



Exercise training ameliorates the impairment of endothelial and nitrergic corpus cavernosum responses in diabetic rats

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ABSTRACT

Aims: The effect of exercise training (ET) on vascular responsiveness in diabetes mellitus has been largely well studied. However, limited studies have investigated the effects of ET on functional responses of the corpus cavernosum (CC) in diabetic animals. Therefore, the aim of this study was to investigate whether prior ET prevents the impairment of erectile function in streptozotocin-induced diabetic rats.

Main methods: Rats were exercised for four weeks prior to the induction of diabetes, and then again for another 4 weeks thereafter. Concentration–response curves to acetylcholine, sodium nitroprusside, Y-27632, BAY 412272 and phenylephrine (PE) were obtained in CC. The excitatory and inhibitory effects of electrical-field stimulation were also evaluated.

Key findings: Plasma SOD levels were markedly decreased in the sedentary diabetic group (D-SD) as compared to control sedentary animals (C-SD), approximately 53% ($P < 0.05$) and this reduction was restored in trained diabetic animals. Physical training restored the impairment of endothelium-dependent and -independent relaxation responses seen in the D-SD group. The potency values for Y-27632 in the CC were significantly reduced in the D-SD group, which was reversed by physical training. The impairment of electrical-field stimulation (EFS)-induced relaxation seen in the D-SD group was restored by physical training. On the other hand, both EFS-induced contractions and concentration–response curves to PE in cavernosal strips were not modified by either diabetes or physical training.

Significance: Practice of regular physical exercise may be an important approach in preventing erectile dysfunction associated with diabetes mellitus by re-establishment of the balance between NO production and its inactivation.

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Introduction

Penile erection is the end result of corpus cavernosum smooth muscle relaxation driven by nitric oxide (NO) production and primary synthesis of cyclic GMP (cGMP). The NO/cGMP signaling pathway plays a key role in mediating erectile function (Burnett et al., 1992). Both endothelial NO synthase (eNOS) and neuronal NO (nNOS) isoforms contribute to the relevant levels of NO production in the corpus cavernosum, which in turn activates the soluble guanylyl cyclase enzyme (sGC) catalyzing the conversion of guanosine triphosphate to cGMP (Andersson, 2001).

Erectile dysfunction (ED) is characterized by a persistent inability to achieve and/or maintain an erection sufficient for satisfactory sexual performance, which has been associated with diminished NO

production and/or its bioavailability to the corporeal smooth muscle. Furthermore, the increase in contractile factors and generation of superoxide anions have been shown to contribute to ED (Andersson and Stief, 1997). A variety of pathological conditions can lead to ED, however diabetes mellitus is a primary cause of this disorder due to the microangiopathy of the cavernosal artery, corporal veno-occlusive dysfunction and autonomic neuropathy (Hakim and Goldstein, 1996). Epidemiological studies have shown that ED is three times more common in diabetics than in non-diabetic men, affecting 35 to 75% of all men with this disease (Braun et al., 2000).

Considerable advances in the treatment of ED have been achieved mainly through type 5 phosphodiesterase inhibition therapy; however, the efficacy of this type of drug in impotent diabetic men is approximately 65% as compared to the general population (Basu and Ryder, 2004). Thus, new pharmacological and non-pharmacological approaches to treat ED associated with diabetes mellitus would be relevant for increasing the quality of life in these patients and their families. Exercise training is considered an important tool in the

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management of chronic diseases such as arterial hypertension and diabetes mellitus (Zanesco and Antunes, 2007; Mostarda et al., 2009). Accordingly, the health-benefit of physical exercise is related to an increase in NO production and/or decrease in NO inactivation. Previous studies from our laboratory have shown that exercise training promotes an up-regulation of superoxide dismutase (SOD) expression and reverses elevations in reactive oxygen species in hypertensive rats (de Moraes et al., 2008; Claudino et al., 2009).

The effect of exercise training on vascular responsiveness in both diabetic patients and experimental models of diabetes has been well documented; however, no studies have investigated the effects of contractile and relaxation responses in isolated corpus cavernosum from trained diabetic rats. Therefore, the aim of the present study was to investigate whether prior exercise training prevents erectile dysfunction in streptozotocin-induced diabetic rats.

Materials and methods

Animals

This study was approved by the Ethical Committee of the Medical School of State University of Campinas (UNICAMP). Male Wistar rats were obtained from UNICAMP Animal Care Facility. They were individually housed at $26 \pm 2^\circ\text{C}$ with food and water delivered ad libitum on a 12 h light:dark cycle with the lights turned on at 6:00 a.m. The animals were divided into three groups: Control sedentary (C-SD, $n = 36$ rats); sedentary diabetic (D-SD, $n = 36$ rats) and trained diabetic (D-TR, $n = 36$ rats).

Physical training program

Rats were submitted to a run training program in a motor-driven treadmill for small animals in sessions of 60 min/day, 5 days/week, at a speed of 0.8–1.2 km/h. The intensity of the run training was determined according to the plasma lactate concentration curves as previously described (Manchado et al., 2005). Only the animals who adapted to the treadmill were used in the present study. No stress or adverse stimuli were used to force the animals to run. Rats were exercised for 4 weeks prior to induction of diabetes and then again for another 4 weeks thereafter. The run training lasted for eight weeks. Both control and sedentary diabetic animals remained in their cages during treadmill exercise sessions of the trained groups.

Induction of type I diabetes mellitus

Diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg, dissolved in citrate buffer, pH 4.5) in both sedentary and trained animals. Control animals were injected with the vehicle alone. Two days following streptozotocin injection, diabetes induction was confirmed by measuring blood glucose levels using a glucometer (Advantage Roche, São Paulo, SP, Brazil). The rats with blood glucose concentrations greater than 280 mg/dl were accepted as being diabetic. Glucose levels were measured at baseline, four weeks after the start of exercise training and at the end of the experimental protocols (eight weeks). Glycated haemoglobin levels were assessed by ion exchange chromatography (Labtest Diag., MG, Brazil). The experimental design is shown below.

Rat isolated corpus cavernosum preparation

The animals were anaesthetized with halothane and exsanguinated. The penis was carefully isolated and removed at the level of attachment of the corporeal body to the ischium and immersed in Krebs solution with the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.17, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2.5, NaHCO_3 25 and glucose 5.6. After removal of the vein and urethra, the penile tissue was cleaned of

connective and adventitial tissue, and the fibrous septum separating the corpora cavernosa was opened from its proximal extremity towards the penile shaft. A slit was made in the tunica albuginea along the shaft to obtain two strips of corpus cavernosum from each animal. The strips of rat corpus cavernosum (RCC) were mounted in 10-ml organ baths containing Krebs solution at 37°C continuously bubbled with a mixture of 95% O_2 and 5% CO_2 (pH 7.4), and vertically suspended between two metal hooks. Isometric force was recorded using a PowerLab 400™ data acquisition system (Software Chart, version 4.2, AD Instrument, MA, USA). The tissues were allowed to equilibrate for 1 h before starting the experiments.

Cumulative concentration–response curves

Following equilibration, potassium chloride solution (KCl 80 mM) was added to the organ baths to verify the tissue viability. After renewal of the bathing solution with regular Krebs and recovery of basal tension, the strips were precontracted with phenylephrine (PE, 10^{-5} M), and cumulative concentration–response curves for the relaxant effects of the muscarinic agonist acetylcholine (10^{-9} to 10^{-3} M), the NO-donor compound sodium nitroprusside (10^{-9} to 10^{-3} M), the inhibitor of Rho-kinase activity Y-27632 (10^{-9} to 10^{-5} M), and the NO-independent sGC stimulator BAY 412272 (10^{-9} to 10^{-5} M) were obtained. Alternatively, in another set of experiments, full concentration–response curves were obtained for the contractile effects of the α_1 -adrenergic agonist PE (10^{-7} to 10^{-4} M). In either case, each strip was used to obtain a single curve.

All concentration–response data were evaluated for fit to a logistics function in the formula:

$$E = E_{\max} / ((1 + (10^c / 10^x)^n) + \Phi)$$

where E is the effect of above basal; E_{\max} is the maximum response produced by the agonist; c is the logarithm of the EC_{50} , the concentration of agonist that produces half-maximal response; x is the logarithm of the concentration of agonist; the exponential term, n is a curve-fitting parameter that defines the slope of the concentration response line, and Φ is the response observed in the absence of added agonist. Nonlinear regression analysis was used to determine the parameters E_{\max} and $\log \text{EC}_{50}$, by using GraphPad Prism (GraphPad Software Inc., San Diego, CA) with the constraint that $\Phi = 0$. The responses for each agonist are showed as the mean \pm SEM of $p\text{EC}_{50}$ and E_{\max} .

Relaxing and contractile responses to electrical-field stimulation (EFS)

Electrical field stimulation (EFS) was applied in strips placed between two platinum ring electrodes connected to a Grass S88 stimulator (Astro-Med Industrial Park, RI, USA). Contractile and relaxation responses to EFS (1–32 Hz) were conducted at 50 V, 1 ms pulse width and trains of stimuli lasting 10 s at varying frequencies.

In order to verify the EFS-induced relaxation, tissues were pretreated with guanethidine (3×10^{-5} M) and atropine (10^{-6} M) to deplete the catecholamine stores of adrenergic fibers and to block muscarinic receptors, respectively. Next, a series of EFS-induced relaxations in PE (10^{-5} M)-precontracted were obtained.

In another set of experiments, contractile responses to EFS were evaluated by obtaining frequency–response curves in the absence of atropine and guanethidine. In all experiments, preparations were allowed to recover completely from each response prior to the next stimulus. Each strip was used to obtain a single EFS curve.

Determination of superoxide dismutase (SOD) activity

Animals were anesthetized with halothane and blood was collected (8 ml) from the abdominal aorta in EDTA containing tubes. Arterial blood was centrifuged ($8000 \times g$, 10 min) and plasma

supernatant was ultrafiltered through microfilter cups (Microcon Centrifugal Filter Units, 10 kDa; Millipore, Bedford, MA, USA). The white buffy layer was removed and discarded. Erythrocytes were lysed 4 times in ice-cold HPLC-grade water and centrifuged at $10,000\times g$ for 15 min at 4 °C. Supernatant was collected and maintained on ice for assaying. Samples, standards, radical detector and xanthine oxidase were prepared and performed as described per the manufacturer's instructions (Superoxide Dismutase Assay Kit, Cayman Chemical Co., Ann Arbor, MI, USA). The assays were performed in duplicate using different dilutions of samples.

Drugs

Acetylcholine chloride, sodium nitroprusside dihydrate, streptozotocin, phenylephrine hydrochloride, Y-27632 dihydrochloride monohydrate, atropine sulphate and guanethidine monosulphate were purchased from Sigma Chemical Co. (St Louis, MO, USA). 5-Cyclopropyl-2-(1-(2-fluorobenzyl)-1H-pyrazolo(3,4-b)pyridin-3-yl)pyrimidin-4-ylamine (BAY 41-2272) was provided by Pharma Research Center, Bayer AG (Wuppertal, Germany).

Statistical analysis

Data are expressed as mean \pm SEM of n experiments. Nonlinear regression analysis to determine the pEC_{50} and E_{max} was performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). One-way ANOVA followed by a Tukey's test was performed using Instat software. Values of $P < 0.05$ were considered statistically significant.

Results

Anthropometric and biochemical parameters

Body weight values at baseline were similar in all groups (286 ± 16 ; 263 ± 10 and 263 ± 8 g for C-SD, D-SD and D-TR, respectively). Diabetes provoked a significant decrease in the body weights in both the sedentary (271 ± 21 g) and trained (268 ± 8 g) groups as compared to control sedentary animals (429 ± 17 g) eight weeks after STZ injection.

Plasma glucose concentrations were similar in all groups at baseline (79 ± 4 ; 76 ± 2 and 72 ± 4 mg/dl, for C-SD, D-SD and D-TR, respectively). A marked increase in the glucose and glycosylated haemoglobin levels was found in the D-SD group (425.2 ± 14 mg/dl and $5.00 \pm 0.13\%$, respectively) as compared to the C-SD group (73.5 ± 2.1 mg/dl and $2.6 \pm 0.07\%$, respectively, $P < 0.05$). Blood glucose levels were slightly reduced, 389.8 ± 14 mg/dl, approximately 6%, in trained diabetic animals as compared to sedentary diabetic group. No changes were found in glycosylated haemoglobin levels in the D-TR group ($5.10 \pm 0.18\%$).

Plasma SOD levels were markedly decreased in the D-SD group ($5.5 \pm .06$ U/mL) as compared to the C-SD animals (9.5 ± 0.6 U/mL; $P < 0.05$), approximately 53% ($P < 0.05$), and this reduction was restored in trained diabetic animals (8.1 ± 0.6 U/mL).

Concentration–response curves to acetylcholine, sodium nitroprusside, BAY 41-2272, and Y-27632

Acetylcholine (10^{-9} to 10^{-3} M, ACh) produced concentration-dependent relaxation responses in isolated RCC in all groups. The potency values at the pEC_{50} level for ACh in the CC were significantly decreased in the D-SD group, approximately 8-fold ($P < 0.05$), as compared to the C-SD group. Physical training restored the impairment of the endothelium-dependent relaxation response seen in the D-SD group (Fig. 1A). Maximal responses for this muscarinic agonist were significantly reduced in the D-SD group as compared to the C-SD group ($P < 0.05$) that was restored with physical training.

Sodium nitroprusside (SNP) produced concentration-dependent relaxation responses in the RCC. The pEC_{50} values for SNP were not different between the C-SD and D-SD groups, however a significant decrease in the maximal responses were seen in the D-SD group as compared to the C-SD group. Physical training caused a leftward shift in the concentration–response curves for this NO-donor agent, approximately 2.8-fold, at the pEC_{50} level (Fig. 1B; $P < 0.05$). Furthermore, physical exercise restored attenuation of the maximal response for SNP seen in the D-SD group.

The NO-independent sGC stimulator BAY 412272 (10^{-9} to 10^{-5} M) produced concentration-dependent relaxation responses in the RCC. NO changes were observed in all groups for this compound (Fig. 1C).

The inhibitor of Rho-kinase activity, Y-27632 (10^{-9} to 10^{-5} μ M) produced concentration-dependent relaxation responses in the RCC in all groups. The potency values at the pEC_{50} level for Y-27632 in the CC were significantly decreased in the D-SD group, approximately 2-fold ($P < 0.05$), as compared to the C-SD group. This reduction in the D-SD group was reversed with physical training (Fig. 1D). No changes were found in the maximal responses for Y-27632 in all groups. The data for acetylcholine, SNP, BAY 412272 and Y-27632 are summarized in Table 1.

Electrical-field stimulation-induced cavernosal relaxation

Electrical-field stimulation (EFS) of cavernosal tissues pretreated with guanethidine (3×10^{-5} M) and atropine (10^{-6} M) caused frequency-dependent relaxation in all groups (Fig. 2). However, a marked decrease in the relaxation of cavernosal tissues from the D-SD group in comparison with C-SD animals was observed at the highest frequency (16 and 32 Hz). Physical training fully restored the impaired EFS-induced relaxation response (Fig. 2).

Contractile responses to EFS and phenylephrine

Electrical-field stimulation produced frequency-dependent contractions in cavernosal strips (1–32 Hz) that were not modified by either diabetes or physical training (Fig. 3A).

Phenylephrine (10^{-7} to 10^{-4} M) induced concentration-dependent contractions in cavernosal preparations in all groups. However, no differences at the pEC_{50} and maximal responses values were found between groups (Fig. 3B).

Discussion

Cross-sectional studies have demonstrated an inverse relationship between ED and the level of physical activity (Esposito et al., 2004; Rosen et al., 2009). Additionally, a follow-up study, by applying questionnaires of sexual function and physical activity, showed that diabetes mellitus was associated with an increased risk for ED whereas high physical activity level was inversely associated with ED (Bacon et al., 2003). However, the cellular and molecular mechanisms by which exercise training promotes beneficial effects on ED have not been studied. Therefore, the present study was designed to evaluate the effects of exercise training on the vascular responses of the corpus cavernosum in a classical diabetes model in rats. Our data showed an impairment of endothelium-dependent and neurogenic relaxation in cavernosal smooth muscle from diabetic rats, confirming previous studies performed in both human and laboratory animals (Hirata et al., 2008; Ângulo et al., 2009).

The beneficial effects of exercise training on endothelium-dependent relaxation responses have been shown in different types of vascular smooth muscle, including corpus cavernosum (de Moraes et al., 2008; Claudino et al., 2009). NO released from nitrergic fibers supplying the erectile tissue is far more important to produce penile erection than endothelium-derived NO (Andersson, 2001); however no study has investigated the effects of exercise training on impairment of neurogenic nitrergic relaxation in the diabetic state. The present study showed that

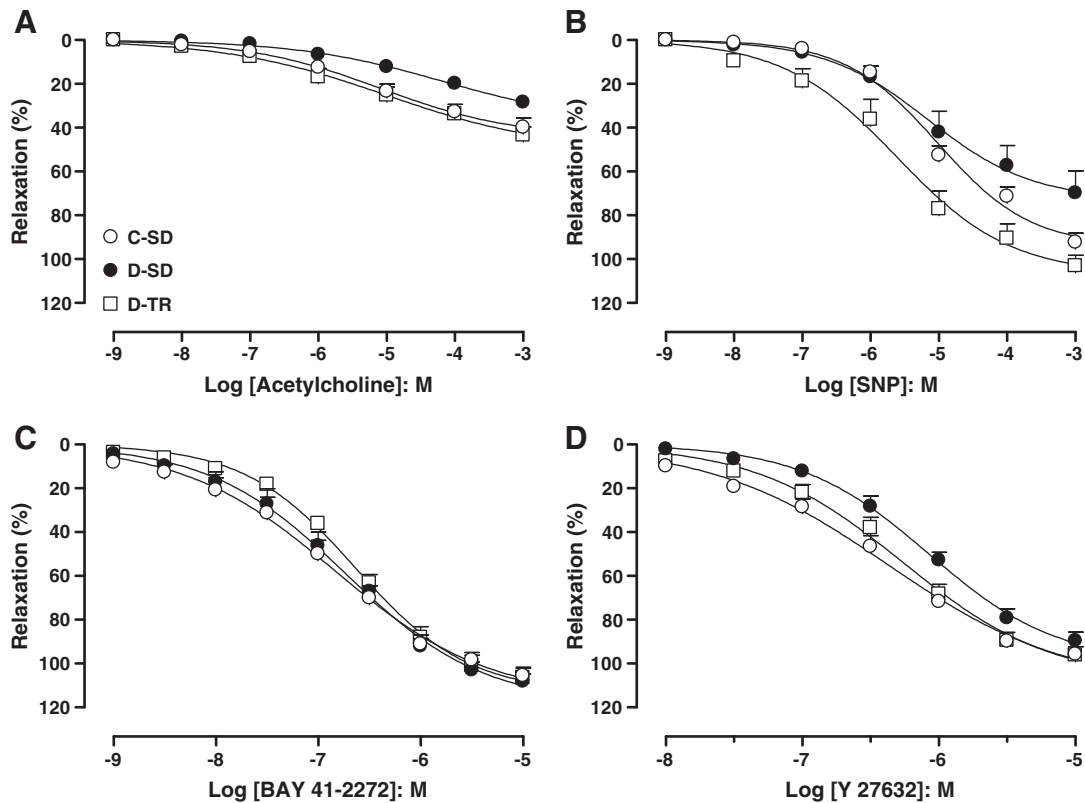


Fig. 1. Concentration–response curves to acetylcholine (panel A), sodium nitroprusside (SNP, panel B), BAY 41-2272 (panel C), and Y-27632 (panel D) in rat corporeal smooth muscle strips for control-sedentary (C-SD), sedentary diabetic (D-SD), and trained diabetic (D-TR) groups. Data were calculated relative to the maximal changes from the contraction produced by phenylephrine (PE, 10^{-6} M) in each tissue, which was normalized to 100%. Data represent the mean \pm SEM from 6 to 8 experiments.

exercise training restores the impairment of endothelium-dependent and neurogenic nitrgic relaxation in diabetic rat corpus cavernosum.

Both acute and chronic exercise promote an improvement in hyperglycemia-induced oxidative stress in diabetic and pre-diabetic states through different cellular signaling pathways. Exercise improves blood glucose clearance via enhanced GLUT 4 translocation as well as improves insulin sensitivity in a variety of tissues. Exercise also stimulates an increase in antioxidant enzyme activity (Mostarda et al., 2009). In our study, we found a slight decrease in blood glucose in trained diabetic rats, suggesting a role for aerobic exercise as an additional therapeutic tool in the management of hyperglycemia induced by diabetes. Oxidant and antioxidant systems play an important role in the regulation of NO concentration in these cells. In the vascular system, antioxidant enzymes including SOD play a critical role in the management of oxidative stress preserving NO bioactivity produced by the endothelium (Darley-Usmar et al., 1995; Kojda and Harrison, 1999). We found that SOD levels were markedly lower in sedentary diabetic rats, suggesting that impairment of endothelium-dependent and neurogenic nitrgic relaxation in the rat corpus cavernosum may result in decreased NO bioavailability. On

the other hand, exercise training restored plasma SOD levels in diabetic animals, suggesting that NO bioavailability to the corporal smooth muscle was normalized. This mechanism may explain the reversal of ED seen in diabetic rats. In fact, SOD has been reported as an important target for gene therapeutic approach for ED as an attempt to increase NO levels into the penis of aged or diabetic rats (Deng et al., 2010).

Nitric oxide can be exogenously supplied to the tissues and cells by various NO generating compounds. The inorganic compound sodium nitroprusside (SNP) is an agent that releases NO in biological systems by non-enzymatic and enzymatic mechanisms (Bates et al., 1992; Feelisch, 1998). Additionally, the NO-independent sGC activator has emerged as a valuable tool to elucidate the NO-sGC-cGMP signaling pathway (Evgenov et al., 2006). BAY 41-2272, known as a potent NO-independent sGC stimulator (Stasch et al., 2001), relaxes vascular smooth muscle (Teixeira et al., 2006), including the corpus cavernosum (Barakat et al., 2003). In our study, SNP-induced cavernosal relaxation was significantly reduced in sedentary diabetic rats, whereas the relaxation responses to BAY 41-2272 remained unchanged. It is likely that increased oxidative stress (superoxide anion production (Scott and King, 2004)) in smooth muscle

Table 1

Potency (pEC_{50}) and maximal responses (E_{max} , %) values obtained from concentration–response curves to acetylcholine (ACh), sodium nitroprusside (SNP), BAY 41-2272, Y-27632, and phenylephrine (PE) in cavernosal smooth muscle from control sedentary (C-SD), sedentary diabetic (D-SD) and trained diabetic (D-TR) groups.

	C-SD		D-SD		D-TR	
	pEC_{50}	E_{max}	pEC_{50}	E_{max}	pEC_{50}	E_{max}
ACh	5.08 ± 0.08	40 ± 4	$4.17 \pm 0.26^*$	$28 \pm 2^*$	$5.00 \pm 0.29^{\#}$	$43 \pm 4^{\#}$
SNP	5.16 ± 0.16	92 ± 4	5.15 ± 0.10	$70 \pm 10^*$	$5.61 \pm 0.16^{\#}$	$103 \pm 5^{\#}$
BAY 41-2272	6.83 ± 0.07	106 ± 4	6.73 ± 0.06	108 ± 3	6.63 ± 0.05	107 ± 5
Y-27632	6.37 ± 0.07	96 ± 1	$6.07 \pm 0.05^*$	90 ± 4	$6.26 \pm 0.10^{\#}$	96 ± 4
PE	5.17 ± 0.04	155 ± 8	5.31 ± 0.04	150 ± 10	5.16 ± 0.05	135 ± 12

Potency is represented as $-\log$ of molar concentration to produce 50% of the maximal response.

Data represent the mean \pm S.E.M. of 6–8 experiments. $^*P < 0.05$ compared to C-SD; $^{\#}P < 0.05$ compared to D-SD (One-way ANOVA followed by the Tukey test).

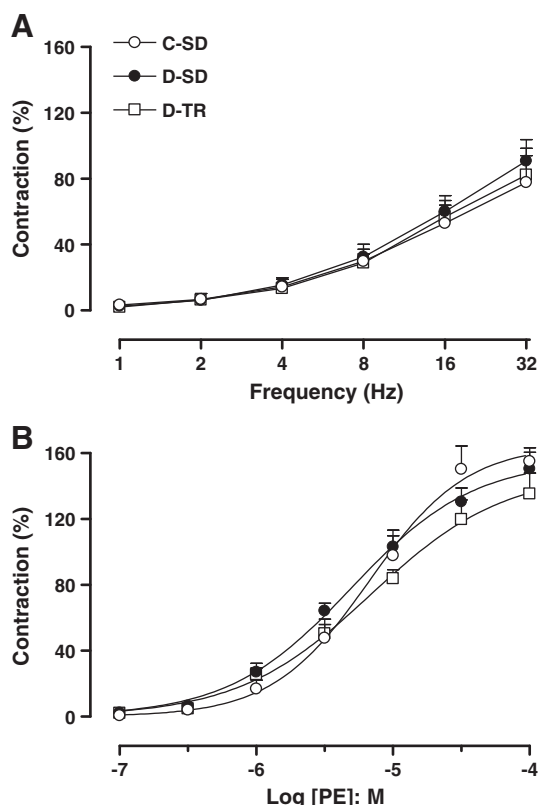


Fig. 2. Relaxation responses to electrical-field stimulation (EFS, 1–32 Hz) in rat corporeal smooth muscle strips for control-sedentary (C-SD), sedentary diabetic (D-SD), and trained diabetic (D-TR) groups. Data are mean \pm SEM from 6 to 8 experiments. * $P < 0.05$ compared to the C-SD group, # $P < 0.05$ compared to the D-SD group.

cavernosal cells of sedentary diabetic rats act inactivates NO (released from SNP) before it reaches sGC, which is consistent with the reduction of SOD activity in these animals. Interestingly, exercise training further augmented corporeal relaxation in response to SNP in diabetic rats, reinforcing the hypothesis that improvement of NO bioavailability is the primary mechanism for its ability to reverse diabetes-related ED. Since the current study did not include an exercised control group, it must be emphasized that the reversal of effects of diabetes seen in CC from exercised diabetics refers specifically to the levels displayed in strips from sedentary controls, even if in some cases there could still be differences in relation to exercised controls (Claudino et al., 2009).

Corpus cavernosum and penile vessels are contracted mainly via stimulation of α_1 -adrenoceptors (Andersson and Stief, 1997). A

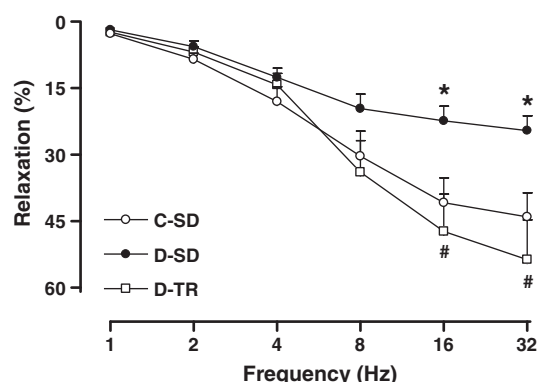


Fig. 3. Contractile responses to electrical-field stimulation (EFS, 1–32 Hz; panel A) and phenylephrine (PE, panel B) in rat corporeal smooth muscle strips from control-sedentary (C-SD), sedentary diabetic (D-SD), and trained diabetic (D-TR) groups. Data are mean \pm SEM from 6 to 8 experiments.

previous study has reported increased sensitivity to the α_1 -adrenergic agonist, phenylephrine, in vascular smooth muscle from hypertensive and diabetic animals (Okon et al., 1995). However, we found no alterations in the contractile machinery in response to phenylephrine or electrical-field stimulation in corporal smooth muscle from sedentary and trained diabetic animals. On the other hand, the inhibitor of Rho-kinase activity Y-27632 provoked a rightward shift in the relaxation responses of the corpus cavernosum smooth muscle from sedentary diabetic rats, suggesting an enhancement of the RhoA/Rho-kinase signaling pathway. The activation of a specific Rho protein named RhoA promotes the stimulation of the enzyme Rho-kinase, which regulates the activity of myosin light chain phosphate increasing Ca^{2+} sensitivity, which in turn has been associated with increased vascular resistance (Lee et al., 2004). Today, the RhoA/Rho-kinase signaling pathway is believed to play an important role in the genesis of hypertension, diabetes, hypogonadism and ED (Linder et al., 2005). Our data are consistent with the hypothesis that RhoA/Rho-kinase and NO/cGMP pathways are physiological antagonists, and possibly an imbalance between these pathways may contribute to detumescence and flaccidity of the penis in our diabetes model (Masumoto et al., 2001). Our findings showed that exercise training restored sensitivity of the corporeal smooth muscle for Y-27632 and re-established the balance between RhoA/Rho-kinase and NO/cGMP pathways.

In conclusion, our study suggests that exercise training prevents the impairment of the relaxation responses in corpus cavernosum from streptozotocin-induced diabetic rats and this beneficial effect was associated with increased plasma SOD levels. These data reinforce the idea that regular physical exercise is an important approach in preventing ED associated with diabetes mellitus through re-establishing the balance between NO production and inactivation.

Conflict of interest statement

The authors declare no conflict of interest.

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