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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.

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

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