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ARTICLE

Exercise and caloric restriction improve cardiovascular and erectile function in rats with metabolic syndrome

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Abstract

The aim of this study is to examine the possible benefits of exercise and caloric restriction (CR) on cardiovascular hemodynamics, erectile function, and antioxidant system in metabolic syndrome (MS). Sixty male Spraque-Dawley rats were divided into five groups; control, MS, MS + CR, MS + exercise (EXC), and MS + CR + EXC. To induce MS, 10% fructose solution was applied for 3 months. Thereafter, in CR groups calorie was restricted 40% and in EXC groups swimming was performed for 6 weeks. Body weight, blood glucose, and blood pressure (BP) levels were measured before and after MS induction and at the end of the experiment. After decapitation, tumor necrosis factor (TNF)-α, adiponectin (ADP), and plasminogen activator inhibitor (PAI)-1 levels were investigated in blood, oxidative stress parameters were examined in heart, aorta, and corpus cavernosum (CC) tissues. Isometric contraction in isolated tissue bath was studied in aorta and CC tissues. Animals subjected to exercise and CR had decreased BP and blood glucose levels. Impaired contraction–relaxation responses in MS group were improved with exercise and CR. MS-induced increase in TNF-α, PAI-1, malondialdehyde (MDA), and decrease in ADP, glutathione (GSH), and superoxide dismutase (SOD) were normalized with exercise and CR. Exercise and CR may be beneficial against changes in cardiovascular hemodynamics caused by MS.

Introduction

Metabolic syndrome (MS) is characterized by coexistence of abdominal obesity, dyslipidemia, high blood pressure (BP), and impaired glucose metabolism [1]. MS generates endothelial dysfunction due to the oxidative stress that results in serious complications [2]. Increased reactive oxygen species reduce nitric oxide (NO) bioavailability. Since NO is the main neurotransmitter for erection, loss of NO limits blood flow to cavernosal tissues and impairs erectile function [3, 4].

Adipose tissue contributes to inflammation by secreting proinflammatory cytokines such as tumor necrosis factor (TNF)-α, while adiponectin (ADP), a cytokine secreted from adipose tissue, has protective effects on the endothelium [5]. In patients with obesity-related coronary artery disease, low plasma ADP levels are closely related to endothelial dysfunction [6]. Plasminogen activator inhibitor (PAI)-1, inhibiting plasmin formation that eliminates fibrinolysis, accelerates the atherosclerotic process in obesity and MS [7].

In developing countries, along with the obesity epidemic [8], MS also reaches a dramatic level and impairs the quality of life [9]. Erectile dysfunction (ED) shares many risk factors seen in systemic inflammatory conditions such as cardiovascular diseases and MS [10]. Exercise (EXC) and calorie restriction (CR) can be protective against metabolic diseases by regulating mitochondrial functions [11].

MS-related animal studies are usually conducted with dietary approaches, such as fructose or fat-enriched diets [12]. MS following high fructose feeding has been a debatable subject, because it was used as an insulin resistance model and resulted in non-significant overweight [13, 14]. However, there are articles that reported successful

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results with fructose-enriched diet on development of MS [15]. Since refined sugar intake is a common reason of MS in humans [16], we preferred this model in the present study. In clinical trials, lifestyle modifications improve ED in obesity and MS through ameliorating oxidative stress and inflammation, improving endothelial NO bioavailability and testosterone levels [17]. The studies investigating the effects of EXC and CR in MS-induced ED animal models are substantially restricted [18] and their effect mechanisms have not been clearly elucidated yet. Accordingly, in the present study, we investigated the effects of EXC and CR on MS-induced changes in cardiovascular hemodynamics and on oxidative tissue damage.

Materials and methods

Animal and experimental design

Male Sprague-Dawley rats (180–200 g, 6 weeks old) obtained from Marmara University Experimental Animal Research Center were housed in an air-conditioned room with 12:12 h light:dark cycles. Temperature (22 ± 2 °C) and relative humidity (65–70%) were kept constant. Experimental protocols were approved by Marmara University Animal Care and Use Committee (82.2012.mar).

Rats were divided into five groups; each consisting of 12 animals: control (C), MS, MS + CR, MS + EXC, and MS + CR + EXC. To induce MS, 10% fructose solution in drinking water was applied to animals for 12 weeks [11]. Thereafter in CR, EXC and CR + EXC groups feeding with 10% fructose solution in drinking water was interrupted. For CR, daily calorie intake was restricted 40% for 6 weeks [15]. In the EXC groups, swimming in a heated water pool (30–32 °C) for 30 min a day and 3 days a week was performed for 6 weeks [15, 19]. Feeding with 10% fructose solution continued over the next 6 weeks in MS group.

Body weight, BP, and blood glucose levels of the animals were measured at baseline, 12 and 18 weeks later. After decapitation, blood and tissue (heart, aorta, and corpus cavernosum (CC)) samples were taken. ADP, TNF-α, and PAI-1 levels were examined in blood samples, malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) as oxidative stress markers were examined in tissue samples. Isometric contraction was performed in isolated organ bath in aortic and CC tissues.

There are 12 animals in each group in the study. Tissue strips for isolated organ bath were taken from six animals.

BP level measurement

BP levels were measured with tail-cuff plethysmography method (MP35 Biopac Systems, Inc. Commat Ltd., Ankara, Turkey). At least three times BP levels were measured in each rat and the average was recorded [20].

Blood glucose level measurement

Blood glucose levels were measured with the glucometer (Accu-Chek, Switzerland) in blood samples taken from the orbital vein under mild ether anesthesia.

Isolated organ bath studies

Surrounding connective tissues of CC and aorta were removed. Aortic segments were cut into rings and CC tissues were cut into strips. All tissues were mounted in organ baths containing Krebs-bicarbonate buffer. The solutions were aerated with 95% O_2 and 5% CO_2 (pH 7.4) at 37 °C. The tissues were equilibrated for 45 min under a resting tension of 0.5 g. Isometric contractions were recorded using a Model FT03 force displacement transducer (Grass Instruments, Quincy, MA, USA) coupled to a Model 7 polygraph (Grass Instruments). After the equilibration period, CC samples were exposed to 124 mM KCl, aorta samples were exposed to 80 mM KCl. The contractile responses of the corporeal tissues and aorta rings to 10^{-9}–10^{-3} M phenylephrine were determined cumulatively and expressed as percentage of the maximal contraction induced by KCl.

In CC and aorta, after a 30 min washout period, submaximal phenylephrine concentrations were applied to obtain relaxation responses. The relaxation responses were evaluated by adding increasing cumulative concentrations of carbachol (10^{-9}–10^{-3} M). The relaxation responses to carbachol are expressed as percentages of the contraction caused by phenylephrine [21].

Blood sample TNF-α, ADP, and PAI-1 levels measurement

Serum TNF-α, ADP, and PAI-1 levels were measured according to the manufacturer's instructions and guidelines of ELISA kits specific for rats (BT Lab, Shanghai Korain Biotech Co Ltd.).

Measurements in tissue samples

MDA levels

A solution containing thiobarbituric acid and trichloroacetic acid was added to the homogenate. According to Beuge and Aust's method, the absorbance of the color of the samples cooled and centrifuged after being kept in a boiling water bath for 15 min was read at 532 nm. Results are expressed in nmol/g tissue [22].

GSH levels

According to Beutler method, Na_2HPO_4 and Ellman solution were added to the sample, the absorbance was read on spectrophotometer at 412 nm. Results are expressed in µmol/g tissue [23].

SOD activity

According to the method of Mylorie et al., with the buffer prepared with potassium phosphate buffer-EDTA, riboflavin and o-dianisinide, the absorbance of color formed in samples kept under fluorescent light and at 37 °C is read at 460 nm. The results are expressed in U/mg protein [24].

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Data were expressed as the mean ± SEM. Biochemical data were analyzed with ANOVA followed by Turkey's multiple comparison tests. Statistical significance was accepted as $p < 0.05$.

Results

Body weights

Body weights increased ($p < 0.001$) after 3 months (T2) in all groups (Fig. 1a). At the end of the experiment (T3), they were higher than baseline (T1) ($p < 0.001$). Body weight of the MS group in T3 was higher ($p < 0.05$) comparing to this period of other groups.

BP levels

BP levels were higher at T2 in MS groups than baseline ($p < 0.001$) (Fig. 1b). EXC and CR decreased BP compared to T2 ($p < 0.001$). In both groups, these values remained higher than T1 ($p < 0.05$). Increase in BP caused by MS (T2, $p < 0.001$) was reduced with CR + EXC ($p < 0.001$).

Blood glucose levels

In MS groups, blood glucose levels increased in T2 compared to T1 ($p < 0.001$) (Fig. 1c). In T3, blood glucose levels reduced in MS + CR, MS + EXC, and MS + CR + EXC groups ($p < 0.001$). Although EXC lowered blood glucose level, it remained higher than baseline values ($p < 0.05$).

Aortic contractions and relaxations

Contractile responses of aortic tissue with cumulatively added phenylephrine in MS group were lower at 10^{-7}–3×10^{-4} M concentrations compared to C (Fig. 2a). Contractile responses in MS + CR and MS + EXC groups were lower than C, whereas in MS + CR + EXC group it was back to C and increased compared to MS group ($p < 0.05$).

EC_{50} of MS, MS + CR, and MS + EXC groups were higher than C (Table 1). EC_{50} of MS + CR + EXC group was close to C and lower than MS group ($p < 0.05$).

Aorta submaximal concentrations are determined as C group 3×10^{-6} M, MS group 10^{-5} M, MS + CR group 10^{-5} M, MS + EXC group 10^{-5} M, MS + CR + EXC 10^{-5} M.

After the contraction given to the submaximal concentration of phenylephrine, the relaxation responses to carbachol at cumulative concentrations were lower in MS group than in C ($p < 0.05$) (Fig. 2b). Relaxation responses in MS + CR and MS + EXC groups decreased in 10^{-7}–10^{-5} M concentrations. In MS + CR + EXC group, there was increase in 3×10^{-8}–3×10^{-4} M concentrations compared to MS group. EC_{50} of the relaxation responses belonging to MS, MS + CR, and MS + EXC groups increased compared to C group, whereas the EC_{50} was similar to the C in MS + CR + EXC group.

CC contractions and relaxations

Cavernous tissue contraction responses with phenylephrine decreased in MS group in the concentrations of 3×10^{-7}–3×10^{-4} M compared to C (Fig. 2c). Contraction response in MS + CR + EXC group was increased at 3×10^{-5}–3×10^{-4} M concentrations compared to MS group ($p < 0.05$). However in MS + CR, MS + EXC, and MS + CR + EXC groups, EC_{50} values elevated.

Corporeal tissues submaximal concentrations are determined as C group 3×10^{-5} M, MS group 10^{-5} M, MS + CR group 3×10^{-5} M, MS + EXC group 3×10^{-5} M, MS + EXC + CR group 3×10^{-5} M.

The relaxation responses to the cumulative carbachol concentration applied after submaximal concentration phenylephrine application showed decrease in the concentrations of 10^{-7}–3×10^{-4} M of MS group compared to C (Fig. 2d). The decrease observed in the relaxation responses of MS + CR and MS + EXC groups occurred at concentrations of 3×10^{-7}–3×10^{-4} M. Relaxation responses of MS + CR and MS + CR + EXC groups showed difference compared to MS group ($p < 0.05$). EC_{50} increased in MS, MS + CR, MS + EXC groups compared to C group and EC_{50} in MS + CR + EXC group decreased compared to MS group and approached to C ($p < 0.05$) (Table 1).

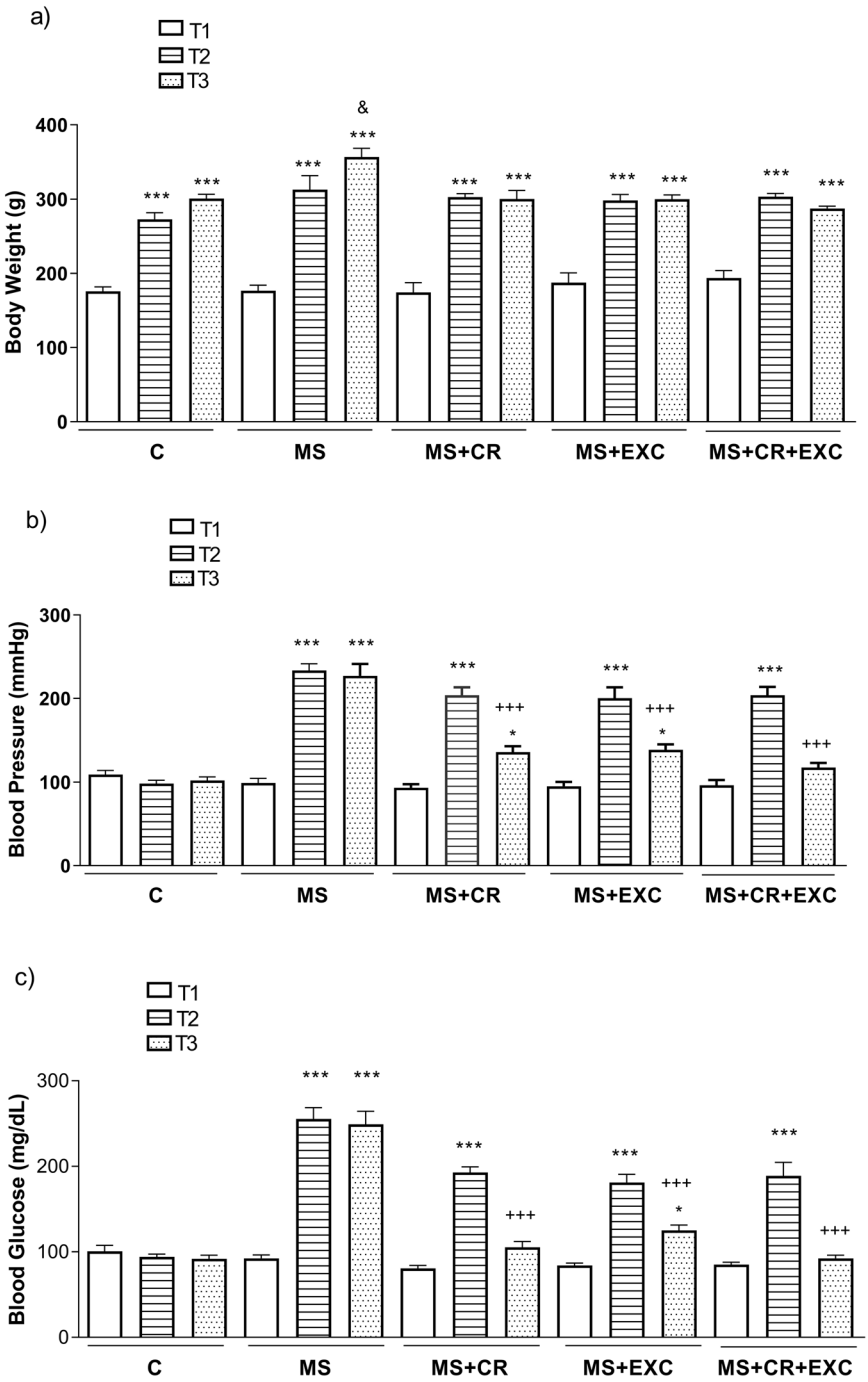

Fig. 1 Body weight, blood pressure and blood glucose levels at different time points. T1: baseline, T2: after 3 months 10% fructose diet administration, T3: at the end of the experiment. C: control, MS: metabolic syndrome, CR: caloric restriction, EXC: exercise. Each group consists of 12 animals. Data are expressed as mean ± SEM. Data are analyzed with variance analysis (ANOVA) followed by Tukey multiple comparison tests. *$p < 0.05$, ***$p < 0.001$ compared to T1, $^{+++}p < 0.001$ compared to T2, $^{\&}p < 0.05$ comparison of all groups' T3.

Blood sample TNF-α, ADP, and PAI-1 levels

While TNF-α levels increased ($p < 0.001$) with MS, this increase was lower in CR and EXC + CR groups ($p <$ 0.05–0.01) (Fig. 3a).

ADP levels were decreased in MS group ($p < 0.001$). CR ($p < 0.01$), EXC ($p < 0.001$), and CR + EXC ($p < 0.01$) increased ADP levels (Fig. 3b).

While the levels of PAI-1 increased with MS ($p < 0.01$), this increase was reversed with CR, EXC, and CR + EXC ($p < 0.001$) (Fig. 3c).

Biochemical measurements in tissues

MDA levels increased in MS group ($p < 0.001$). EXC, CR, and CR + EXC reduced MDA levels by

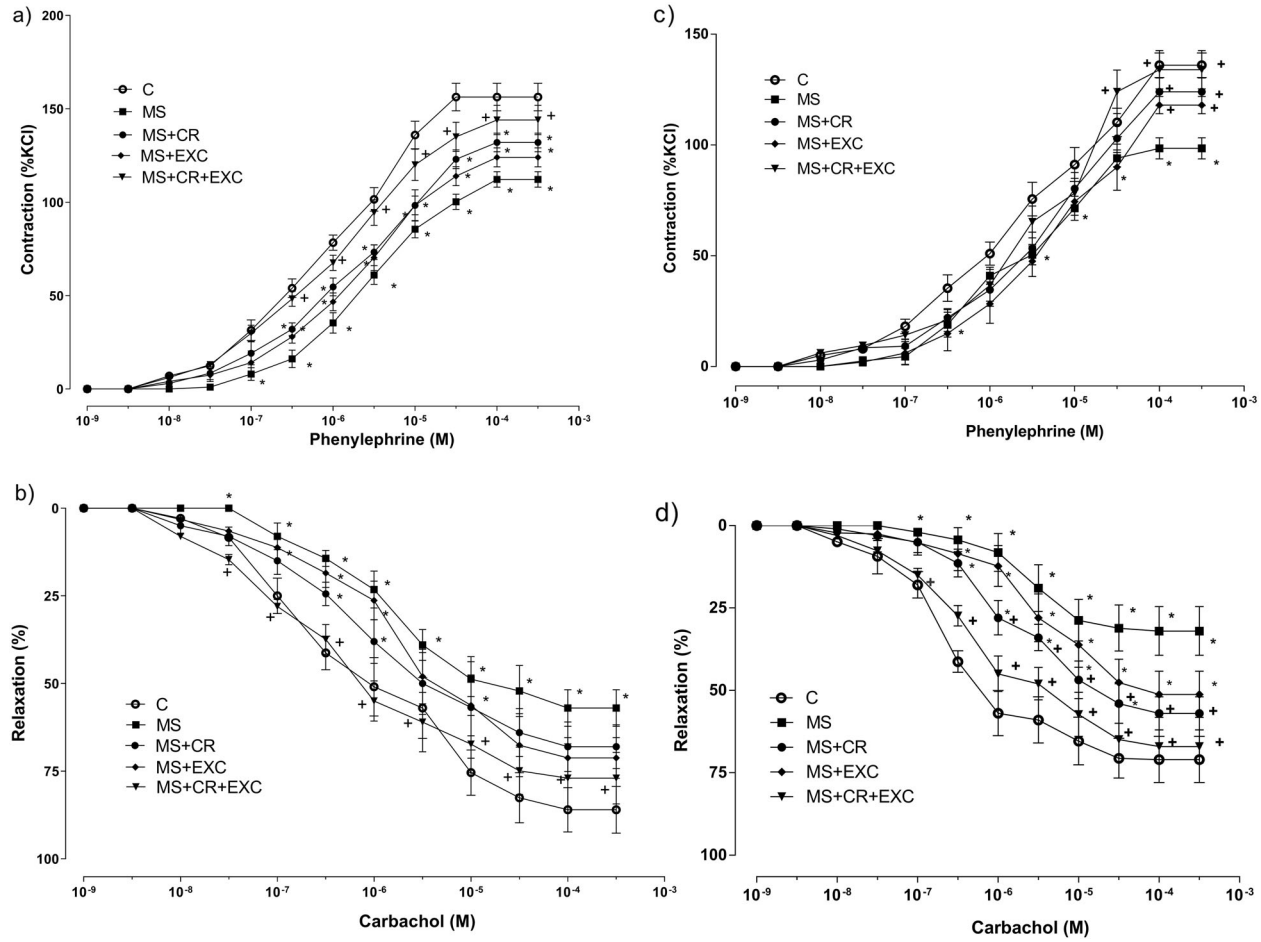

Fig. 2 Concentration-response curves of all groups. C: control, MS: metabolic syndrome, CR: caloric restriction, EXC: exercise. Tissue samples of six animals were used from each group. Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA) and Tukey post hoc test were used for statistical analysis. *T*-test was used in binary comparisons of concentrations. **a** The concentration–response curve obtained by applying cumulative phenylephrine in rat aortic tissue. Contractions are expressed as % of KCl (80 mM) contraction. **b** The concentration–response curve obtained by applying cumulative carbachol in rat aortic tissue. Relaxations are expressed as % of the contraction response with a submaximal concentration phenylephrine. **c** The concentration–response curve obtained by applying cumulative phenylephrine in rat corpus cavernosum tissue. Contractions are expressed as % of KCl (124 mM) contraction. **d** Concentration–response curve obtained by applying cumulative carbachol in rat corpus cavernosum tissue. Relaxations are expressed as % of the contraction response with a submaximal concentration phenylephrine. $^{*}p < 0.05$ compared to control group, $^{+}p < 0.05$ compared to MS group.

Table 1 EC_{50} values in contractile responses obtained with phenylephrine applied cumulatively to aortic and corpus cavernosum tissues, EC_{50} values in relaxation responses obtained with carbachol applied cumulatively to aortic and corpus cavernosum tissues.

	C	MS	MS + CR	MS + EXC	MS + CR + EXC
EC_{50} Phenylephrine applied to aortic tissue	1.06×10^{-6}	2.5×10^{-6}*	2.0×10^{-6}*	2.1×10^{-6}*	$1.1 \times 10^{-6+}$
EC_{50} Carbachol applied to aortic tissue	4.5×10^{-7}	1.3×10^{-6}*	6.9×10^{-6}*	1.7×10^{-6}*	$2.8 \times 10^{-7+}$
EC_{50} Phenylephrine applied to corpus cavernosum tissue	2.2×10^{-6}	2.3×10^{-6}	4.6×10^{-6}*	5.2×10^{-6}*	4.7×10^{-6}*
EC_{50} Carbachol applied to corpus cavernosum tissue	2.4×10^{-7}	2.3×10^{-6}*	1.4×10^{-6}*	3.3×10^{-6}*	$4.8 \times 10^{-7+}$
E_{max} 80 mM KCl applied to aortic tissue	1346 ± 114	1305 ± 135	1332 ± 119	1328 ± 131	1341 ± 127
E_{max} 124 mM KCl applied to corpus cavernosum tissue	3532 ± 145	3289 ± 125	3428 ± 116	3367 ± 129	3512 ± 133

E_{max} values (mg) for 80 mM KCl for aortic tissue and 124 mM KCl for corpus cavernosum. Tissue samples of six animals from each group were used. Data are analyzed with variance analysis (ANOVA) followed by Tukey multiple comparison tests.

C control, *MS* metabolic syndrome, *CR* caloric restriction, *EXC* exercise.

$^{*}p < 0.05$ compared to control group; $^{+}p < 0.05$ compared to MS group.

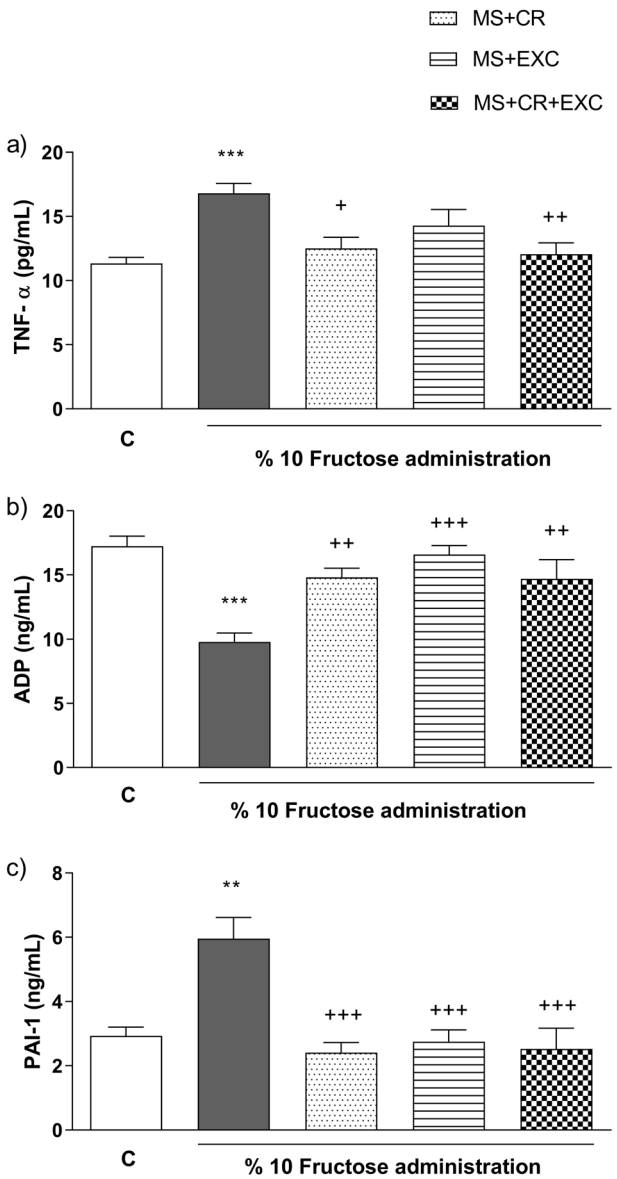

Fig. 3 Serum tumor necrosis factor (TNF)-α, adiponectin (ADP) and plasminogen activator inhibitor (PAI)-1 levels. C: control, MS: metabolic syndrome, CR: caloric restriction, EXC: exercise. Each group consists of 12 animals. Data are expressed as mean ± SEM. Data are analyzed with variance analysis (ANOVA) followed by Tukey multiple comparison tests. $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control group, $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ compared to MS group.

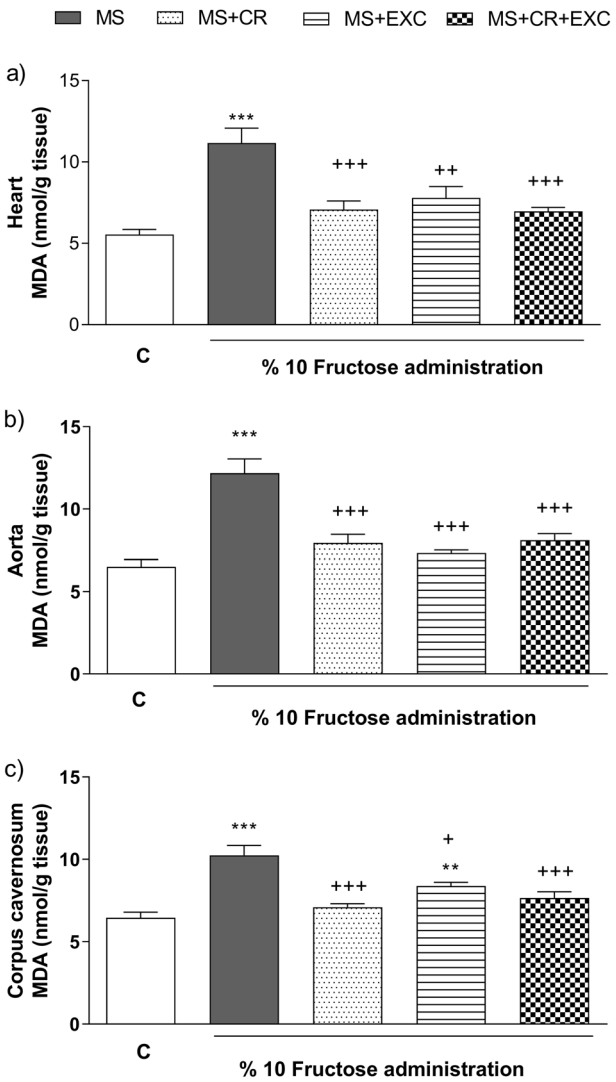

Fig. 4 Malondialdehyde (MDA) levels in the heart, aorta, and corpus cavernosum tissues. C: control, MS: metabolic syndrome, CR: caloric restriction, EXC: exercise. Each group consists of 12 animals. Data are expressed as mean ± SEM. Data are analyzed with variance analysis (ANOVA) followed by Tukey multiple comparison tests. $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control group, $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ compared to MS group.

reducing oxidative damage in tissues ($p < 0.05$–0.001) (Fig. 4).

GSH levels decreased ($p < 0.05$–0.001) in MS group, whereas CR increased GSH levels ($p < 0.05$–0.01). GSH levels were elevated with EXC in the aorta and with CR + EXC in the heart tissue ($p < 0.05$–0.01) (Fig. 5).

The SOD activity decreased ($p < 0.001$) in MS group. SOD activity increased in MS + CR group ($p <$ 0.05–0.001). SOD levels in heart and cavernous tissue increased with EXC ($p < 0.05$–0.001). EXC + CR increased SOD activity in the tissues ($p < 0.05$–0.001); in the aorta and CC tissues, these increases were higher than that of CR or EXC applied alone ($p < 0.05$–0.001) (Fig. 6).

Discussion

The present study demonstrates that MS caused oxidative damage as evidenced by increased MDA levels and decreased GSH and SOD levels. MS reduced contraction–relaxation responses in aorta and CC tissues. The findings of the present study showed that EXC and CR

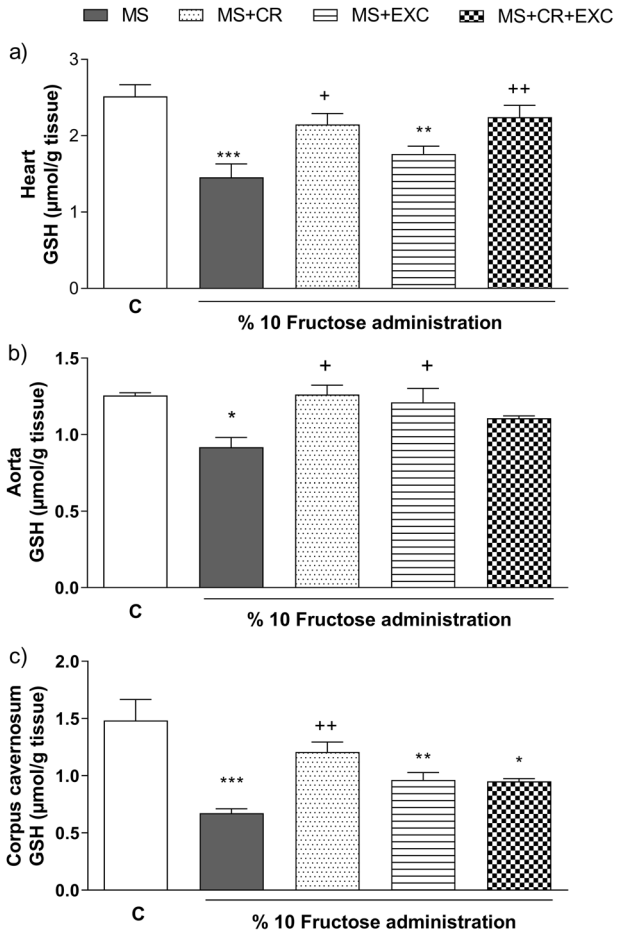

Fig. 5 Glutathione (GSH) levels in the heart, aorta, and corpus cavernosum tissues. C: control, MS: metabolic syndrome, CR: caloric restriction, EXC: exercise. Each group consists of 12 animals. Data are expressed as mean ± SEM. Data are analyzed with variance analysis (ANOVA) followed by Tukey multiple comparison tests. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control group, $^{+}p < 0.05$, $^{++}p < 0.01$ compared to MS group.

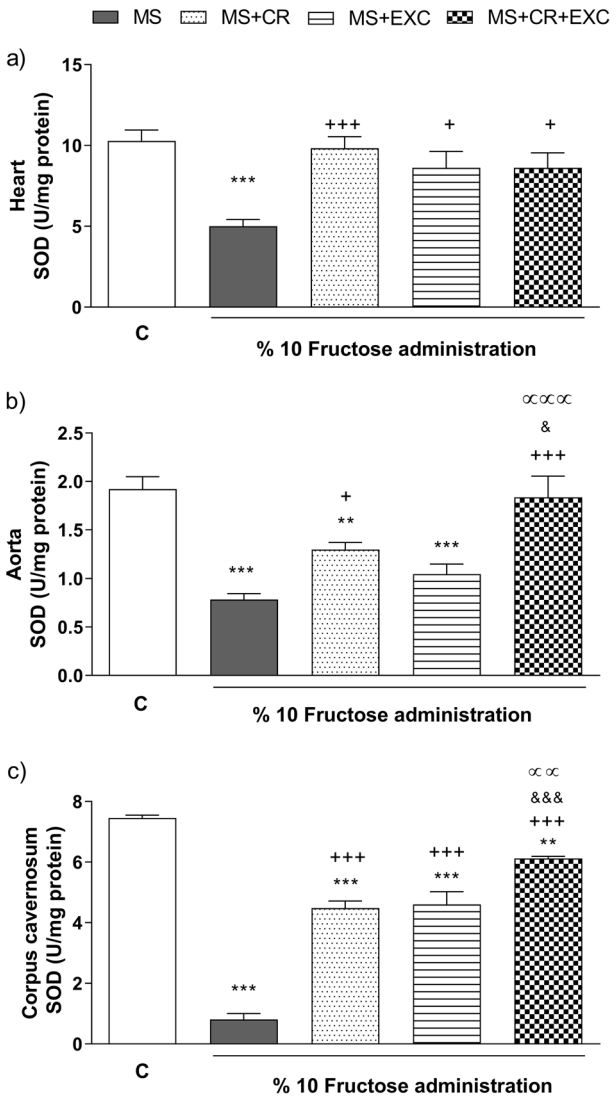

Fig. 6 Superoxide dismutase (SOD) levels in the heart, aorta, and corpus cavernosum tissues. C: control, MS: metabolic syndrome, CR: caloric restriction, EXC: exercise. Each group consists of 12 animals. Data are expressed as mean ± SEM. Data are analyzed with variance analysis (ANOVA) followed by Tukey multiple comparison tests. $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control group, $^{+}p < 0.05$, $^{+++}p < 0.001$ compared to MS group, $^{\&}p < 0.05$, $^{\&\&\&}p < 0.001$ compared to MS + CR group, $^{\alpha\alpha}p < 0.01$, $^{\alpha\alpha\alpha}p < 0.001$ compared to MS + EXC group.

can reverse these changes and protect cavernosal tissue against fructose-induced MS damage.

Refined sugar intake is a common reason of MS in humans [16] and ED is associated with permanent disability and reduced life expectancy [17]. Patients with MS [25] and ED [26] represent an important financial burden on healthcare systems. In addition to common pathological features of MS such as hypertension and dyslipidemia [1], ED is also an important problem affecting quality of life in men with MS [17].

Studies examining the effect of MS on ED are limited to high-fat diet triggered MS animal model [27]. Previous studies have not drawn attention to fructose-induced MS as a separate causative factor. MS needs to be evaluated with its effects on cardiovascular hemodynamics such as blood glucose and BP levels, contraction–relaxation responses of aortic and CC tissues and oxidative stress parameters. Treatment of ED caused by MS should aim to control the cardiovascular hemodynamic and metabolic parameters and suppress oxidant damage. Lifestyle modifications offer a solution for MS [28] and ED [17]. Accordingly, we evaluated effects of EXC and CR on ED in MS rats.

Elevated blood glucose and BP levels have important effects on the endothelium in erectile tissues. Endothelial injury causes ED by impairing the relaxation of cavernosal tissues and vascular smooth muscles [29, 30]. In our study, EXC and CR provided weight loss and improved blood glucose levels and BP, which can play an important role in tissue protective activity. In EXC group, blood glucose

levels were higher than baseline values. Only in CR + EXC group, BP levels were not higher compared to baseline values, demonstrating these two lifestyle modifications are needed to improve MS-induced ED.

Since NO is an important mediator for relaxation process in both vascular and penile smooth muscles [31], reduction in NO levels would be reasonable for increase in BP [32] and ED [33] due to endothelial damage. Although NO levels were not determined in this study, we have concluded that MS caused elevation in oxidative stress. NO is combined with superoxide anion radicals and converted to peroxynitrite [34]. There are many studies showing that CR and EXC prevent endothelial damage [35, 36].

Oxidative stress impairs contractile and relaxation responses of tissues to various agents due to endothelial damage [37]. We determined that corporeal and aortic tissues of rats with MS had attenuated responses to relaxant and contractile agents comparing to C group. Since the endothelial layer regulates the tone of vascular structures by secreting many vasoactive autocoids [38], in our study, the impaired responses may be caused by the loss of endothelial integrity due to increased oxidant damage due to MS. EXC and CR improved the contraction–relaxation responses in the aortic and CC tissues.

Regular EXC plays an important role in preventing cardiovascular system diseases, delaying the progression of preexisting diseases, or reducing the complications of diseases. However, inappropriate EXC has opposite effects and creates shear-force in the circulatory system [39]. EXC type, frequency, intensity, and experimental model determine the effects of EXC [38]. In the swimming EXC, rats can swim comfortably, unless there is any power requirement, such as pulling the weight with rope attached to tail. Kramer et al. emphasized the importance of duration and intensity of swimming EXC in their experimental study. They demonstrated that SOD activity in erythrocytes did not reduce after EXC, unless emotional stimulation was created in the rats [40]. Low and moderate EXC is protective against stress-induced multiorgan damage and high-fat diet-induced tissue damage with the antioxidant effects [39, 41, 42]. Since ED is related to cardiovascular risk factors such as obesity and MS [43], physical activity can be a solution to ED. In the present study, low intensity swimming ameliorated the damage caused by MS. Supportively, Macit et al. reported beneficial effects of low intensity swimming program on the aortic and corporeal tissue aging [44].

During oxidative reactions, measurable end product levels such as MDA elevate [45]. In our study, MDA levels which are the indicator of lipid peroxidation were increased in the heart, aortic, and CC tissues in MS group. EXC, CR, and CR and EXC depressed the increase in MDA levels in the tissues. Although MDA levels were determined higher in CC tissues of EXC group compared to control animals, they were lower than MS group's levels.

In the MS group, GSH levels and SOD activity reduced in all tissues. CR restored the GSH levels and SOD activity in the tissues. With EXC, GSH levels increased in aortic tissue, and SOD levels elevated in the heart and cavernous tissues. In EXC and CR group, GSH levels elevated in heart tissue while SOD activity increased in all three tissues. These increases in SOD activity in the tissues of aorta and CC were higher than those of individual CR or EXC.

ADP is a remarkable parameter in the follow-up of obesity [46]. Lemos et al. demonstrated the increase in ADP levels in the rats that performed regular swimming EXC [19]. In the present study, levels of ADP, which is a protective cytokine in serum, decreased in MS group. It was observed that both CR and EXC increased serum ADP levels.

PAI-1 affects the vascular homeostasis by inhibiting the activation of plasminogen that reduces fibrinolytic activity and increases cardiovascular risk. In an animal study, obesity and insulin resistance were prevented in mice with PAI-1 deficiency [47]. In the present study, while serum PAI-1 levels increased in animals with MS, this elevation was reversed with CR and EXC.

TNF-α is an apoptotic cytokine which generates insulin resistance by reducing the number of insulin receptors. Elevated TNF-α blood levels in obese people with diabetes were observed [48]. In the present study, TNF-α levels increased with MS, whereas this increase was decreased in CR and EXC–CR groups.

In MS, when comparing three different lifestyle modifications considering all parameters, the best results are provided by CR and EXC combination. Moreover, as vascular and cavernoal tissues showed structural similarity and have common contraction–relaxation mechanism [49], CR and EXC did not have different impacts in the aortic and cavernosal functions. In conclusion, lifestyle modifications can be useful approach for cardiovascular diseases and ED seen in MS with hypotensive, hypoglycemic, anti-inflammatory, and antioxidant effects.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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