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ORIGINAL ARTICLE

Regular swimming exercise performed either before or after the induction of renovascular hypertension alleviates oxidative renal injury in rats

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Abstract

Epidemiological studies have shown that regular exercise and increased aerobic fitness are associated with a decrease in all-cause mortality and morbidity, including diseases related with high blood pressure. However, whether exercise has an anti-inflammatory impact on the pathogenesis of hypertension was not elucidated yet. In the present study, to investigate the potential protective and therapeutic effects of exercise training (swimming for 30 min/day, 5 days/week for 9 weeks) on renovascular hypertension (RVH) 10-week-old male Wistar albino rats were divided into 4 groups as sham-operated sedentary control group, sedentary group with RVH (2-Kidney, 1-Clip Goldblatt) and two exercised RVH groups, which had 9-week training either before the surgery or after the surgery. Systolic blood pressures (SBP) were measured by the tail-cuff method on a weekly basis and at the end of 12 weeks, rats were decapitated to obtain kidneys. SBP were significantly higher in the sedentary RVH group than the control group, whereas in the trained RVH group measurements were not different than those of the control animals. In the renal tissues of the sedentary RVH group, malondialdehyde and myeloperoxidase levels were increased with a concomitant decrease in glutathione levels, while in the trained RVH group the levels were not different than those of the control group. Moreover, in the trained RVH group, superoxide dismutase and catalase levels measured as antioxidant parameters, were also significantly increased as compared with those of the sedentary RVH group. Current results demonstrate that regular moderate training controls high blood pressure in RVH, while RVH-induced oxidative damage in renal tissue is ameliorated through the modulation of oxidant-antioxidant balance. Exercise training does not only improve the circulatory functions, but it also initiates an anti-inflammatory process to defend against the angiotensin-II-induced renal injury.

Keywords: Exercise, hypertension, kidney, oxidative stress

INTRODUCTION

Renovascular hypertension (RVH) is described among the most prevalent and important causes of secondary hypertension and renal dysfunction, which may be mostly caused by atherosclerotic disease, reduced perfusion pressure, including fibromuscular diseases and renal infarction (1). It was suggested that RVH develops from the activation of a variety of pressor signals, including the renin-angiotensin system, oxidative stress and sympathoadrenergic pathways (1). Higashi et al.(2) have shown in patients with RVH that oxidative stress parameters were increased in parallel with increased plasma angiotensin (Ang) II levels. Following renal angioplasty plasma renin activity, plasma Ang II concentration, as well as serum malondialdehyde-modified low-density lipoprotein concentration and urinary 8-hydroxy-2'-deoxyguanosine excretion were decreased in these patients with RVH, verifying the involvement of oxidative stress in the ethiopathogenesis of RVH. Accordingly, several potent antioxidants were investigated to be used as antihypertensive agents and were shown to reduce blood pressure by improving

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baroreflex sensitivity, down-regulating vascular matrix metalloproteinases or scavenging the oxygen radicals that would inactivate vasodilatory action of nitric oxide (NO)(3,4,5). Similar to antioxidants, aerobic exercise training was shown to reduce oxidative stress and inflammation and to increase anti-oxidant defenses by enhancing the resistance against reactive oxygen-induced lipid peroxidation and DNA damage (6,7).Voluntary running exercise in spontaneously hypertensive rats was shown to reduce plasma renin activity and plasma renin concentration (8) and improve microcirculatory profile in different tissues (9,10), making exercise a recommended non-pharmacological treatment modality in hypertension (11,12).

Although exercise is known to lower blood pressure (13) and hyperlipidemia (14)in humans, and has long been accepted as an important component in the treatment of diabetes (15), not much is known about the effects of exercise on renal disease. Despite that exercise endurance is lowered in patients with renal dysfunction (16), physical training is proven to improve quality of life and nutrient metabolism in patients with kidney disease (17). Recently, it was shown in hypertensive rats induced with an inhibitor of NO synthesis, exercise training had hypotensive effect in rats and physical training could protect against oxidative renal injury caused by hypertension (18). Similarly, it was shown in rats with chronic renal disease that exercise exerted a positive influence on oxidative stress parameters (19). Based on these observations, current study was aimed to elucidate the protective and therapeutic effects of exercise on renovascular hypertension-induced oxidative injury of the kidneys.

MATERIALS AND METHODS

Animals and experimental design

Male Wistar albino rats (300-350 g, 10 weeks old, n=55), supplied by the Marmara University (MU) Animal Center (DEHAMER; approval no: 22.2009.mar), were housed in a temperature-controlled (22 ± 2°C) room with relative humidity (65-70%) and standardized light/dark (12/12 hour) cycles. Rats were fed with standard rat pellets and tap water ad libitum. All experimental protocols were approved by the MU Animal Care and Use Committee.

Rats were randomly assigned to sham-operated control (n=10) and renovascular hypertension (RVH) groups (n=45). RVH rats were either kept sedentary throughout the 12-week follow-up period of the experiment (n=15) or had swimming exercise for 9 weeks either before (n=15) or after (n=15) RVH surgery. In order to produce a unilateral Ang-dependent RVH (2-kidney-1-clip, 2K1C) model, briefly a silver clip (internal diameter 0.25 mm) was placed around the left renal artery of rats, which were anaesthetized with ketamine (100 mg/kg) and chlorpromazine (0.75 mg/kg) given intraperitoneally (ip) (20). In the sham-operated control group, rats had similar surgical procedures except the clip placement.

Rats in the exercise condition were subjected to a moderate load swimming exercise (21) in which they swum for 30 min per day and 5 days per week for nine consecutive weeks. Swimming was chosen as the exercise model, because it is a natural ability of the rats and has advantages over treadmill running (22). The swimming sessions were made in a glass pool (100 cm x 50cm) with a 50 cm height filled with lukewarm water (32± 28ºC) to a depth of 35 cm. Sedentary rats were placed in the bottom of a separate tank with shallow water (5 cm) where they could stand on their feet.

Blood pressure measurements

Before the surgery and at weekly intervals, blood pressure measurements were made by the tail-cuff method. It was previously described in the RVH model that hypertension develops and plasma renin and angiotensin II content increase during the first 3 or 4 weeks (23). After the 9 weeks, hypertension becomes renin-independent, because both plasma renin and angiotensin II content return to normal. In the current study, at the end of the 12-week experimental procedure, exercised-before-RVH group has had a 3-week-RVH, because RVH was induced following 9 weeks of exercise. Exercised-after-RVH group has had a 12-week-RVH at the end of the experimental procedures, because 9 weeks of exercise was started 3 weeks after the induction of RVH.

Indirect blood pressure measurements were made by the tail-cuff system (Biopac MP35 Systems, Inc. COMMAT Ltd. Ankara, Turkey). Initially, the rats were placed for 10 min in a chamber heated to 35°C. Then the rats were placed in individual plastic restrainers and the cuffs equipped with pneumatic pulse sensors were wrapped around their tails. Blood pressure recording for each measurement period was averaged from at least three consecutive readings on that occasion obtained from each rat.

Biochemical measurements

At the end of the 12-week experimental procedure, rats were decapitated. The kidneys were rapidly removed and the non-clipped left kidneys were removed and weighed. Kidney samples obtained from each animal were stored at –80°C until the determination of tissue myeloperoxidase (MPO) activity, malondialdehyde (MDA), glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) levels.

Measurement of renal myeloperoxidase (MPO) activity

Since tissue MPO activity was shown to correlate significantly with the number of neutrophils determined histochemically, it is frequently used to estimate tissue neutrophil accumulation in the inflamed tissues (24). The method used for the assay of MPO activity was similar to that was previously described by others (24). Kidney samples (0.2–0.3 g) were homogenized in 10 volumes of ice-cold potassium phosphate buffer (50 mm K_2HPO_4, pH 6.0) containing hexadecyltrimethylammonium bromide (HETAB; 0.5%, w/v). The homogenate was centrifuged at 41,400 g for 10 min at 4°C, and the supernatant was discarded. The pellet was then re-homogenized with an equivalent volume of 50 mm K_2HPO_4 containing 0.5% (w/v) HETAB and 10 mm EDTA (Sigma Chemical Co., St. Louis, MO, USA). MPO activity was assessed by measuring the H_2O_2-dependent oxidation of o-dianisidine.2HCl. One unit of enzyme activity was defined as the amount of MPO present per gram of tissue weight that caused a change in absorbance of 1.0 min^{-1} at 460 nm and 37°C.

Measurement of renal malondialdehyde (MDA) and glutathione (GSH) levels

Samples of kidney tissues were homogenized in 10 volumes of ice-cold 10% trichloracetic acid in an Ultra Turrax tissue homogenizer. Homogenized tissue samples were centrifuged at

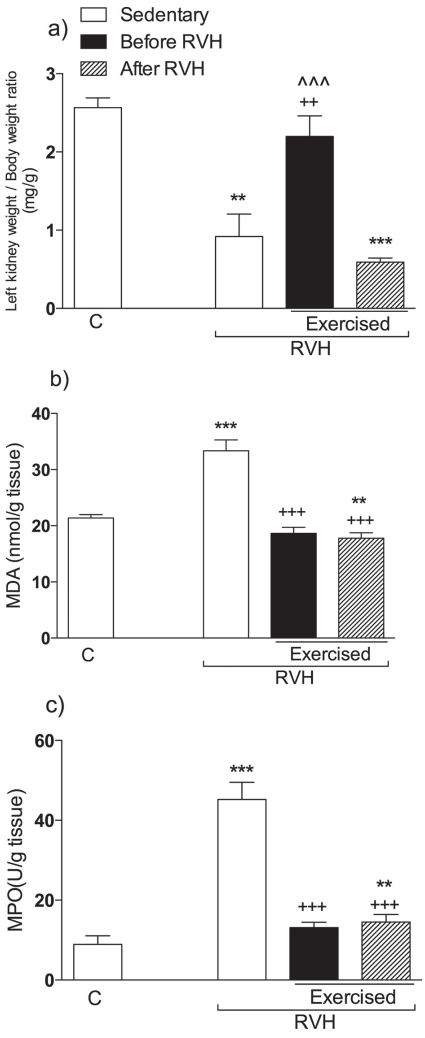

Figure 1. a) Left kidney weight to body weight ratio (mg/g), b) Malondialdehyde (MDA, nmol/g) levels, c) Myeloperoxidase (MPO, U/g) activity in the sham-operated control (C), sedentary renovascular hypertension (RVH), exercised-before- RVH and exercised-after-RVH groups.
** p<0.01, *** p<0.001 compared with the control group. $^{++}$ p<0.01, $^{+++}$ p<0.001 compared with the sedentary RVH group.

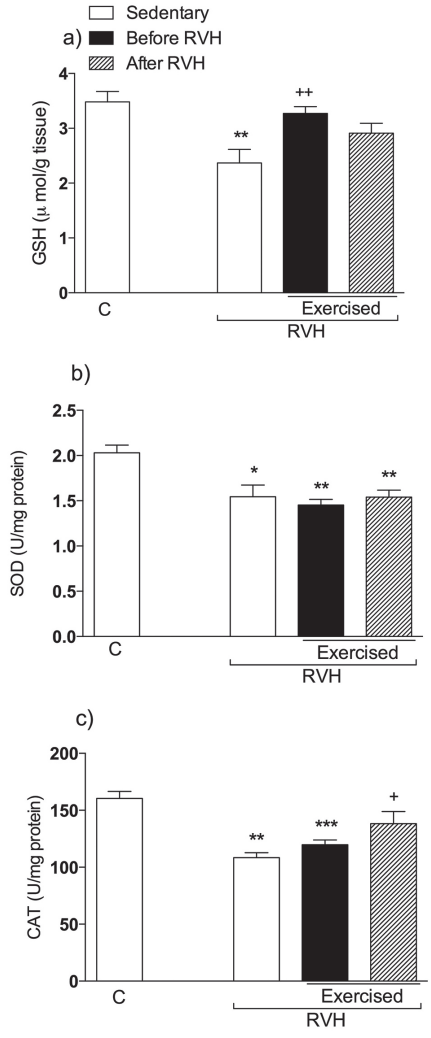

Figure 2. a) Glutathione (GSH; mmol/g) levels, b) Superoxide dismutase (SOD; U/mg protein) levels, c) Catalase (CAT; nmol/g) levels in the sham-operated control (C), sedentary renovascular hypertension (RVH), exercised-before-RVH and exercised-after-RVH groups.
*p<0.05, ** p<0.01, *** p<0.001 compared with the control group. $^{+}$ p<0.05, compared with the sedentary RVH group.

2,000 g for 15 min at 4°C. The supernatant was removed and re-centrifuged at 41 400 g for 8 min. GSH measurements were performed using a modification of the Ellman procedure (25). Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid-reactive substances as previously described (26). Lipid peroxide levels were expressed in terms of malondialdehyde (MDA) equivalents using an extinction coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$.

Measurement of superoxide dismutase (SOD) and catalase (CAT) activity in the kidneys

SOD activity in kidney samples was measured according to a previously described method (27). Briefly, measurements were performed in cuvettes containing 2.8 ml 50 mM potassium phosphate (pH = 7.8) with 0.1 mM EDTA, 0.1 mM 0.39 mM riboflavin in 10 mM potassium phosphate (pH 7.5), 0.1 ml of 6 mM o-dianisidin.2 HCl in deionized water, and tissue extract (50, 100 ml). Cuvettes with all their components were illuminated with 20-W SlylvaniaGro-Lux fluorescent tubes (Sylvania GRO-LUX F18W/GRO, Erlangen, Germany) that were placed 5 cm above and to one side of cuvettes maintaining a temperature of 37°C. Absorbance was measured at 460 nm with a Schimadzu UV-02 model spectrophotometer (Schimadzu, Tokyo, Japan). A standard curve was prepared routinely with bovine SOD (S-2515-3000 U; Sigma Chemical Co, St Louis, MO, USA) as reference. Absorbance readings were taken at 0 and 8 min of illumination and the net absorbance was calculated. The method for the measurement of CAT activity is based on the catalytic activity of the enzyme that catalyzes the decomposition reaction of H_2O_2 to

Table 1. The effect of exercise on blood pressure and heart rate of experimental groups with renovascular hypertension.

	Sham-operated control	Sedentary RVH	Exercised before RVH	Exercised after RVH
Blood pressure (mmHg)				
Before surgery	126 ± 2.6	122 ± 1.9	120 ± 0.8	123 ± 2.6
12th week	129 ± 2.5	175 ± 6.7***	139 ± 1.5***, +++	175 ± 2.7***
Heart rate (BPM)				
Before surgery	322 ± 8.2	323 ± 7.3	321 ± 9.3	318 ± 6.9
12th week	329 ± 7.9	408 ± 7.6	384 ± 10.5	422 ± 4.6

Blood pressure (mmHg) and heart rate (beats per minute; BPM) measurements in the sham-operated control (n=6), sedentary renovascular hypertension (RVH; n=8), exercised-before-RVH (n=8) and exercised-after-RVH groups (n=8).

*** $p<0.001$ compared with the sham-operated control group. $^{+++}$ $p<0.05$ compared with the sedentary RVH group.

give H_2O and O_2 (28). Briefly, the absorbance of the tissue samples containing 0.4 ml homogenate and 0.2 ml H_2O_2 was read at 240 nm and 20°C against a blank containing 0.2 ml phosphate buffer and 0.4 ml homogenate for about 1 min.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). All data are expressed as means ± SEM. Groups of data were compared with an analysis of variance followed by Tukey's multiple comparison tests and Student's t test. Values of $p<0.05$ were regarded as significant.

RESULTS

The effect of swimming exercise on blood pressure

The basal blood pressures that were recorded before surgery were not different among the experimental groups (Table 1). At the end of the 12 weeks, the mean systolic blood pressures in all the RVH groups were significantly ($p<0.001$) elevated as compared to sham-operated control group. However, in the exercised-before-RVH group that had a shorter duration (3-week) of RVH following the swimming training, elevation in blood pressure was significantly lower ($p<0.05$) as compared to the sedentary RVH group or to the group that had swimming exercise 3 weeks after the induction of RVH. Blood pressures measured at the 3rd weeks in the exercised-after-RVH (142.6±2.3 mmHg; $p<0.05$) and sedentary RVH (165±7.2 mmHg; $p<0.001$) groups were significantly higher than the pressure measured at the 12th week of exercised-before-RVH group (139±1.5 mmHg). On the other hand, no significant differences were observed among the heart rate recordings of the groups.

The effect of swimming exercise on renal atrophy and injury

Although body weights of the rats increased during the 12-week follow-up period, these alterations were not different among the experimental groups (data not shown). Morphological atrophy of the kidney was determined by the ratio of the kidney weight to the body weight measured on the day of decapitation. Indicating renal atrophy, the ratio calculated from the kidneys in the sedentary RVH group was significantly decreased when compared with the ratios of the control group ($p<0.01$; Fig. 1a). Similarly, in the exercised-after-RVH group, renal atrophy ratio was significantly lower ($p<0.001$). On the other hand, the ratio calculated in the exercised-before-RVH rats was not different than that of the control rats, suggesting that no renal atrophy is evident as seen in the other RVH groups.

In the sedentary RVH group, renal levels of MDA, an index of lipid peroxidation, were found to be significantly higher than those of the sham–operated control group ($p< 0.001$), while exercise training, either performed before or after the induction of RVH, abolished the elevations in renal MDA levels ($p<0.001$, Fig. 1b). In accordance with that, MPO activities in the renal tissue samples were significantly increased in the sedentary RVH group as compared with the control group ($p < 0.001$; Fig. 1c), indicating increased neutrophil infiltration to the kidney. However, in the RVH rats, which have exercised for 9 weeks either before or after the surgery, elevations in MPO activity were significantly suppressed ($p < 0.001$; Fig. 1c).

The effect of swimming exercise on renal antioxidant enzymes

Induction of RVH in the sedentary rats caused significant reductions in GSH levels and SOD and CAT activities of the kidneys when compared with those of the sham-operated control rats ($p<0.05$-0.01, Fig. 2). Reduced SOD activity was not altered in either group that had exercised before or after RVH. Antioxidant GSH content was replenished significantly ($p<0.01$) in the exercised-before-RVH group, while the elevation in GSH in the exercised-after-RVH did not reach a statistical significance. On the other hand, RVH-induced reduction in CAT was significantly reversed in the group that has exercised after RVH ($p<0.05$), but not in the group that has exercised in the beginning of the protocol.

DISCUSSION

In the present study, the findings demonstrate that regular swimming exercise performed for 9 weeks, either completed before the induction of RVH or started by the 3rd week of RVH induction, alleviated oxidative renal injury as evidenced by suppressed lipid peroxidation and neutrophil infiltration along with increased levels of GSH, SOD and CAT in the kidney.

Despite extensive evidence demonstrating that exercise training should be considered as an important intervention in protecting against chronic diseases such as diabetes, heart failure and end-stage renal disease (29, 30, 31), it has not been explored thoroughly for the treatment of patients at the earlier phases of renal disease with hypertension. In a rat model of diabetes- and obesity-associated nephropathy, treadmill running of moderate intensity significantly ameliorated early renal injury (32). Current data demonstrate that, in contrast to sedentary or post-RVH exercised groups, no renal atrophy was observed when regular exercise was performed before RVH was induced. This group represented an early (3-week) phase of the RVH model with high plasma levels of angiotensin II (23). In the sedentary and exercised-after-RVH groups that had atrophy, a long-term renin-

independent hypertension was present. However, the current results further demonstrate that exercise training whether performed before or after the occurrence of RVH, protected the renal tissue against oxidative injury. Mainly for its cardioprotective effect, use of exercise training was considered as an essential component in the treatment of patients with chronic renal disease (33).It appears that the protective impact of exercise training is also evident directly on the injured kidney tissue.

Exercise was shown to increase antioxidant activity (e.g. superoxide dismutase, glutathione peroxidase and catalase) in the heart and skeletal muscle (34). High SOD and GPx activities in the sera of elite rugby players were suggested to be due to the presence of oxidative stress of exercise (35). Similarly, after a 12-week endurance training in healthy humans, SOD and GSH peroxidase (GPx) activities in the erythrocyte membrane were increased, but CAT activity was proved to be unchanged (36). In parallel to human data, swimming exercise in rats for 10 weeks increased SOD activity in the diaphragm and kidney (37). Treadmill exercise in rats with either chronic hypertension (38) or chronic renal disease was shown to increase SOD and GPx activity, but not the antioxidant effects of CAT, implicating that the protective effect of regular aerobic exercise on kidney damage occurs by up-regulating SOD and GPx activity (19). In the current study, exercise training did not alter RVH-induced depletion in renal SOD levels. However, depletion in renal GSH was blocked in rats that have exercised before RVH, while the reduction in CAT was abolished only in the exercised-after-RVH group. When taken with previous studies, it appears that exercise when performed either before or after RVH has a stimulatory effect on the generation of antioxidants or it inhibits their depletion by the oxidants. That is, regularly performed swimming exercise before RVH is likely to be interpreted as an oxidative stress, which might precondition the renal tissue and prepare the antioxidant stores against other oxidant insults, while exercise after RVH might have a provocative effect on the antioxidants.

Activated neutrophils release chemotactic substances, which further promote neutrophil migration to the tissue, exacerbating tissue injury through the production of oxygen metabolites, initiating the deactivation of antiproteases and the activation of cytotoxic enzymes, such as elastase, proteases, lactoferrin and MPO (39). MPO, released into the extracellular fluid as a response to various stimulatory substances, is indicative of neutrophil infiltration to the injured tissue. We have previously demonstrated that swimming exercise for 8 weeks reversed the elevated MPO activities in the cardiac muscle, liver, stomach, and brain of the stressed rats (21). In the current study, regular exercise made either before or after renal injury effectively abolished RVH-induced high MPO activity, suggesting that the protective effects of exercise training is mediated in part by blocking renal neutrophil infiltration.

Extensive evidence from animal studies suggests that exercise in all its forms has a positive impact on several aspects of health. Moderate and regular exercise has attenuated aging-associated mitochondrial dysfunction (40), improved cognitive performance (41) and memory (42) and positively affected emotional aspects of behavior, resulting in better responses to acute or chronic stressor exposures (43).It was shown that exercise training in rats improved cardiac function following myocardial infarction (44), attenuated endotoxemia-induced lung damage (45) and reduced the progression of renal disease (46). In accordance with these, swimming exercise ameliorated RVH-induced oxidative damage of the kidneys via the inhibition of neutrophil infiltration. In conclusion, regular moderate exercise may represent an effective non-pharmacologic approach in preventing or reducing renal oxidative damage induced by renovascular hypertension.

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Renovasküler hipertansiyon oluşturulmasının öncesi veya sonrasında yapılan düzenli yüzme egzersizi sıçanlarda oksidatif böbrek hasarını hafifletir

Özet

Epidemiyolojik çalışmalar düzenli egzersizin ve artmış aerobik zindeliğin mortalite ve morbidite oranlarında azalmaya neden olduğunu aynı zamanda yüksek kan basıncını düşürdüğünü göstermiştir. Egzersizin hipertansiyon patogenezi üzerine anti inflamatuvar etkileri net olarak ortaya konmamıştır. Çalışmada, egzersizin (30 dk/gün, 5 gün/hafta, 9 hafta yüzme egzersizi) hipertansiyondaki olası iyileştirici ve koruyucu etkilerini ortaya koymak için 10 haftalık erkek Wistar albino sıçanlarda renovasküler hipertansiyon (RVH) modeli oluşturuldu. Sıçanlar sedanter bırakılan taklit-cerrahi grubu (kontrol), sedanter RVH (2-böbrek 1-klip, Goldblatt metodu), RVH cerrahisi öncesi veya sonrasında 9 hafta egzersiz yapmış 2 ayrı RVH grubu olmak üzere 4 gruba ayrıldı. Sistolik kan basınçları (SKB) her hafta ölçüldü ve 12. hafta sonunda sıçanlar dekapite edilerek biyokimyasal analizler yapılmak üzere böbrek dokuları alındı. Sonuçlar GraphPad Prism 6.0 paket programı ile analiz edildi. Varyans analizleri Tukey çoklu karşılaştırma ve Student'ın t testi ile yapıldı $p<0,05$ anlamlı kabul edildi. SKB kontrol grubuna göre sedanter RVH grubunda anlamlı derecede yüksek bulunurken egzersiz yapan grupların ölçümlerinde kontrol hayvanlarından farklı değildi. Böbrek dokusunda kontrol grubuna göre sedanter RVH grubunda malondialdehit ve miyeloperoksidaz seviyelerinde artış görülürken glutatyon seviyelerindeki azalma egzersiz gruplarında ortadan kalkmıştır. Ayrıca, antioksidan parametre olarak ölçülen süperoksit dismutaz ve katalaz seviyeleri de sedanter gruba kıyasla egzersiz yapan her iki RVH grubunda da artmıştır. Bu sonuçlar orta şiddette düzenli egzersizin kan basıncını kontrol ederek böbrek dokusunda RVH kaynaklı oksidatif hasarda oksidan-antioksidan dengenin modülasyonu ile iyileştirme yaptığını ortaya koymaktadır. Egzersiz yalnızca kardiyak fonksiyonları geliştirmekle kalmaz aynı zamanda anjiotensin-II-kaynaklı böbrek hasarına karşı savunmak için bir anti-inflamatuvar süreci başlatır.

Anahtar kelimeler: Egzersiz, hipertansiyon, böbrek, oksidatif stres

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