiac remodeling, myocardial dysfunction, low-grade inflammation, and reduced cardiac adiponectin in patients with type 1 diabetes mellitus (T1DM). Alternatively, physical exercise is an important strategy for the management of diabetes. This study aimed to investigate the influence of low-intensity swimming training in cardiac cytokines, structural remodeling, and cardiomyocyte contractile dysfunction in growing rats with untreated experimental DM. Thirty-day-old male Wistar rats were divided into four groups ($n = 14$, per group): sedentary control (SC), exercised control (EC), sedentary diabetic (SD), and exercised diabetic (ED). Diabetes was induced by streptozotocin (60 mg kg^{-1}, i.p.). Animals from exercised groups swam (5 days/week, 90 min/day, loading up to 5% body weight around the animal's chest) for 8 weeks. The left ventricle (LV) was removed for molecular, morphological, and cardiomyocyte mechanical analysis. Diabetic animals presented cardiac remodeling with myocardial histoarchitectural disorganization, fibrosis, and necrosis. The capillary density was lower in diabetic animals. LV cardiomyocytes from diabetic animals exhibited more prolonged time to the peak of contraction and time to half relaxation than those from control animals. The cardiac levels of interleukin 10, nitric oxide, and total and high molecular weight (HMW) adiponectin were significantly decreased in diabetic animals. Exercise training reduced the level of TNF-α, increased capillary density, and attenuated the histopathological parameters assessed in diabetic rats. In conclusion, the cardiac structural remodeling coexists with reduced levels of total and HMW adiponectin, inflammation, and cardiomyocyte contractility dysfunction in experimental DM. More important, low-intensity swimming training attenuates part of these pathological changes, indicating the beneficial role for exercise in untreated T1DM.

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Abbreviations: BG, blood glucose; BW, body weight; CaMKII, Calcium/calmodulin-dependent protein kinase II; CRP, C-reactive protein; CVD, cardiovascular disease; DM, diabetes mellitus; EC, exercise control; ECG, electrocardiogram; ED, exercise diabetic; eNOS, endothelial nitric oxide synthase; GLUT, glucose transporter; HMW, high molecular weight; HR, heart rate; ICAM-1, intercellular adhesion molecule 1; IL-, interleukin; LV, left ventricle; NCX, Na/Ca exchange; NO, nitric oxide; PLB, phospholamban; RyR2, Ryanodine receptor 2; SC, sedentary control; SD, sedentary diabetic; SEM, standard error of the mean; SERCA2, sarcoplasmic reticulum Ca 2+ ATPase; STZ, streptozotocin; T1DM, type 1 diabetes mellitus; TNF-α, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule-1; VW, ventricular weight.

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1. Introduction

Cardiovascular disease (CVD) is one of the major complications of diabetes mellitus (DM) that commences in childhood [1] and greatly affects mortality and morbidity [2,3].

Type 1 DM (T1DM) is primarily a disease of insulin deficiency from pancreatic beta-cell destruction [4], and CVD may be linked to insulin resistance in this form of diabetes [5,6]. The chronic hyperglycemia has been related to low-grade inflammation and micro- and macrovascular complications in adults [7] and children with DM [8]. Moreover, low-grade inflammation has been associated with an increase in pro-inflammatory circulating proteins, such as tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), and C-reactive protein, and a decrease in the anti-inflammatory proteins, such as IL-10 [7,9].

Adiponectin majorly affects the pathogenesis of insulin resistance, diabetes, and vascular injury [10,11] and plays an important role in glucose and lipid metabolism [6,12]. Adiponectin is a plasma protein mainly secreted by adipocytes [13], with antidiabetic, anti-inflammatory, and antiatherogenic properties [12,14]. Moreover, cardiomyocytes are also capable of synthesizing adiponectin [15,16]. Studies suggest that adiponectin, in particular, the high molecular weight (HMW) adiponectin isoform [17], is a potent immunomodulatory and cardioprotective molecule [18,19]. In fact, this protein protects the heart from ischemic injury, cardiomyopathy, and systolic dysfunction [20]; inhibits pathological cardiomyocyte hypertrophy and myocardial fibrosis [16,21]; reduces oxidative and nitrative stress [20,22]; reduces TNF-α and IL-6; and increases the expression of IL-10 in the heart [16,20,21].

Adiponectin improves cardiomyocyte contractile function in db/db diabetic obese, possibly by alleviating endoplasmic reticulum stress [23]. In addition, in diabetes, endothelial nitric oxide synthase (eNOS) protein expression is progressively reduced in the myocardium, and nitric oxide (NO) content is decreased [22,24].

Regular physical exercise is an important strategy for the management of DM because of beneficial health effects, especially CVD prevention [25]. Aerobic exercise training decreases inflammation and cardiovascular risks [25,26], reduces insulin resistance, improves glucose and lipid metabolism [27], and attenuates morphological changes [28] and contractile dysfunction in both animals and human with DM [3,29,30].

The regulation of glucose or lipid metabolism by adiponectin through exercise training has been investigated [12,13]; however, inconsistent findings have emerged, mainly on total circulating and HMW adiponectin levels [31].

So far, few studies have examined the relationship of factors influencing adiponectin levels in children and adolescents with DM, and the results of those studies are very inconsistent [32–34]. Recent studies showed a decrease in the cardiac adiponectin expression in streptozotocin (STZ)-induced diabetic rats [35]. However, the relationship between cardiac adiponectin in growing rats with DM and the effects of a low-intensity swimming training has not been investigated yet.

Therefore, we hypothesized that low-intensity swimming training can reduce the effects of STZ-induced diabetes on the cardiac histopathology, cytokines, NO, capillary density, and cardiomyocyte contractile dysfunction.

2. Materials and methods

2.1. Animals and experimental groups

Male Wistar rats weighing 90.0 ± 5.0 g, 30 days old, were obtained from the animal facility at the Federal University of Viçosa, Brazil, and were randomly divided into four groups (n = 14, per group): sedentary control (SC), exercised control (EC), sedentary diabetic (SD), and exercised diabetic (ED). Rats were maintained on 12 h dark/12 h light cycle at 22 °C, humidity at 60%–70%, housed in groups of five, and fed standard commercial rodent chow and water ad libitum. All procedures followed the Guidelines for Ethical Care of Experimental Animals and were approved by the Ethics Committee on Animal Experimentation (CEUA) from the Federal University of Viçosa (protocol number 46/2011).

2.2. Diabetes induction

Severe diabetes was induced in the animals by the intraperitoneal injection of STZ (Sigma–Aldrich, St. Louis, MO) dissolved in 0.1 M citrate buffer solution (0.1 M, pH 4.5) at a dose of 60 mg kg^{-1} body weight (BW) [36]. Equivalent volume (1 mL kg^{-1}) of vehicle was injected into the rats assigned to the control groups. Animals were fasted overnight for 12 h before STZ administration. Water and food were available immediately after dosing. Diabetes was determined 7 days after STZ injection. Glycemia (>300 mg dL^{-1}) [37] was dosed using the Accu-Chek Advantage glucometer (Boehringer Mannheim Corporation, Indianapolis, IN) after a fasting period of 12 h overnight. Body weights and blood glucose levels were recorded once a week throughout the study. All animals were euthanatized 8 weeks after diabetes induction by intraperitoneal injection of sodium pentobarbital (120 mg kg^{-1}).

2.3. Exercise protocol

After 7 days of diabetes induction and confirmation of consistent hyperglycemia, animals from the exercised groups (ED and EC) were submitted to a swimming training program (adapted from Gomes et al., 2006 [38]) for 8 weeks. The water tank, measuring 65 cm in height and 75 cm in diameter, was filled with warm water (28 °C to 30 °C) at a depth of 45 cm. Rats were placed in the tank and forced to swim. Animals were then dried and returned to the home cage. Training intensity varied by changing the load that was placed around the animal's chest from 0% to 5% of its BW. Briefly, in the first week, animals swam for 10 to 50 min, with no load, while duration was increased by 10 min/day. In the second week, animals remained exercising with no load and with the duration incremented by 10 min/day until a maximum of 90 min of continuous swimming. From the fourth week, animals began swimming with a load until the end of the training program (8 weeks). The load was progressively increased by 1% of the animal's BW from the fourth week on, such that at the eighth week, animals swam with a total load of 5% of their BW. During the swimming sessions, animals from the sedentary group (SD and SC) were placed in a polypropylene box containing warm water (28 °C to 30 °C) with a depth of 10 cm.

2.4. Electrocardiogram

Four animals per group randomly selected were anesthetized in an induction chamber flushed with 2% isoflurane and 100% oxygen at a constant flow of 1 L min^{--}. Once unconscious, they were placed on a platform in dorsal recumbency, with the four limbs fixed. Isoflurane was maintained at a concentration sufficient for restrain (0.5% to 1.0%), and animals were able to maintain spontaneous breathing during the electrocardiogram (ECG). A trichotomy of approximate 1 cm^2 was performed in the forelimbs and left hindlimb for electrode insertion. Derivation II of the ECG was recorded using the data acquisition system PowerLab (AD Instruments, São Paulo, Brazil), and data analysis was done with the program LabChart Pro (ADInstruments LabChart 7, São Paulo, Brazil). ECGs were performed at the end of the experiments by

an experienced fellow blind to the study groups and treatments. Resting heart rate (HR) was derived from the ECG, following established guidelines [39].

2.5. Histological processing and histochemistry

After euthanasia, the heart of five animals per group randomly selected was excised, washed with saline solution, dissected, and weighed separately. The left ventricle (LV) was dissected and weighed separately. Half of the LV tissue was rapidly frozen in liquid nitrogen and stored at −80 °C for subsequent assay, and the other half of the LV was fixed by immersion in 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2–7.4, for 24 h. LV fragments were obtained through the orientator method to define the isotropic and uniform random sections required in the stereological study [40]. These fragments were dehydrated in ethanol, cleared in xylol, and embedded in paraffin or glycolmethacrylate resin (Historesin, Leica). Blocks were cut into 5 or 2 μm sections and mounted on histological slides. The LV sections were stained by Sirius red (for total collagen), Gomori's trichrome (for microcirculation), and toluidine blue/sodium borate 1% to evaluate the series of histopathological changes in the diabetic myocardium. Digital images were captured using a light microscope (mod. Primo Star; Carl Zeiss AG, Oberkochen, Germany) connected to a digital camera (AxioCam ERc5s; Carl Zeiss AG).

2.6. Analysis of myocardial microcirculation

To quantify intramyocardial capillaries, LV was evaluated in histological sections 5 μm thick stained with Gomori's trichrome. The distribution of capillary density was analyzed using the image analysis software Image Pro-Plus 4.5 (Media Cybernetics, Silver Spring, MD, USA). In this analysis, 12 microscopic fields from five rats per group were randomly investigated (magnification × 400). A 208-point grid was superimposed on each image, and the total of 2496 points were assigned for each rat. The area was determined by adding these points then dividing the result by the total points for the cross section. Results were expressed as mean ± SEM.

2.7. Sirius red and toluidine blue/sodium borate staining

The pathological analysis of the LV was performed using toluidine blue/sodium borate 1% and Sirius red stain. In this analysis, 12 microscopic fields from five rats per group were randomly investigated (magnification × 400). Tissue sections of LV were stained with Sirius Red for the detection of collagen accumulation, perivascular fibrosis and interstitial fibrosis, and toluidine blue for general pathological alterations.

2.8. Analysis of myocardial collagen

To quantify interstitial fibrosis, LV was evaluated in histological sections 5 μm thick stained with Sirius Red (Sirius Red F3B, Mobay Chemical Co., Union, NJ, USA). The distribution of total collagen content was analyzed using the image analysis software Image Pro-Plus 4.5® (Media Cybernetics, Silver Spring, MD, USA). In this analysis, 12 microscopic fields from five rats per group were randomly investigated (magnification × 400). A 208-point grid was superimposed on each image, and the total of 2496 points was assigned for each rat. The area was determined by adding up these points, then dividing the result by the total points for the cross-section. Results were expressed as mean ± SEM.

2.9. Enzyme-linked immunosorbent assay-based cytokine detection assay

The levels of IL-10, TNF-α, adiponectin, and HMW adiponectin were measured from the LV homogenate by enzyme-linked immunosorbent assay (ELISA). Cytokines IL-10, TNF-α, and adiponectin were assayed using USCN Life Science Inc. (Wuhan, China) kits and HMW adiponectin from BioVendor Co. (Heidelberg, German). The ELISA procedure was performed according to the manufacturer's protocol. The cytokine concentrations were determined with reference to a standard curve for serial twofold dilutions of the rat recombinant cytokines. The inflammatory balance was calculated from the ratio of the pro-inflammatory cytokine TNF-α with anti-inflammatory cytokine IL-10, provides important information about the state of inflammation.

2.10. NO production

NO production was quantified by the standard Griess reaction [41]. Briefly, 50 μl of supernatants from the LV homogenates was incubated with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthalene diamine dihydrochloride, and 2.5% phosphoric acid) at room temperature for 10 min. The absorbance was measured at 550 nm in a microplate scanning spectrophotometer (Power Wave X; Bio-Tek Instruments, Inc., Winooski, VT). The conversion of absorbance into micromolar concentrations of NO was deduced from a standard curve using a known concentration of sodium nitrite.

2.11. Cardiomyocyte isolation

Nine animals from each group were used to cardiomyocyte contractile function measurement. Two days after the last training session, the rats were weighed and euthanized by cervical dislocation under resting conditions, and their hearts were quickly removed. Left ventricular myocytes were enzymatically isolated as previously described [42]. Briefly, the hearts were mounted on a Langendorff system and perfused for approximately 5 min, with a modified HEPES-Tyrode solution of the following composition (in mM): 130 NaCl, 1.43 $MgCl_2$, 5.4 KCl, 0.75 $CaCl_2$, 5.0 HEPES, 10.0 glucose, 20.0 taurine, and 10.0 creatine, pH 7.3 at 37 °C. The perfusion solution was changed for the calcium-free solution with ethyleneglycotetraacetic acid (0.1 mM) for 6 min. Afterward, the hearts were perfused for 15–20 min with a solution containing 1 mg mL^{-1} collagenase type II (Worthington, USA). The digested heart was then removed from the cannula. The LV was separated, weighed, and cut into small pieces. The LV fragments were placed into small conical flasks with collagenase-containing solution supplemented with 1% bovine serum albumin. The cells were dispersed by agitating the flasks at 37 °C for periods of 5 min. Then, single cells were separated from the nondispersed tissue by filtration. The resulting cell suspension was centrifuged and resuspended in a HEPES-Tyrode solution. Nondispersed tissue was subjected to further enzyme treatment. The isolated cells were stored at 5 °C until use. Only calcium-tolerant, quiescent, rod-shaped cardiomyocytes showing clear cross striations were studied. The isolated cardiomyocytes were used within 2–3 h of isolation.

2.12. Measurements of cell contractility

Cell contractility was evaluated as previously described [43]. Briefly, isolated cells were placed in a chamber with a glass coverslip base mounted on the stage of an inverted microscope (Nikon Eclipse-TS100, USA). The chamber was perfused with HEPES-Tyrode solution at 37 °C. Steady-state 1 Hz contractions were elicited via platinum bath electrodes (Myopacer, Field Stimulator;

Table 1
Effect of swimming training on biometric and functional parameters of control and diabetic rats.

Parameters	SC	EC	SD	ED
Baseline BW, g	90.80 ± 3.37	89.40 ± 5.01	86.20 ± 5.67	91.20 ± 6.87
Final BW, g	320.60 ± 26.05	334.60 ± 13.57	164.60 ± 5.89[a]	170.60 ± 5.18[b]
WG, g	229.80 ± 25.42	245.20 ± 13.17	75.20 ± 7.69[a]	79.40 ± 4.76[b]
VW, mg	339.20 ± 17.45	379.20 ± 19.09	211.80 ± 6.67[a]	222.40 ± 4.04[b]
VW/BW, mg g^{-1}	1.06 ± 0.30	1.13 ± 0.63	1.29 ± 0.51[a]	1.30 ± 0.35[b]
Baseline BG, mg dL^{-1}	73.20 ± 5.35	68.20 ± 5.47	74.20 ± 4.33	73.60 ± 8.54
BG Post-STZ, mg dL^{-1}	72.60 ± 3.31	65.40 ± 2.48	429.20 ± 33.65[a]	461.18 ± 11.07[b]
Final BG, mg dL^{-1}	98.20 ± 7.93	89.80 ± 2.71	567.80 ± 19.78[a]	533.00 ± 6.25[b]
Resting HR, bmp	356.40 ± 7.39	300.50 ± 20.44[a]	262.98 ± 7.39[a]	274.35 ± 20.53

Data are presented as mean ± SEM (resting HR, four animals per group, other parameters $n = 14$ animals per group). SC, sedentary controls; EC, exercised controls; SD, sedentary diabetics; ED, exercised diabetics; BG, blood glucose; BW, body weight; VW, ventricular weight; WG, weight gain; HR, heart rate.

[a] Different from SC.

[b] Different from EC ($p < 0.05$).

Ionoptix, Milton, MA, USA) with 5 ms duration voltage pulses and an intensity of 20 V. Cells were visualized on a PC monitor with an NTSC camera (Myocam; Ionoptix) in partial scanning mode. This image was used to measure cell shortening (our index of contractility) in response to electrical stimulation using a video motion edge detector (IonWizard; Ionoptix). The cell image was sampled at 240 Hz. Cell shortening was calculated from the output of the edge detector using an IonWizard A/D converter (Ionoptix). Cell shortening (expressed as a percentage of resting cell length), time to peak shortening, and time to half relaxation were calculated.

2.13. Statistical analysis

Results were expressed as mean ± SEM. Statistically significant differences were evaluated by two-way ANOVA and the post hoc Tukey test was applied for multiple comparisons. Cell contractility properties of isolated cardiomyocytes were compared by one-way ANOVA on ranks followed by the Dunn test. The analysis was performed using the Sigma Stat software, version 3.0, and statistical significance was defined as $p \leq 0.05$.

3. Results

Seven days after the application of STZ and at the end of the study, the glycemic levels for the diabetic groups (SD and ED) were significantly greater compared to control animals (Table 1). At the baseline, there were no differences in blood glucose (BG) levels between diabetic (SD vs. ED) and control rats (SC vs. EC) (Table 1). BG levels were 34 mg dL^{-1} lower in ED animals compared to SD animals; however, this was not statistically different.

The mean BW at baseline was similar across all groups (Table 1). Diabetic rats had lower BW gain compared with normal rats (SC and EC). Similarly, the LV weighted significantly less in the diabetic rats (SD and ED). STZ-induced diabetes increased the index of LV hypertrophy (VW/BW ratio) observed in SD versus SC and ED versus

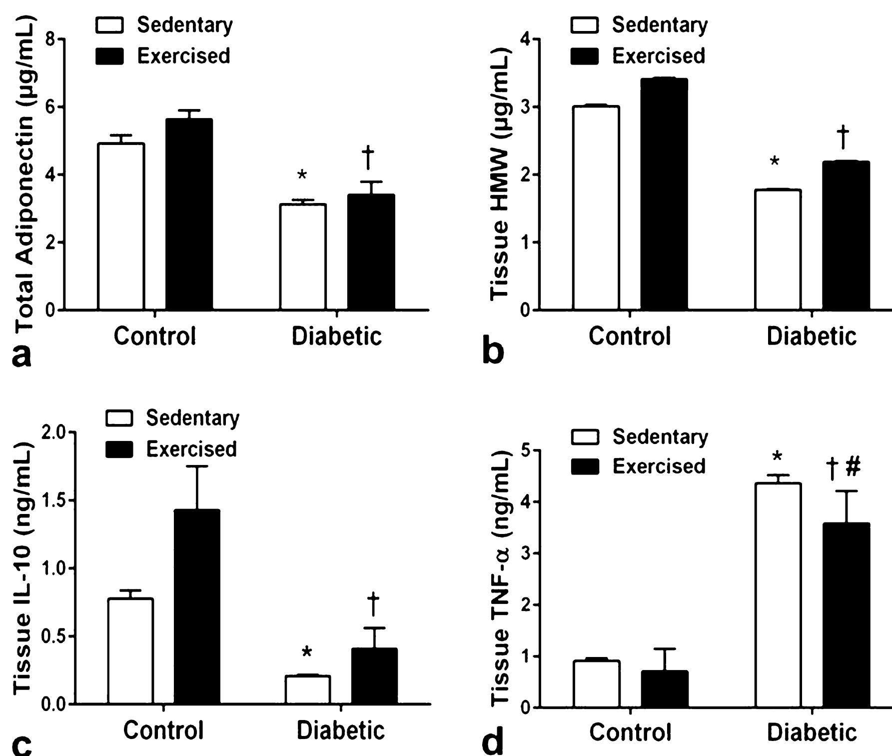

Fig. 1. Effect of swimming training on cytokine levels in the left ventricle (LV) of control and diabetic rats. Graphs show the quantitative levels of (a) total adiponectin, (b) HMW adiponectin, (c) IL-10, and (d) TNF-α of the indicated sample groups. Quantitative data are displayed as mean ± SEM ($n = 5$ animals per group). *Significant difference from sedentary control (SC). †Significant difference from exercised control (EC). #Significant difference from sedentary diabetic (SD) ($p < 0.05$).

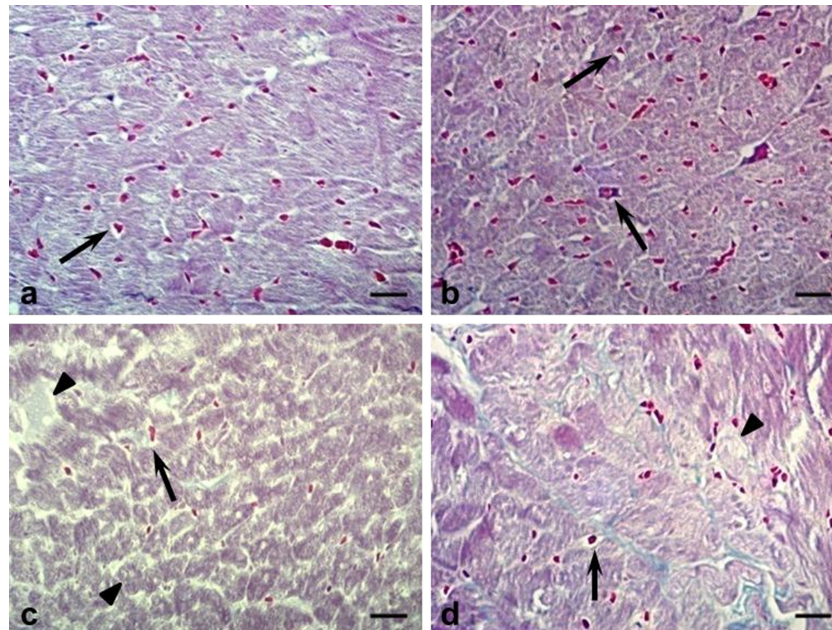

Fig. 2. Effect of swimming training on blood vessels morphology in the left ventricle (LV) of control and diabetic rats. Histological sections of myocardium subjected to the Gomori's trichrome technique. (a) Sedentary control, (b) exercised control, (c) sedentary diabetic, and (d) exercised diabetic groups. Arrows = capillaries. Note the increased level of capillary density in panel b and its reduction in the panels' c and d. The arrowhead in panels c and d indicates cardiomyocyte with vacuolar sarcoplasm (magnification × 400, bar = 30 μm).

EC rats (Table 1). The index of hypertrophy did not differ between ED and SD rats (Table 1).

Diabetes significantly reduced resting HR in SD versus SC rats. However, resting HR did not differ between trained animals (Table 1). The swimming training program reduced the resting HR in EC group compared to SC animals.

As shown in Fig. 1, total adiponectin tissue levels were significantly lower in the diabetic rats compared with controls. Similarly, the HMW adiponectin level was significantly lower in the SD versus SC rats and ED versus EC rats. The swimming training program promoted a trend to increase levels of HMW adiponectin in the EC ($p = 0.06$) and ED ($p = 0.07$) animals.

The tissue levels of IL-10 were significantly lower in the diabetic compared with control rats. Diabetes increased TNF-α levels in the LV of diabetic compared with control rats. Exercise promoted a trend to increase levels of IL-10 levels in both groups, control ($p = 0.08$) or diabetic animals ($p = 0.09$) (Fig. 1c), and reduced TNF-α levels in the diabetic group (Fig. 1d). Similarly, the balance pro/anti-inflammatory (ratio TNF-α/IL-10) were significantly higher in the diabetic rats (SD = 20.77 ± 6.99 and ED = 8.74 ± 1.07) compared with the control rats (SC = 1.16 ± 0.37 and EC = 1.49 ± 0.06). However, this increased ratio shows that the inflammatory response was significantly controlled by the exercise in animals with induced diabetes (SD vs. ED, $p < 0.05$).

Diabetic animals presented a lower total capillary density compared with SC animals (Fig.2a-d and 3a). The capillary density of the LV was greater in the ED and EC groups compared with the SD and SC groups (Fig. 3a). Diabetes was associated with reduced myocardial levels of NO compared to the control groups (Fig. 3b). The exercise training increased NO levels in EC rats compared with SC. Swimming training increased the NO level by 31% in the diabetic group (SD vs. ED), but this did not reach statistical difference. In addition, a qualitative analysis of the LV revealed greater amounts of collagen fibers around blood vessels characterizing perivascular fibrosis in SD animals; however, this was found to a lesser degree in ED animals (Fig. 4).

In SC and EC rats, cardiomyocytes were well organized, with myofibrils symmetrically disposed (Fig. 5a, b). Pathological characteristics from the LV of diabetic rats were observed. Diabetic LV myocardium demonstrated collagen accumulation, perivascular fibrosis (Fig. 4c) and intramyocardial fibrosis ($p < 0.05$). Collagen content was significantly greater in SD rats compared with SC and in ED compared with EC rats. The exercise training reduced collagen accumulation in the LV of diabetic animals (SD vs. ED, $p < 0.05$). However, this was not true in the nondiabetic animals (Fig. 4).

Others pathological characteristics such as architectural disorganization along with the degeneration and atrophy of cardiomyocytes (Fig. 5c), and evidence of inflammatory cell infiltration (not showed in Fig. 5c) were observed from LV of diabetic myocardium. In addition, cardiomyocytes from DM rats presented irregular hypertrophy, vacuolar sarcoplasm, reduction of myofibrils, contraction band necrosis, and nuclei with irregular shape (Fig. 5c). Pathological characteristics from the LV of diabetes were attenuated by the swimming training program (Fig. 4d and d).

3.1. Cardiomyocytes contractility

The analysis of cell contractility showed marked changes in the mechanical properties of isolated cardiomyocytes from diabetic animals (Fig. 6). The LV cardiomyocytes of animals in the SD group had a significant prolongation of the time to peak of contraction compared with the SC group (300.93 ± 15.26 ms vs. 231.25 ± 7.30 ms, respectively, $p < 0.05$). The swimming training did not reduce the time to peak contraction in diabetic animals (SD = 300.93 ± 15.26 ms vs. ED = 290.65 ± 4.8 ms, $p > 0.05$). The effect of the swimming program was also not detected regarding the time to peak contraction in the LV cardiomyocytes of control animals (EC = 227.35 ± 10.93 ms vs. SC = 231.25 ± 7.30 ms, $p > 0.05$). In addition, the SD versus SC groups (3.05 ± 0.24 and 2.23 ± 0.20, respectively) and the ED versus EC groups (2.52 ± 0.22 and 2.60 ± 0.21, respectively) did not exhibit statistically significant differences in cell shortening. Time to half relaxation was greater in the SD group compared with the SC group (SD = 174.26 ± 12.65 ms vs. SC = 146.46 ± 10.06 ms, $p < 0.05$). The swimming training program did not affect the time to half relaxation of cardiomyocytes in diabetic animals (SD = 174.26 ± 12.65 ms

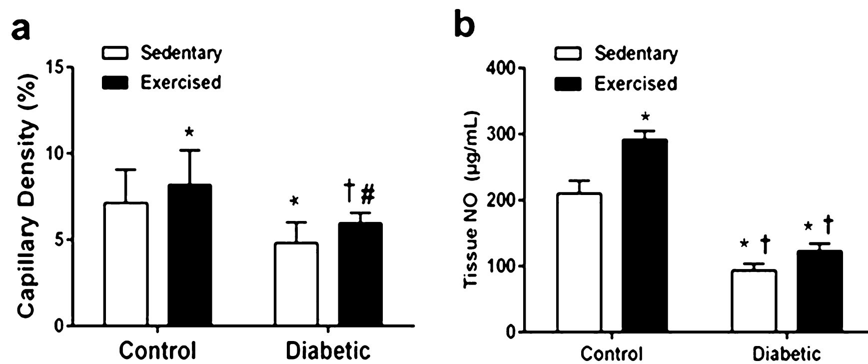

Fig. 3. Effect of swimming training on blood vessels distribution and nitric oxide (NO) levels in the left ventricle (LV) of control and diabetic rats. (a) capillary density and (b) NO levels. Data are displayed as mean ± SEM (n = 5 animals per group). *Significant difference from sedentary control. †Significant difference from exercised control. #Significant difference from sedentary diabetic (p < 0.05).

vs. ED = 209.78 ± 13.37 ms, p < 0.05). In addition, the same occurred in the control animals (SC = 146.46 ± 10.06 ms vs. EC = 139.12 ± 12.22 ms, p < 0.05).

4. Discussion

In the present study, we evaluated the effects of low-intensity swimming training on the cardiac structural remodeling, cardiac cytokines, and cardiomyocyte contractile function in growing rats with untreated experimental DM. The results showed that STZ-induced diabetes promoted marked myocardial morphological reorganization and cardiomyocyte contractile function impairment. The LV of diabetic rats demonstrated pathological characteristics such as histoarchitectural disorganization, collagen accumulation, fibrosis, reduced blood vessels, inflammatory infiltrate, necrosis, and irregular hypertrophy. Total adiponectin, HMW adiponectin, IL-10, and NO tissue levels were significantly lower in diabetes, but LV TNF-α levels and the ratio TNF-α/IL-10 were increased. However, our exercise training protocol induced a trend toward enhanced IL-10 levels and attenuated the TNF-α level, the inflammatory imbalance and the pathological characteristics of the LV in diabetes.

Animals with DM presented a marked reduction of total adiponectin and HMW adiponectin levels in the LV. As far as we know, this is the first study investigating the levels of cardiac adiponectin in growing rats with diabetes. As for the three major isoforms of adiponectin, low molecular weight, middle molecular weight, and HMW adiponectin [44], the HMW is thought to be the major active isoform in peripheral tissues [16,45]. Consistent with previous studies on adult animals, the total and the HMW adiponectin levels were inversely associated with DM [22,35]. Wang et al. [24] showed decreased cardiac adiponectin levels and increased inflammatory cytokines TNF-α and IL-6 in diabetic rats. This runs parallel with our results showing that DM rats exhibited reduced cardiac adiponectin levels and local inflammatory response as compared with the control.

In response to swimming training, total and HMW adiponectin levels were similarly unchanged in both diabetic and control groups. These suggest that even if low-intensity aerobic exercise affects the metabolism, probably the production and secretion of total and HMW adiponectin levels are not locally affected in

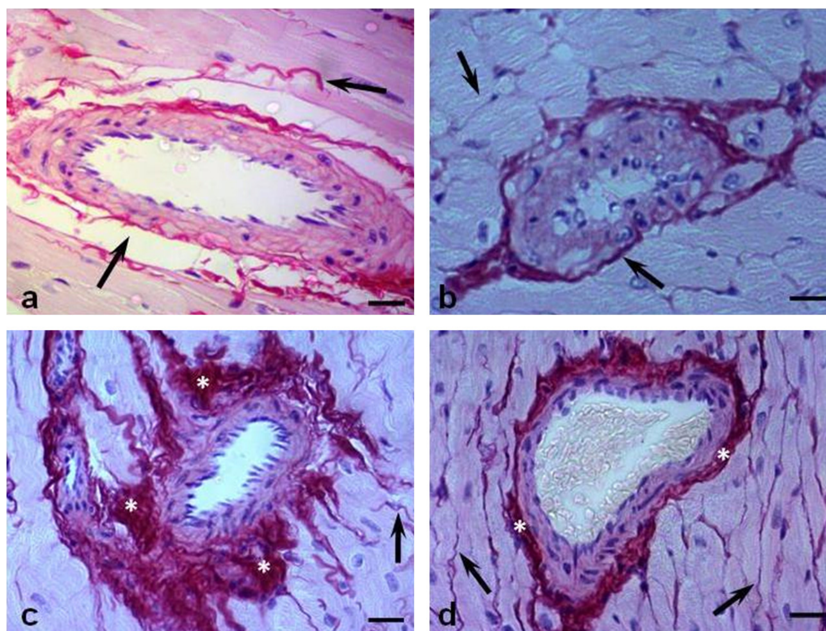

Fig. 4. Effect of swimming training on collagen content in the left ventricle (LV) of control and diabetic rats. The red color of Sirius red staining under light microscopy indicates total collagen deposition. (a) Sedentary control, (b) exercised control, (c) sedentary diabetic, and (d) exercised diabetic groups. Arrows = collagen fibers. Note the increased amount of collagen fibers in panel c and its reduction in panel d. *Perivascular fibrosis (magnification ×400; bar: 30 µm).

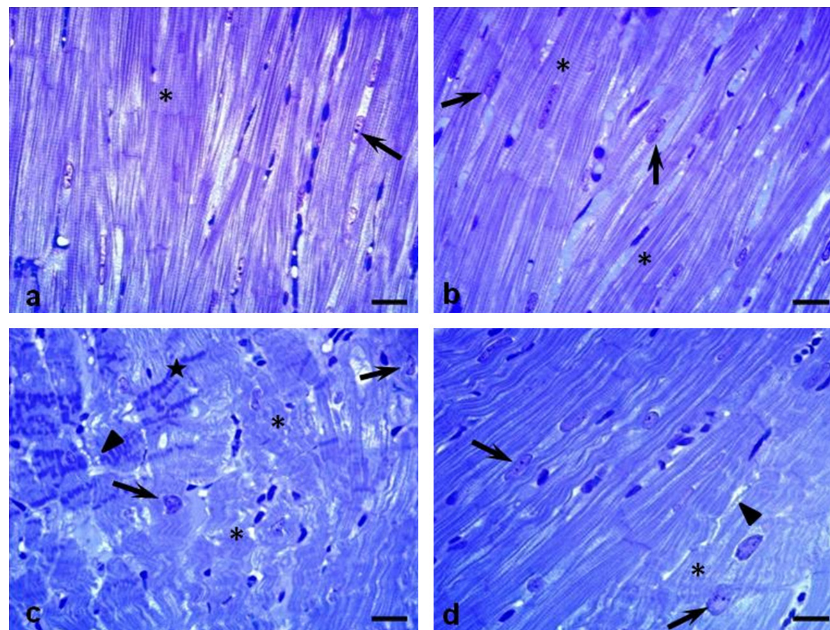

Fig. 5. Effect of swimming training on the left ventricle (LV) morphology of control and diabetic rats (toluidine blue/sodium borate 1% staining). (a) Sedentary control, (b) exercised control, (c) sedentary diabetic, and (d) exercised diabetic groups (magnification ×400, bar = 30 μm). Asterisks = cardiomyocytes, arrows = cardiomyocytes nucleus, arrowhead = vacuoles, and star = contraction band necrosis. Note that pathological characteristics from the LV of diabetic animals were attenuated by swimming training in panel d (magnification ×400, bar = 30 μm).

the heart of growing rats. Thus, the effect of exercise training on adiponectin remains to be elucidated.

In the present study, we observed that DM caused microvascular damage and reduced NO levels in the LV. Interestingly, swimming training increased capillary density. The attenuation of microcirculation impairment in the ED rats was evidenced by increased capillary density and reduced perivascular fibrosis. These results are exciting as adiponectin is known to exert protective effects through its vasodilator, antiapoptotic, anti-inflammatory, antiatherogenic, and antioxidative activities in both cardiac and vascular cells [44,46]. Our findings demonstrated a reduced density of myocardial capillaries in DM rats, which is in concert with previous reports [47,48]. In the current study, DM affected cardiac levels of NO, as shown previously [24]. Evidences suggest that in DM, the impairment of endothelial function may involve reactive oxygen species, such as superoxide, that readily react with NO to form peroxynitrate, which results in decreased NO bioavailability [49]. Wang et al. [24] demonstrated that STZ-induced diabetic rats displayed reduced cardiac endothelial nitric oxide synthase (eNOS) activation, which decreased cardiac NO content [50]. This result was associated with reduced cardiac adiponectin and increased inflammatory cytokines.

In the present study, the exercise program significantly attenuated the reduction in capillary density, perivascular fibrosis and interstitial fibrosis in diabetic rats. These are exercise benefits to the myocardium inasmuch as the capillary network participates in maintaining the supply of oxygen and energy substances to the heart. However, in our study, these changes were independent of adiponectin, suggesting that exercise plays an effective role in restoring the capillary net in the myocardium of rats. In addition, exercise training was capable of stimulating angiogenesis in EC animals compared with SC animals. These findings are in agreement with those reported by Ellison et al. [51], who demonstrated that exercise training induced the vascular remodeling of the cardiac muscle by increasing capillary density. Exercise training can also decrease oxidative stress and improve the antioxidative capacity of the vascular wall; thus, it appears to be beneficial in the prevention and improvement of microvascular dysfunction in DM [49]. Abnormalities in the endothelium-NO pathway have been reported in human and animal models of DM in both micro- and macrovessels [50,52]. However, the mechanisms underlying the morphological and functional changes of blood vessels are complex and only partially understood.

We observed an inflammatory imbalance, increased TNF-α and decreased IL-10 levels in the LV of diabetic rats. In addition, cardiac collagen accumulation (total collagen), local inflammation, degeneration and atrophy of cardiomyocytes, necrosis, and myocardial fibrosis were also increased in these animals. Interestingly, our exercise training program attenuated the TNF-α production, myocardial fibrosis, inflammatory imbalance and left ventricular pathological remodeling in diabetic animals. Our findings are consistent with previous reports in which intramyocardial inflammation was evidenced by increased levels of cardiac TNF-α [2,3,53,54] and decreased levels of cardiac IL-10 [54,55]. TNF-α is one of the major mediators of inflammation [9], and endogenous TNF-α plays a central role in initiating and sustaining the inflammatory response [46,56]. The cardiac production of TNF-α has been related to multiple detrimental effects on the heart, including cardiomyocyte hypertrophy, myocardial contractile dysfunction, fibrosis, apoptosis, pathologic heart remodeling, and reduction of adiponectin, leading to heart failure in DM [3,46,53]. A recent study revealed intramyocardial inflammation in unmanaged diabetic rats 7 weeks after STZ injection, as evidenced by the enhanced activity and expression of nuclear factor kappa B, thus leading to increased levels of cardiac pro-inflammatory cytokines (TNF-α and IL-1β), enhanced expressions of cell adhesion molecules (ICAM-1 and VCAM-1), and activated invading immunocompetent cells, such as macrophages (CD^{68+} cells) and T lymphocytes (CD^{3+} cells), and cardiac collagen accumulation [53]. The consequence of these abnormal structural alterations in diabetic rats leads to impaired cardiac performance, that is, increased left ventricular dimensions, reduced ejection fraction and fractional shortening [53]. The highest VW/BW ratio of the diabetic rats in this study reflects the increased dimensions of the LV.

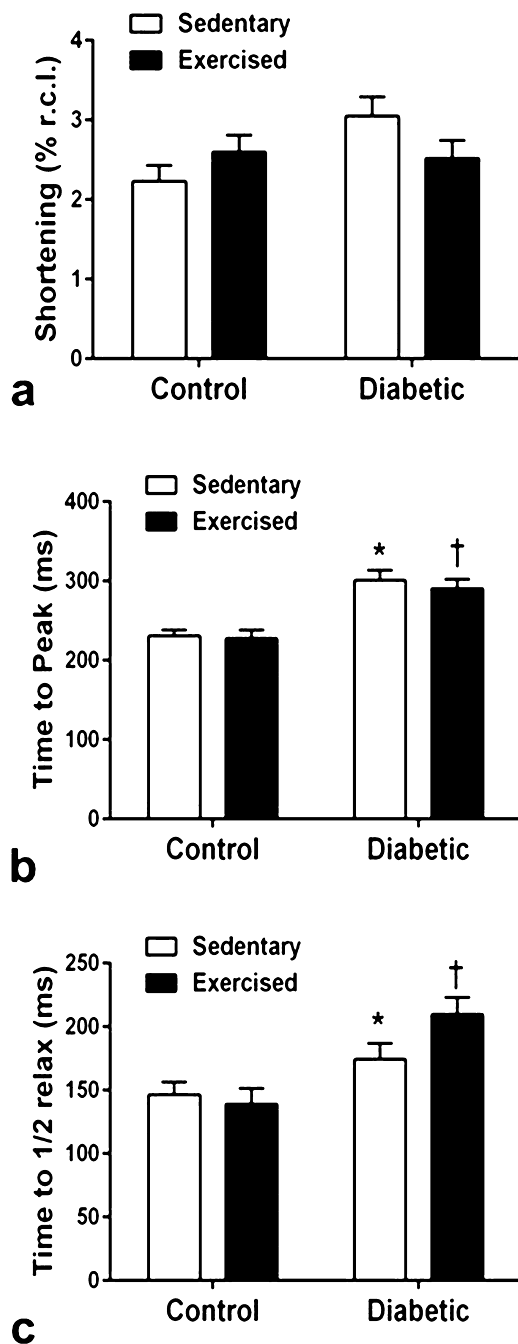

Fig. 6. Effect of swimming training on the contractility parameters of left ventricular cardiomyocytes form control and diabetic rats. (a) Cell shortening expressed as a percentage of resting cell length, (b) time to peak, and (c) time to half relaxation. Data are represented as mean ± SEM of 46–68 cells in each group; % r.c.l., percentage of resting cell length. *Significant difference from sedentary control. †Significant difference from exercised control ($p < 0.05$).

The increase in pro-inflammatory cytokines in diabetes could also have influenced the levels of cardiac adiponectin and contractile dysfunction in the present study. Inflammatory cytokines can attenuate cardiomyocyte contractility directly through the immediate reduction of the Ca^{2+} transient, through alterations in the sarcoplasmic reticulum function and indirectly through the attenuation of myofilament calcium sensitivity, by NO-dependent attenuation [53,57]. However, the relationship of these molecular changes with myocardial structural remodeling and the cellular inflammatory mechanisms in diabetic cardiomyopathy requires further investigation. We also found that TNF-α production was attenuated in the ED animals, demonstrating a potential cardioprotective effect provided by exercise training against future cardiovascular injuries. Evidences support the idea that regular exercise may suppress proinflammatory cytokines TNF-α, CRP, and IL-6 levels; also, it increases anti-inflammatory cytokines such as IL-10, IL-4, and transforming growth factor β [58,59].

In the present study, DM prolonged the time required for peak cell contraction and the time to half relaxation of left ventricular cardiomyocytes, which is in concert with recent studies conducted in our laboratory [60]. Although the molecular basis associated with cardiomyocytes contractile insufficiency has not been investigated in the present study, there are evidences that cardiomyocytes of diabetic animals exhibit reduced expression of regulatory proteins such as CaMKII, NCX, RyR2, SERCA2, and phospholamban (PLB). These changes impair the control of Ca^{2+} dynamics and affect the cardiac function of diabetic rats [29,61–63]. On the contrary, in our study left ventricular cardiomyocyte shortening was not altered by DM. Previous studies show inconsistent results because unchanged [37,64] and reduced cardiomyocyte shortening [60,61] are demonstrated in diabetic rats. Although a decreased sensitivity of contractile myofilaments to Ca^{2+} and a reduced intracellular Ca^{2+} levels has been suggested as potential mechanisms involved in the reduction cell shortening [63], this issue is poorly understood and requires further investigations.

The swimming training used here did not affect the cardiomyocytes contractile properties in control or diabetic rats. In fact, the effectively of exercise training on cardiomyocytes function in DM is controversial [36,42,60]. However, it has been indicated that exercise training can restore the cardiomyocytes contractile function in diabetic animals [62,65]. Apparently, the main beneficial cardiomyocytes adaptations induced by exercise training is the increase in Ca^{2+}availability, rate of ATP hydrolysis, sensitivity of contractile myofilaments to Ca^{2+}, and upregulation in RyR2 expression and activity [65]. However, even in the absence of contractile adaptations in cardiomyocytes, it has been described that beneficial effects of exercise training on myocardial function may be associated to secondary effects, including improved glucose metabolism and/or alterations in the collagen content of the heart and its associated effects on compliance [28,60,66]. Moreover, as specific characteristics of exercise training (i.e. intensity, duration, frequency, and metabolic overload [aerobic and anaerobic]) influence directly the effects of exercise programs, the absence of cardiomyocytes contractile adaptations may be related to the design of the training protocol applied here, aspects that should be considered in further investigations in order to ensure sufficient overload levels to induce cardiovascular adaptations.

In this study, exercise training had no significant effect on body mass in either diabetic or control rats, which is consistent with previous studies [28,36,60]. Pathological cardiac hypertrophy induced by experimental diabetes has been related in previous studies. In addition, the apparent absence of an effect of exercise on body mass in either control or diabetic rats probably is due to the anabolic and catabolic effects of the exercise regime on muscle protein and fat metabolism, respectively.

Physical exercise is an important factor to improve the balance between glycemic control, inflammation, and cardiovascular risk in DM [26]. However, we observed that after a 12-hour overnight fast blood glucose was not altered by exercise in either diabetic or control rats. These results are consistent with other reports [28,60]. It is possible that there has been an increase in glucose uptake through GLUT1 and GLUT4 transporters via the activation of AMPK in ED animals, and its counterregulatory action of glucagon has helped in the maintenance of hyperglycemia [67]. Studies have demonstrated that exercise was able to improve glucose metabolism in diabetic rats with the reduction of blood glucose levels [68,69]. However, it is not clear whether the apparent absence of an effect

of exercise on blood glucose level in diabetic rats may be due to the duration and intensity of exercise protocol, the type and severity of diabetes in the animal model, or the age of the animals.

In this study, DM reduced the resting HR in growing rats, but no statistical difference between ED and SD animals was found, which is in concert with previous studies what reported a reduced HR in adult [70,71], adolescent [72], and growing rats with DM [28], possibly due to autonomic dysfunction. In fact, bradycardia is a very early indication of diabetic cardiomyopathy [71].

5. Conclusion

In summary, our results demonstrated that the left ventricle of growing rats with STZ-induced diabetes displayed decreased level of total and HMW adiponectin and inflammatory response along with pathological structural remodeling. Low-intensity swimming training promoted a nonsignificant increasing trend in the levels of IL-10, attenuated TNF-α, myocardial fibrosis, inflammatory imbalance and pathological remodeling, and increased capillary density in the left ventricle of these animals. These positive changes coexisted with cardiomyocyte contractile dysfunction and reduced HMW adiponectin level. These results provide insight into the beneficial effects of exercise on the myocardial complications caused by DM.

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