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Research Article

Voluntary Exercise Protects Heart from Oxidative Stress in Diabetic Rats

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Abstract

Purpose: Oxidative stress plays a key role in the onset and development of diabetes complications. In this study, we evaluated whether voluntary exercise could alleviate oxidative stress in the heart and blood of streptozotocin - induced diabetic rats.

Methods: 28 male Wistar rats were randomly divided into four groups (n=7): control, exercise, diabetes and exercise + diabetes. Diabetes was induced by injection of streptozotocin in male rats. Rats in the trained groups were subjected to voluntary running wheel exercise for 6 weeks. At the end of six weeks blood and heart tissue samples were collected and used for determination of antioxidant enzymes (including SOD, GPX and CAT activities) and MDA level.

Results: Exercise significantly reduced MDA levels both in the heart tissue ($p<0.01$) and blood samples ($p<0.05$). In addition, exercise significantly increased SOD ($p<0.05$), GPX ($p<0.001$) and CAT ($p<0.05$) in the heart tissue. Voluntary exercise also significantly increased SOD ($p<0.01$), GPX ($p<0.05$) and CAT ($p<0.001$) in the blood.

Conclusion: Voluntary exercise diminishes the MDA level in blood and heart tissue of diabetic rats. It also accentuates activities of SOD, GPX and CAT. Therefore, it may be considered a useful tool for the reduction of oxidative stress in diabetes.

Introduction

Diabetes mellitus, a chronic and progressive metabolic disorder, is a challenging public health problem and nowadays, diabetes-related complications are one of the most important contributing mortality factors in the world.[1] The risk of CVD in patients with diabetes mellitus is increased more than 3-fold and is the major cause of mortality and morbidity in diabetic patients. Oxidative stress, an imbalance between production and detoxification of oxygen/nitrogen free radicals, plays a key role in the onset and development of diabetes complications. Peroxidation or glycation of lipids, proteins, and DNA, reduction of antioxidants defenses and progression of tissues inflammations are some disturbances which are induced by oxidative stress.[2] Several studies demonstrate that neutralization of reactive molecules has significantly been able to inhibit the development of endothelial dysfunction, cardiomyopathy, retinopathy, nephropathy, and neuropathy in patients with DM.[3] In order to neutralize ROS, cells are equipped with antioxidant defense mechanisms capable of combating oxidative stress. Intriguingly, compared to other tissues, ß-cells have a lower abundance of antioxidant defense enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX). Therefore because of the low antioxidant defense status of islets, excessive ROS lead to oxidative stress during ß-cell dysfunction.

Many beneficial effects of exercise such as increasing insulin sensitivity, improved glucose uptake and alleviating diabet complications in diabetic animals and patients are reported.[4-8] Exercise is a modifiable behavioral factor which can produce several beneficial effects, including improved cardiac functions and diabetes complications.[9] Exercise training has been reported to increase SOD[10,11] and also partially reverses oxidative stress in the brains of ethanol-exposed rats.[12] Malondialdehyde plasma levels were significantly reduced in streptozotocin-induced diabetic rats submitted to forced swimming test and that the associated treatment of insulin and clonazepam.[13]

In the animal model of voluntary exercise the animal has free access to a running wheel and uses the wheel according his physiological threshold for physical activity. So, voluntary exercise is ranged in mild / moderate exercise.[14] The aim of this study is to investigate the effect of voluntary exercise on oxidative stress in the heart and blood of diabetic male rats.

Materials and Methods

Animals and experimental design

Animals used in this study were provided by the colony of our university. Male Wistar rats (200 - 250 g) were randomly assigned to a sedentary or voluntary exercise group. Twenty eight male wistar rats were divided into four groups (n= 7),: control, exercise, diabetes, and exercise + diabetes.

Both diabetic groups were injected with streptozotocin, toxic to islet ß-cells. Streptozotocin (Sigma, St. Louis,

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Mo, USA) with dose of 50 mg/kg body weight (freshly dissolved in 10 mM sodium citrate, pH 4.5, with 0.9% NaCl) was administered to induce diabetes. Control animals were injected with 0.4 mL of sodium citrate buffer, pH 4.5. Animals in the two diabetic groups were identified as having diabetes when blood glucose levels reached greater than 300 mg/dL. Blood glucose level was measured by glucometer (Elegance, Model:no:CT-X10 Germany).

All animals were housed in a temperature-controlled facility (21 -23°C) maintained on a 12:12-h light-dark cycle with food and water provided ad libitum. Rats in the voluntary exercise group were housed individually in cages with stainless-steel running wheels (1.00 m circumference, Tajhiz Gostar) and were allowed free access to the wheel 24 h per day for 6 weeks. Running distance was monitored daily. Sedentary rats were housed in standard holding cages without running wheels for the same period.

At the end of the 6th week the rats were anesthetized with pentobarbital sodium (35 mg/kg, i.p.) and blood samples were collected from the inferior vena cava and were stored in tubes for determination of erythrocyte SOD, GPX and catalase activities by commercial specific Kits .

Tissue processing and homogenate preparation

Hearts were excised, frozen in liquid nitrogen and stored at deep freeze (-70 °C) for later measurements. For antioxidant activities measurement the heart samples were homogenized in 1.15% KCl solution. The homogenates were centrifuged at 1000 rpm for 1 min at 4°C. The tissue homogenate was then stored at −20 °C for determination of lipid peroxides and activities of catalase, SOD, MDA and GPX.[15]

Determination of Antioxidant Enzymes

Erythrocyte lysates were used for determination of GPX and SOD. Superoxide dismutase (SOD) activity was determined using a RASOD labratory kit (Randox Crumlin, UK) according to Delmas-Beauvieux et al.[16] SOD activity was measured at 505 nm by a spectrophotometer (Pharmacia Biotech; England). In this method, xanthine and xanthine oxidase were used to generate superoxide radicals that react with 2-(4-iodophenyl)-3 (4-nitrophenol)-5-phenyl tetrazolium chloride (ITN) to form a red formazan dye. Concentrations of substrates were 0.05 mmol/L for xanthine and 0.025 mmol/L for ITN. SOD activity was measured by the degree of inhibition of this reaction. After calculating the percent of inhibition by using related formula, SOD activity value was calculated by comparing with the standard curve and was expressed as U/mg protein.

Glutathione peroxidase (GPX) activity was determined using a RANSEL labratory kit (Randox Crumlin, UK) according to the method of Paglia and Valentine.[15] GPX catalyses the oxidation of glutathione (at a concentration of 4 mmol/L) by cumene hydroperoxide. In the presence of glutathione reductase (at a concentration ≥ 0.5 units/L) and 0.28 mmol/L of NADPH, oxidized glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to NAD+. The decrease in absorbance at 340 nm (37°C) was measured using a spectrophotometer (Pharmacia Biotech; England), and then GPX concentration was calculated from the following formula:

GPX U/L of sample = 8412 × ΔA 340 nm/min

ΔA = difference of blank value from sample value

GPX U/mg protein = GPX U/ml/protein concentration/ml

Malondialdehyde, Catalase Assessment

Malondialdehyde (MDA) levels were measured using the thiobarbituricacid reactive substances (TBARS) method.[17] Catalase activity was measured using the Aebi method.[18] According to this method, measurement was performed based on dissociation rate of H2O2 in 240 nm at 20°C. Myocardial homogenate aliquots were centrifuged at 1000 g for 10 min at 4°C. The adequate amount of supernatant (60 µL equivalent to 1.5 mg tissue wet weight) was added to a reaction mixture that contained 0.002% Triton X-100, 0.1 mM EDTA, 0.5 M potassium phosphate buffer, and 15 mM H2O2 in 1 mL final volume at pH 7.0. Activity was calculated within the initial 15s decomposition rate. The initial absorbance was recorded (A240 at t = 0). Then, it was mixed well with a plastic paddle and decrease in absorbance was recorded again for about 15 sec (A240 at t = 15) and catalase activity (K) was calculated by the related formula and was expressed as U/mg protein: K =0.153 (log A240 at t = 0/A240 at t = 15)

Statistical analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. The significant level was set at $p < 0.05$. Results are expressed as means ± S.E.M.

Results

The exercised diabetic and exercised control rats were given unlimited access to voluntary running wheels for 6 weeks. The average running distance was 1455 ± 145 m/day for the exercised diabetic group and 2147± 215 m/day for the exercised control group ($P < 0.05$).

There was a significant decrease in body weight gain and a significant increase in blood glucose in diabetic group compared to control group ($p < 0.001$) (Table 1). However no significant difference was observed in body weight gain and blood glucose between diabetes and diabetes+exercise groups after 6 weeks.

Lipid peroxidation

In the heart tissue, the level of MDA increased in diabetes group ($p < 0.05$), and decreased in diabetes + exercise group ($p < 0.01$), but the levels of MDA showed no change in exercise group compared to control group. Lipid peroxidation levels in all the blood samples

showed a similar trend to that of the heart samples (Figure 1).

Table 1. Effects of voluntary exercise on body weight and blood glucose concentrations in Streptozotocin-induced diabetic rats at first and after 6 weeks (means ± S.E.M).

Variants	Groups			
	Control	Exercise	Diabetes	Diabetes + Exercise
Initial BW	230±5.6	238±5.2	241±3.9	243±3.5
After 6 weeks BW	267±8.3	295±7.1	180±8.6 ***	191±15.3
Initial BG	118±2.1	121±4.5	383±17.9 ***	351±11.6
After 6 weeks BG	115±1.6	115±3.05	434±19.8 ***	491±22.7

*** P<0.001 compared with control group. BW, body weight, BG, blood glucose.

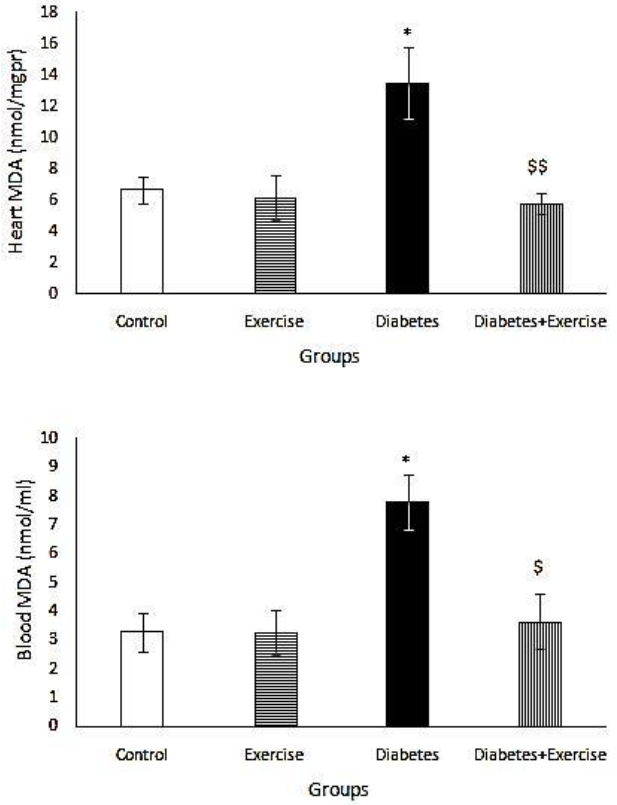

Figure 1. Effects of diabetes and exercise on MDA levels in the heart tissue and blood of different groups of rats. The values represent means ± S.E.M (n=7).*p<0.05 versus control group. $ p<0.05, $$ p<0.01 versus diabetic group.

Antioxidant enzymes

Figure 2 depicts that non-diabetic exercised animals have higher SOD activity in the heart tissues than control group (p<0.01) and also the level of SOD increased in diabetes + exercise group compared to diabetes group (p < 0.05). Moreover, the activity of SOD in the blood samples were enhanced in exercised group compared to the control (p < 0.05) and it was significantly increased in diabetes + exercise group than diabetes group (p < 0.01).

Figure 3 shows that the levels of GPX in exercise and diabetes + exercise group increased in the heart tissue than control and diabetes group respectively (p<0.001). In parallel, GPX activities in blood of the exercise and diabetes + exercise groups showed a significant (p<0.05) increase in comparison to control and diabetic rats respectively.

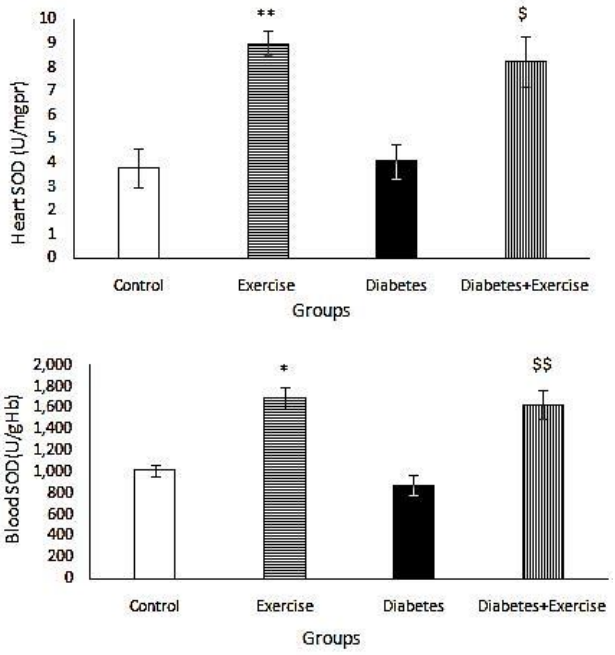

Figure 2. Effects of diabetes and exercise on SOD levels in the in the heart tissue and blood of different groups of rats. The values represent means ± S.E.M (n=7).).*p<0.05, **p<0.01 versus control group. $ p<0.05, $$ p<0.01 versus diabetic group.

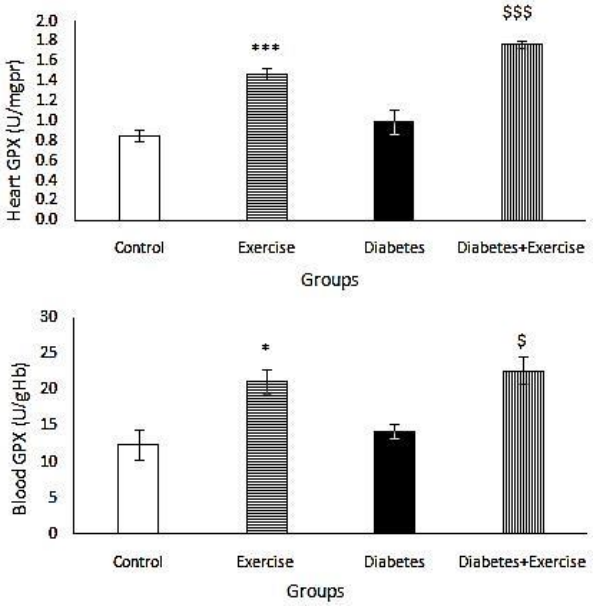

Figure 3. Effects of diabetes and exercise on GPX levels in the in the heart tissue and blood of different groups of rats. The values represent means ± S.E.M (n=7). *p<0.05, ***p<0.001 versus control group. $ p<0.05, $$$ p<0.001 versus diabetic group.

Figure 4 indicates that CAT activities in the heart samples enhanced in exercise group versus control group and also the levels of CAT were higher in diabetes + exercise group compared to diabetes group ($p < 0.05$). In addition a significant elevation of CAT in blood was observed in exercise group compared to the control group ($p<0.05$) and also this elevation was seen in diabetes + exercise group compared to the diabetes group ($p<0.001$).

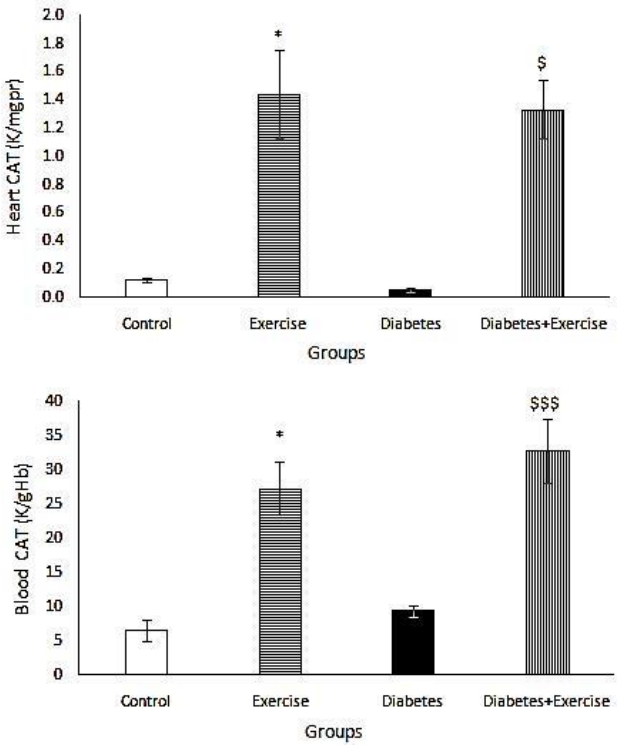

Figure 4. Effects of diabetes and exercise on CAT levels in the heart tissue and blood of different groups of rats. The values represent means ± S.E.M of (n=7).*p<0.05 versus control group. $ p<0.05, $$$ p<0.001 versus diabetic group.

Discussion

Our results indicate that voluntary exercise reduced oxidative stress in the blood and heart of streptozotocin - induced diabetic rats. We evaluated lipid peroxidation, which is a well known marker of oxidative stress, and also antioxidant enzymes including SOD, GPX and CAT. Diabetes induced oxidative stress demonstrated by high level of MDA and also diminished antioxidant enzymes activities which is reversed by voluntary exercise. Therefore we have demonstrated that voluntary exercise protects heart from oxidative stress in diabetic rats. Previously, it was shown that increased oxidative stress and altered antioxidant pool have been found in diabetic heart.[19-21] This was in conjunction with depletion of superoxide scavenger SOD, GPX, CAT and increase in lipid peroxidation product MDA.

Since antioxidant enzymes may be activated selectively during exercise depending on the oxidative stress imposed on the specific tissues as well as the intrinsic antioxidant defense capacity, there are conflicting data about relationship between exercise and the changes of antioxidant enzyme activities.

In rodents, exercise training (forced treadmill running or swimming) has been shown to enhance antioxidant enzyme activity[11,22-25] adaptations that would be expected to reduce tissue oxidative damage. Also it has been reported that the myocardial antioxidant levels were improved greatly in exercise training in hamsters.[26] There is also evidence that exercise may reduce oxidative damage and increase antioxidant enzyme activities in a variety of tissues.[27-29] Conversely Judge etal.reported that voluntary exercise resulted in a significant reduction in MnSOD activity although there was no effect of wheel running on GPX and CAT activity in the heart. Moreover there were no changes in cytosolic SOD, GPX, or CAT activities. Also no significant differences in MDA from sedentary rats and runners were observed.[30] No differences were observed in antioxidant enzyme activities, except in heart total superoxide dismutase activity with short-term voluntary wheel running in the heart of a model small mammal species.[31] Vaanholt LM et al. reported that Long-term elevations in voluntary exercise did not result in elevations in antioxidant enzyme activities.[32]

Our study showed that SOD, GPX and CAT activities were increased in non-diabetic exercised animals compared to control group. MDA levels showed no difference between non diabetic exercised and control rats which indicate that voluntary exercise could not affect the peroxidation index.

Although exactly how exercise improves SOD, GPX and CAT activities is still unclear, several possible mechanisms can be put forward to explain this effect. It has been suggested that taurine has beneficial effects in various physiological and pathological conditions by diminishing production of reactive oxygen species (ROS) that increase in exercise. Taurine may be a mechanism that reduce the lipid peroxides and also restore the levels of SOD in the diabetic hearts.[19]

Another mechansin may be Nrf-2 that is a transcription factor and increase in phosphorylation of Nrf-2 by exercise was reported in physical activity.[33] Therefore activation of Nrf-2 in exercise provides a mechanism of antioxidative protection by binding to the antioxidant response elements (ARE), which are present in the promoter regions of several antioxidative enzymes, including MnSOD.

Furthermore, exercise training induces increased SIRT1 activity (a cluster of proteins composed by seven homologues that regulate cellular biology and metabolism through deacetylation of histones and other cellular factors such as NFkB, HSF1, p53, FOXOs, and PGC- 1) that is responsible both of raised activation of antioxidants system (by MnSOD and Catalase) and cell cycle arrest to promote DNA repair. and this finding could be linked to increased NOS expression.[34] Also Eksakulkla et al.[35] suggested that physical activity could improve endothelial dysfunction by increasing NO bioavailability.

A difference between the exercise protocols may explain the discordant results. It is difficult to directly compare our results with other studies that have used exercise training (treadmill or swimming), since the duration and intensities of those types of exercise are typically much greater than voluntary wheel running and are generally performed over much shorter time periods.

In conclusion, our findings indicate that voluntary exercise is a proper method for protection of heart with positive changes in MDA, SOD, GPX and CAT activities. The present study shows that voluntary exercise decrease oxidative stress and lipid peroxidation in the heart and blood of diabetic rats. It may therefore be a beneficial tool for the reduction of oxidative stress in patients with diabetes.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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