

SWIMMING ATTENUATES BLOOD PRESSURE AND OXIDATIVE STRESS IN HYPERTENSIVE RATS

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ABSTRACT

Hypertension presents one of the main risk factors for cardiovascular diseases which are the leading cause of morbidity and mortality worldwide. Structural and mechanical changes of the heart and blood vessels as well as overproduction of reactive oxygen species may occur due to the increased blood pressure. Therefore, the goal of our study was to estimate the effects and duration of swimming as a possible therapy approach on blood pressure and oxidative stress parameters in normotensive and hypertensive rats. The study was conducted on 60 male Wistar albino rats divided into two groups, normotensive and hypertensive rats. Each of these groups was divided into three subgroups according to the swimming protocol. The swimming training was kept constant (60 min/day, for five days a week) with two days of rest. After six or nine weeks of the swimming protocol, blood pressure and oxidative stress markers were measured. The control group rats were put in water for one minute a day, in order to avoid water-induced stress. Training significantly reduced systolic blood pressure in hypertensive rats, while diastolic pressure did not change in the group that swam six or nine weeks. The results showed that swimming increases the activity of all measured antioxidative parameters, while values of prooxidants varied depending on the training protocol. Our results confirmed that swimming, as an aerobic exercise, decreases blood pressure and has time-dependent positive system adaptations, especially on the antioxidant parameters.

Keywords: Antioxidant protection, hypertension, oxidative stress, rats, swimming.



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INTRODUCTION

Despite significant progress in the pathophysiology understanding and available effective treatment strategies, hypertension is still the leading risk factor in developing many diseases, especially cardiovascular ones (1). Hypertension leads to numerous changes such as a structural and mechanical modification of the heart and/or blood vessels (2). Furthermore, hypertension has been related to an imbalance between oxidants and antioxidants in favor of oxidants, which consequently causes tissue damage, vascular disorders and diseases (3). The beneficial effects of exercise are highlighted in cardiovascular diseases and many authors emphasize its importance in the treatment of hypertension (4).

Regular physical exercise is considered to be one of the crucial factors for a healthy lifestyle due to its ability to diminish the risk of osteomuscular, endocrine, cardiovascular and immune system disorders (5). Exercise, especially aerobic, represents an important and necessary part of everyday life since it may prevent and treat various diseases and pathological conditions (6).

Mechanisms responsible for beneficial effects of training on blood pressure are not quite revealed. Nevertheless, various papers have been speculating about peripheral mechanisms responsible for antihypertensive effect, such as vascular resistance and endothelium dependent relaxation (7). Previous investigations reported the role of oxidative stress during exercise in terms of enhancing the prooxidants. On the other hand, some researchers suggested that exercise may also enhance antioxidant enzymes activity. However, it should be taken into consideration that oxidative stress response to exercise can be affected by type, duration and frequency of training which is of great importance in stimulation of adaptive processes of the antioxidative system, especially in patients with cardiovascular problems (8, 9).

Swimming represents the aerobic type of exercise where the motion of the body and all muscles induces adaptation of the cardiovascular system. As a total-body workout, it increases flexibility and leads to an improvement in blood circulation, superior systolic and diastolic function and less cardiac fibrosis (1). Considering that swimming leads to suppression of the sympathetic nervous system and the renin-angiotensin system, as well as to lower vascular resistance, it should be recommended to patients with hypertension (2, 10).

In past few decades, an increasing number of scientists invested great efforts to find exercise which is ideal for patients with cardiovascular diseases. Given the fact that influence of oxidative stress in swimming is not still clarified, we aimed to reveal the effects and different duration of swimming training on blood pressure and systemic oxidative stress parameters in normotensive and salt-induced hypertensive rats.

MATERIALS AND METHODS

Ethical approval

All experimental procedures were carried out in the Laboratory for Cardiovascular Physiology of the Faculty of Medical Sciences, the University of Kragujevac. It was approved by Ethics Committee of the institution as well as according to EU Directive for welfare of laboratory animals (86/609/EEC) and principles of Good Laboratory Practice (GLP).

Animals

Our research included sixty male *Wistar albino* rats (six weeks old) received from the Military Medical Academy, Belgrade, Serbia, placed under controlled conditions: temperature of 22 ± 1 °C with twelve hours automatic illumination daily. Food and tap water or solution of NaCl were available to rats which were randomly divided into two groups: normotensive (NT) and hypertensive (HT) animals, while each group consisted of three subgroups depending on the swimming protocol. Normotensive rats were separated into: normotensive rats subjected to swimming for six weeks (NT-6-ST, n=10); normotensive rats exposed to swimming for nine weeks (NT-9-ST, n=10) and sedentary control rats (NT-C, n=10).

To induce hypertension, rats from HT group were drinking 8% high sodium (NaCl) mixture for four weeks (11). Hypertension was assessed on the day after completing the swimming protocol by using the tail-cuff (*Rat Tail Cuff Method Blood Pressure Systems (MRBP-R), IITC Life Science Inc. USA*) (12). After the confirmation of hypertension, animals were divided into three subgroups according to swimming sessions: hypertensive rats exposed to six weeks of swimming (HT-6-ST, n=10); hypertensive rats subjected to weeks of swimming (HT-9-ST, n=10) and sedentary control rats (HT-C, n=10).

Swimming training protocol

Rats were practicing in a specially constructed glass swimming pool with following dimensions: $80 \times 60 \times 100$ cm. Water temperature (37 ± 1 °C) was preserved by an electric heater, while waves were made by pump. Animals were abstaining from food during the night prior to the swimming protocol. The swimming training protocol was chosen according to a recent investigation (13) and was maintained five days a week at 9:00–10:00 am for all exercise sessions. The adaptation protocol includes ten minutes of constant swimming exercise on the first day and slowly enhanced daily until reaching sixty minutes on the fifth day. After accomplishing the adaptation, rats were subjected to the continuous swimming protocol: one hour daily during five days with two days of rest. In order to accomplish similar water-induced stress, control group rats (NT-C and HT-C) were in water one minute per day for five days a week during all exercise sessions. Between every procedure animals rested for

two days. Supervisor was continuously present during swimming.

Biochemical analysis

Blood from all experimental groups was collected for the determination of redox status after establishing blood pressure. Quantification of the index of lipid peroxidation via reactive thiobarbituric substances (indirect, measured as TBARS), nitrites (NO_2^-), superoxide anion radical (O_2^-), and hydrogen peroxide (H_2O_2) were performed in plasma samples, while superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were determined in erythrocytes samples. All biochemical analyses were carried out using the spectrophotometric method (UV-1800 Shimadzu UV spectrophotometer, Japan) by repeatedly confirmed methods used in our previous studies (14).

Determination of the index of lipid peroxidation measured as TBARS

Products of the reaction with thiobarbituric acid were used for determination of the index of lipid peroxidation. Briefly, 0.4 ml of plasma samples and 0.2 ml of 28% trichloroacetic acid were vortexed, incubated for fifteen minutes on ice and centrifuged (6000 rpm) for fifteen minutes. Afterwards, 0.4 ml of supernatant and 0.1 ml of 1% thiobarbituric acid were incubated at 100°C for fifteen minutes and measured at 530 nm spectrophotometrically. The distilled water was used as blank control.

Determination of nitrites (NO_2^-)

Nitric oxide (NO) quickly resolves into nitrites/nitrates. Therefore, nitrites (NO_2^-) are used as an index of NO production via a spectrophotometric method. For NO_2^- determination in plasma 0.1 ml 3 N PCA (perchloric acid), 0.4 ml 20 mM ethylenediaminetetraacetic acid (EDTA), and 0.2 ml plasma were put on ice for fifteen minutes, centrifuged for fifteen minutes at 6,000 rpm. After pouring off the supernatant, 220 μl K_2CO_3 was added. Detection of nitrites was performed at 550 nm. Distilled water was used as a blank probe.

Determination of superoxide anion radicals (O_2^-)

Superoxide anion radical concentration was measured using the NTB (Nitro Blue Tetrazolium) reagent in assay mixture (TRIS buffer) with plasma samples. Wavelength for determination of O_2^- was 530 nm. Blank control was assay mixture.

Determination of hydrogen peroxide (H_2O_2)

Measurement of H_2O_2 is based on phenol red oxidation by H_2O_2 in a reaction catalyzed by horseradish peroxidase (HRPO). A total of 0.2 ml of sample was precipitated with 0.8 ml of freshly prepared phenol red solution, followed by the addition of 10 μl of (1:20) HRPO (made *ex tempore*). An adequate volume of distilled water solution was used in blank probes. The concentration of H_2O_2 was detected at 610 nm.

Determination of antioxidant enzymes (SOD, CAT)

SOD activity was measured by mixing 0.1 ml lysate, 1 ml carbonate buffer and 100 μl of epinephrine. Detection was performed at 470 nm. Distilled water was used as a blank probe. Isolated RBCs were washed three times with three volumes of ice-cold 0.9 mmol/l NaCl and hemolysates containing about 50 g Hb/l were used for the determination of CAT activity. Then 50 μl CAT buffer, 100 μl sample, and 1 ml 10 mM H_2O_2 were added to the samples. Spectrophotometric measurement was at 360 nm.

Determination of reduced glutathione (GSH)

Based on GSH oxidation via 5,5-dithiobis-6,2-nitrobenzoic acid, we determined the level of GSH spectrophotometrically. Combination of 0.1 ml 0.1% EDTA, 0.4 ml hemolysate, and 0.75 ml precipitation solution was mixed on the vortex machine and extracted on ice for fifteen minutes. Afterwards, the mixture was centrifuged on 4000 rpm for ten minutes. Measuring was performed at 412 nm. Distilled water was used as a blank probe.

Statistical analysis

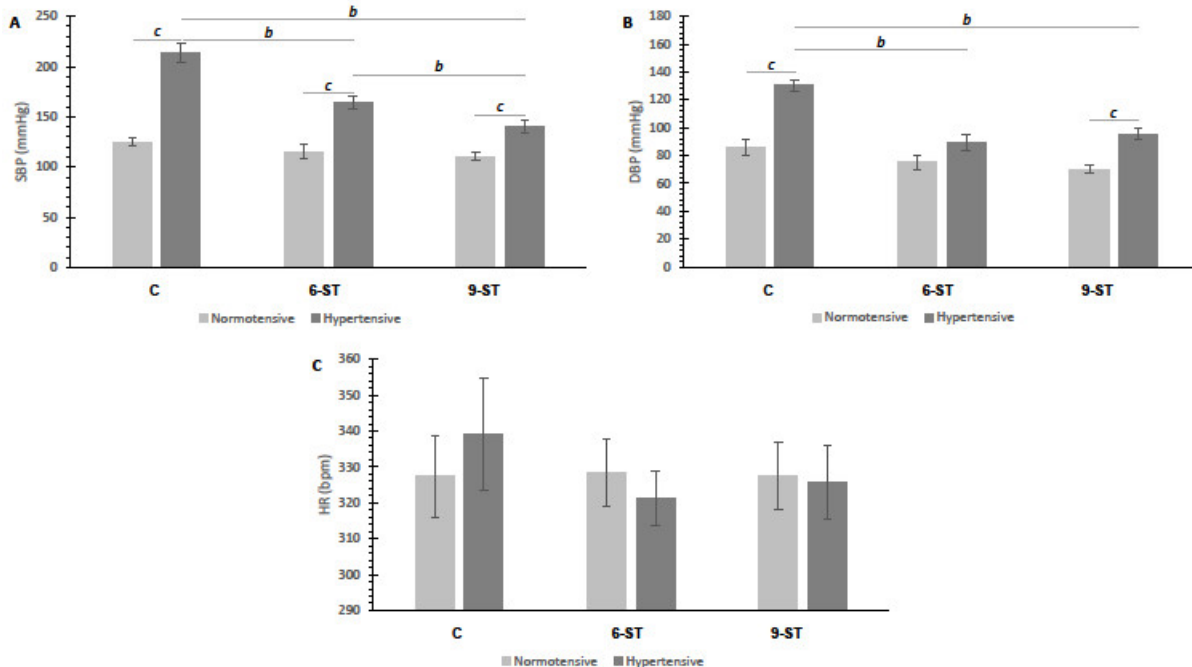
Statistical analyses were performed by using SPSS 23.0 software. Data are presented as the mean values $\pm$ standard deviations of the mean with statistical significance. The Shapiro–Wilk test was used for determination of normality of parameter's distribution. We used a parametric Friedman's ANOVA test or a non-parametric Mann–Whitney U test (Kruskal–Wallis test) for comparison of groups. Values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Blood Pressure and Heart Rate

Training did not significantly affect blood pressure in the NT group of rats, while hypertensive trained rats had a significantly reduced blood pressure compared to the sedentary group. Systolic blood pressure was significantly lower after nine weeks relative to six weeks of training (HT-9ST vs. HT-6ST). On the other hand, diastolic blood pressure didn't significantly change after the prolongation of training. Although the training significantly decreased the blood pressure of hypertensive rats, values were actually still significantly higher after six or nine weeks of swimming than in normotensive rats (Figures 1A, 1B). The heart rate was similar in all groups, regardless of blood pressure or training (Figure 1C).

Figure 1. Time-dependent swimming training-induced alterations in blood pressure: (A) systolic blood pressure; (B) diastolic blood pressure; (C) heart rate. Each bar represents the mean $\pm$ standard deviation, *a* statistically significant difference between normotensive rats; *b* statistically significant difference between hypertensive rats; *c* statistically significant difference between normotensive and hypertensive rats. ($p < 0.05$)



Systemic redox state

Levels of TBARS

The TBARS level was increased in the hypertensive sedentary group (HT-C) compared to the normotensive sedentary group (NT-C) while values of TBARS didn't significantly change comparing hypertensive and normotensive rats during different training sessions (Figure 2A).

Levels of NO_2^-

Hypertensive rats which swam six and nine weeks had a significantly increased level of NO_2^- compared to sedentary rats (HT-C). Statistically higher levels of NO_2^- were noticed in the hypertensive sedentary group relative to the normotensive sedentary group (NT-C vs. HT-C). Normotensive rats had lower level of NO_2^- than hypertensive rats who swam six as well as nine weeks (NT-6ST vs. HT-6ST and NT-9ST vs. HT-9ST) (Figure 2B).

Level of O_2^-

The level of O_2^- significantly decreased after nine weeks of swimming relative to sedentary normotensive rats as well as to hypertensive sedentary rats. Nine weeks of exercise significantly reduced the level of O_2^- relative to six weeks of swimming both in the normotensive and hypertensive group (NT-9ST vs. NT-6ST and HT-6ST vs. HT-9ST). In hypertensive rats, six weeks of swimming significantly reduced the

level of O_2^- relative to the sedentary hypertensive group (HT-6ST vs. HT-C). Statistically higher levels of O_2^- were recorded in the hypertensive rather than in the normotensive sedentary groups (HT-C vs. NT-C) (Figure 2C).

Levels of H_2O_2

A significantly reduced level of H_2O_2 was observed after six and nine weeks of swimming comparing to sedentary normotensive and hypertensive rats. Exposure to nine weeks of training led to a significant reduction of H_2O_2 in both normotensive and hypertensive rats. Statistically increased levels of H_2O_2 in hypertensive relative to normotensive rats were observed in the sedentary groups, while after nine weeks of swimming, statistically lower levels were noticed in hypertensive comparing to normotensive rats (Figure 2D).

Activity of superoxide dismutase (SOD)

Swimming statistically increased SOD activity in both the normotensive and hypertensive groups after six (NT-C vs. NT-6ST and HT-C vs. HT-6ST) and nine (NT-C vs. NT-9ST and HT-C vs. HT-9ST) weeks of training relative to the sedentary group. Nine weeks of exercise induced a higher activity of SOD relative to six weeks of training. There was no difference in this parameter between hypertensive and normotensive rats (Figure 2E).

Levels of reduced glutathione (GSH)

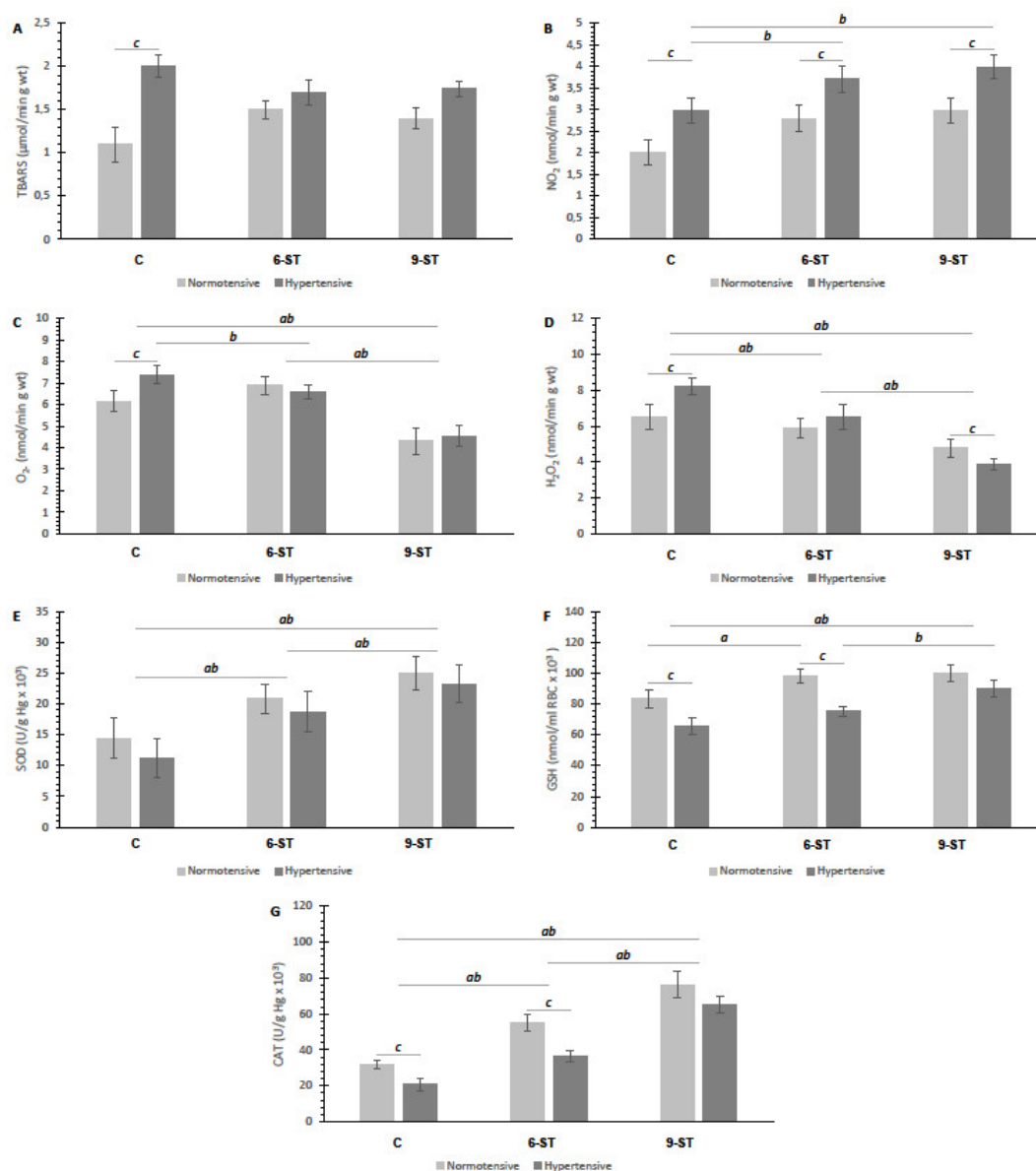
Nine weeks of the swimming protocols significantly increased the value of GSH relative to the sedentary normotensive and hypertensive groups. Normotensive rats had a higher level of GSH after six weeks of training than in the control group. Higher levels were noticed in hypertensive rats after nine weeks of swimming compared to six weeks. Hypertensive sedentary rats as well as hypertensive rats after six weeks of swimming had statistically lower values of GSH compared to the normotensive group and six-week-trained normotensive rats (Figure 2F).

Activity of catalase (CAT)

The activity of CAT increased after six and nine weeks of training both in the normotensive and hypertensive group relative to the control group. Furthermore, the nine-week training protocol (NT-9ST, HT-9ST) had significantly higher values of CAT than the six-week training protocol (NT-6ST, HT-6ST). Sedentary and six-week-trained hypertensive rats had a statistically lower value of CAT than sedentary and six-week-trained normotensive rats (Figure 2G).

Figure 2. Time-dependent swimming training-induced alterations in systemic redox status: (A) TBARS; (B) NO₂; (C) O₂⁻; (D) H₂O₂; (E) SOD; (F) GSH; (G) CAT.

Each bar represents the mean $\pm$ standard deviation, *a* statistically significant difference between normotensive rats ($p < 0.05$); *b* statistically significant difference between hypertensive rats ($p < 0.05$); *c* statistically significant difference between normotensive and hypertensive rats ($p < 0.05$)



DISCUSSION

Swimming, as aerobic training, has been proposed as a convenient model for studying the physiological changes and stress response to exercise. In addition, it presents one of the non-pharmacological therapy approaches for treating hypertension (15). However, available information regarding the time-dependent benefits is deficient. Therefore, the present study aimed to estimate the effects of six and nine weeks of swimming protocols on systemic oxidative stress markers and blood pressure in hypertensive rats.

In the current study, we confirmed the previous findings that swimming lowers blood pressure which might be the consequence of vascular resistance reduction (16). Blood pressure, especially systolic blood pressure was decreased in correlation with training duration in hypertensive groups (HT-6ST and HT-9ST). These experimental findings indicate that swimming might be useful in a combination with suitable antihypertensive agents. Additionally, prolongation of exercise enhances the beneficial effect of swimming on arterial blood pressure which might be due to reduced sympathetic activity after physical activity. The findings from earlier preclinical and clinical investigations are in line with our results (17). Gilbert and co-workers (17) showed that physical activity significantly affected the endothelial dysfunction occurring in hypertension and observed that aerobic physical activity led the reduction of vasoconstriction in rats (18). Recent findings demonstrated that twelve weeks of swimming aerobic exercise is effective in evoking lower blood pressure thus improving vascular function and arterial rigourousness in prehypertensive or hypertensive subjects at the first stage (19).

Another part of our investigation was focused on the ability of swimming to affect the redox status of both hypertensive and normotensive rats. In that sense, we measured the markers of systemic oxidative stress and activity of antioxidative enzymes. Although numerous studies were mainly concentrated on the effects of anaerobic exercises (treadmill) on the redox status, we decided to use the swimming model, as a natural ability of rats (20). The results of our study clearly showed that over-production of ROS is linked to hypertension as well as that swimming training led to a decrease of almost all pro-oxidants and an increase of almost all antioxidants measured in the blood of both normotensive and hypertensive rats. Generally viewed, duration of swimming affected the values of redox markers. Actually, nine weeks of swimming indicated more benefits than six weeks of the training protocol. Although as a consequence of the adaptive cell response to exercise, an increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can occur (21), oxidative damage didn't happen in our investigation. That was probably due to the moderate intensity swimming which was long enough to allow the organism adjustment to stress.

Lipid peroxidation indicates the oxidative damage mainly to the membrane lipids thus, TBARS is expected to increase

in hypertensive rats (22). Our results are in accordance with an earlier study carried out by Hu and authors (21), who didn't notice changes in the lipid peroxidation level in the rat liver and heart after seven days of swimming (23). Moreover, some authors (22) revealed no modifications in the levels of malondialdehyde (MDA) in male rats after eight weeks of swimming (24).

We noticed that swimming training reduced the NO_2^- level in a time-dependent manner, which was more pronounced in hypertensive than in normotensive rats. These results are in correlation with the previous findings suggesting that moderate exercise on the treadmill increased total nitrates/nitrites in spontaneously hypertensive rats, thus inducing relaxation and subsequent vasodilatation. In fact, this is one of the most commonly proposed mechanisms which explain reduction of blood pressure due to training (25). A large body of evidence indicates that aerobic exercise improves vascular function in the blood vessels of hypertensive patients and animals primarily through increase in NO production and/or decrease in NO inactivation by oxidative stress (26).

Reduced values of the prooxidants, especially O_2^- and H_2O_2 were supported by elevation of SOD and CAT activity. Actually, SOD eliminates O_2^- acceleration of its dismutation to H_2O_2 , while CAT as a ferric hem protein can catalyze the degradation of H_2O_2 (21). Bearing in mind that O_2^- can react quickly with several radicals and iron-sulfur clusters in the protein, as well as that H_2O_2 has cytotoxic properties (27), the reduction of these free radicals directly indicates the benefits of swimming in hypertension. According to the several lines of evidence, a moderate physical exercise has antioxidative effects (28), but training duration necessary to achieve these benefits is still controversial. However, there are only few studies dealing with this problem in hypertension. In this study, it was found that six weeks of swimming were sufficient to increase statistically significantly SOD, GSH and CAT in healthy, respectively SOD and CAT in rats with hypertension. On the other hand, all of the measured antioxidative parameters were enhanced in both normotensive and hypertensive rats after nine weeks of swimming. Literature data revealed that the duration and intensity of physical activity are directly related to the antioxidant levels of enzyme activity (29). Additionally, based on our results, nine weeks of swimming were sufficient to achieve the antioxidant effects in hypertensive animals.

Our research illustrated that the moderate intensity swimming training reduced blood pressure values in hypertensive conditions, which was more featured in the nine-week swimming group. Lifestyle modifications in sense of starting swimming, afterdeveloping hypertension, may be beneficial as non-pharmacological co-therapy. Moreover, swimming has a positive influence on the system adaptation, especially in controlling the redox status, which is unbalanced in hypertension.

ACKNOWLEDGMENTS

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ETHICS APPROVAL

Research was approved by Ethics Committee of the institution. All research procedures were done according to EU Directive for welfare of laboratory animals (86/609/EEC) and principles of Good Laboratory Practice (GLP).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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