Effects of Regular Treadmill Exercise on a DNA Oxidative-Damage Marker and Total Antioxidant Capacity in Rat Hippocampal Tissue

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\textbf{Background and Purpose} Regular exercise can result in changes in the levels of oxidative stress in the hippocampus; however, little attention has been paid to physical-activity-induced neuronal protection to exposure to lead compounds. This study investigated the effects of regular treadmill exercise on a DNA oxidative-damage marker [8-hydroxy-2'-deoxyguanosine (8-OHdG)] and the total antioxidant capacity (TAC) of hippocampal tissue in lead-acetate exposed rats.

\textbf{Methods} This study investigated the effects of 8 weeks of regular treadmill exercise on 8-OHdG and the TAC of hippocampal tissue in lead-acetate-exposed rats. Wistar rats were randomly divided into four groups: baseline, sham (control), lead, and exercise+lead. The exercise program involved running on a treadmill with increasing intensity five times a week for 8 weeks. Animals in the lead and exercise+lead groups received lead acetate at 20 mg/kg body weight intraperitoneally three times weekly for 8 weeks. Animals in the sham group received solvent (ethyl oleate) at 30 mg/kg body weight three times weekly for 8 weeks. TAC and 8-OHdG were measured by spectrophotometric and ELISA techniques, respectively. Data were analyzed by ANOVA and Tukey’s post-hoc test with a significance cutoff of \(p \leq 0.05\).

\textbf{Results} The level of 8-OHdG and the TAC were significantly higher and lower, respectively, in the lead group than in the baseline and sham groups (\(p<0.01\)). However, the 8-OHdG level and TAC value in hippocampal tissue were significantly decreased and increased, respectively, in the exercise+lead group relative to the lead group (\(p<0.05\)).

\textbf{Conclusions} The TAC of hippocampal tissue may be directly associated with neural protection mechanisms of exercise following lead acetate injection, and the beneficial effects of regular exercise in preventing hippocampal neuronal damage could be due to decreased hippocampal oxidative stress such as reflected by a lower 8-OHdG level and increased TAC.

\textbf{Key Words} regular treadmill exercise, total antioxidant capacity, 8-OHdG, hippocampus, lead acetate, oxidative stress.

\textbf{INTRODUCTION}

Studies performed in recent decades have indicated that environmental factors such as stress and exposure to drugs and pharmacologically toxic substances in the prenatal period can have profound effects on brain development, leading to lasting negative effects on brain function and an increased risk of mental disorders later in life.\textsuperscript{1} Lead is a common environmental pollutant with a long history of toxicity in several body tissues, especially those of systems characterized by gradual development, such as the nervous system.\textsuperscript{2,3} The very high sensitivity of the nervous system to lead raises societal health concerns about the quality of life. Neurodegenerative diseases such as Alzheimer’s and Parkinson’s diseases are examples of diseases associated with lead exposure.

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of these problems; White et al.\textsuperscript{2} reported that even small amounts of lead in the blood (10–15 μg/mL) are associated with reduced cognitive and behavioral abilities. In addition, our previous studies have revealed the ability of lead to induce oxidative stress, and there is growing evidence for the role of oxidative stress in the pathophysiology of lead toxicity.\textsuperscript{3,4} The existing data show that physical activity may reduce age-related cognitive disorders,\textsuperscript{4} and so this has been suggested as a therapeutic strategy for the prevention and/or regression of neurodegenerative diseases.\textsuperscript{5} Leeuwenburgh and Heinecke\textsuperscript{6} demonstrated that acute exercise decreases the levels of antioxidant substances, oxidative stress increases in people who do not exercise, and long-term exercise increases antioxidant enzyme activity.

On the other hand, few studies have addressed the relationship between the total antioxidant capacity (TAC) and inflammation indices in plasma following acute and chronic exercise. Ficicilar et al.\textsuperscript{7} revealed that severe acute exercise can cause platelet aggregation in rats, with no significant difference in platelet count but a remarkable reduction in TAC, with there being a significant negative correlation between these two indicators. That study measured platelet function and the TAC in rats following the implementation of a training protocol including running at 65% maximum oxygen consumption for 60 min on a treadmill at 30 m/min and a 0% incline; their results showed that acute strenuous exercise increased platelet aggregation and the platelet count, and decreased the TAC.\textsuperscript{7}

Ficicilar et al.\textsuperscript{8} evaluated platelet function and the TAC after a short-term training protocol comprising 30 min of running on a treadmill at 25 m/min and a 0% incline for three consecutive days, and found that this increased TAC and decreased platelet aggregation and function. However, no previous study has investigated the effects of regular physical activity on a DNA oxidative-damage marker [8-hydroxy-2'-deoxyguanosine (8-OHdG)] and the TAC of hippocampal tissues in rats exposed to lead acetate. Therefore, the present study investigated the effects of 8 weeks of a treadmill-running exercise on the 8-OHdG level and the TAC value as indicators of oxidative stress in the hippocampus of lead-acetate-exposed rats.

### METHODS

#### Research methodology

The study population consisted of 50-day-old male Wistar rats raised at the Pasteur Institute, Tehran, Iran. Forty rats with an average weight of 270 g were purchased, and after their transfer to the laboratory, acclimatization, and familiarization with the mode of treadmill exercise, they were randomly divided into four groups: baseline, control (sham), lead, and exercise+lead (Table 1). During the experimental period the rats were housed in groups of four in transparent polycarbonate cages (15 cm×15 cm×30 cm; Razi Institute, Tehran, Iran) at 20–24°C, 45% to 55% humidity, and a 12-h/12-h light/dark cycle, with free access to water and standard pellet food (Behparvar, Tehran, Iran) at 10 g/100 g body weight according to weekly weight measurements.

A protocol that included injecting some of the animals with lead or solvent (ethyl oleate) was conducted three times weekly for 8 weeks. The lead solution was prepared by first weighing 2 g of lead acetate with a 0.001 accuracy scale, and placing this in a graded container and gradually adding 100 mL of distilled water. According to recent findings, a 20-mg dose of lead acetate can affect the oxidative stress induced in rats.\textsuperscript{9} Therefore, in the present study, lead acetate solution was injected intraperitoneally at 20 mg/kg body weight three times weekly for eight consecutive weeks in the lead and exercise+lead groups. Moreover, considering the probability of an injection inducing oxidative stress and consequently interfering with the results of the study, a sham procedure was performed on a control group. Thus, concurrent with the injection of lead acetate into the exercise+lead and lead groups, the sham group was injected intraperitoneally with solvent (ethyl oleate) at 30 mg/kg body weight three times weekly for 8 weeks.\textsuperscript{9} In addition, to determine the possible effects of exercise on reducing the harmful effects of lead, a “lead” group was included to demonstrate the effects of lead on the TAC.

#### Exercise training

The animals were familiarized with the mode of activity in

| Group            | Parameter                              | Body weight before regular exercise (g) | Body weight after regular exercise (g) | Wet brain weight (g) | No. |
|------------------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------|-----|
| Baseline         |                                        | 267.8±16.4                             | 342.3±24.6                             | 1.74±0.15            | 10  |
| Sham (control)   |                                        | 252.1±18.5                             | 341.7±34.1                             | 1.70±0.18            | 10  |
| Lead             |                                        | 295.0±17.1                             | 307.2±23.4                             | 1.62±0.19            | 10  |
| Exercise+lead    |                                        | 284.8±19.6                             | 328.4±20.3                             | 1.77±0.21            | 10  |
| Total            |                                        |                                        |                                        | 1.74±0.15            | 40  |

Data are mean±SD values.
five sessions of activity on a treadmill at a speed of 5 to 8 m/min and a 0% incline for 5 to 10 min. The training program also included running on the treadmill, which was specially designed for rodents, in which exercise was implemented based on the principle of progressive overload for 25–64 min at a speed of 15–22 m/min in five weekly sessions for 8 weeks. To warm up, the animals ran for 3 min at 7 m/min at the beginning of the experiment, and then the treadmill speed was increased in increments of 2 m/min until the desired speed was reached. To progressively cool the body down at the end of each training session, the treadmill speed was decreased in the opposite manner until it reached the initial speed. The whole training program was conducted on a treadmill with no incline.

**Sampling and tissue collection**

All procedures were performed according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA). All chemicals were purchased from Sigma Chemical (USA).

To avoid the effect of age on changes in the indices used in the study, all animals were killed at the end of the study under a similar condition, by injecting 3 units of ketamine and xylazine at a ratio of 5:2 in the resting state (24 h after the last training session or 24 h after the last injection of lead acetate or solvent). The brain (including the hippocampus) was removed and immediately placed in liquid nitrogen and subsequently stored at -70°C until being analyzed. For sample preparation, the hippocampal tissue was powdered in liquid nitrogen and then homogenized in a buffer containing 20 mM Tris HCl (pH 8.0), 137 mM NaCl, 1% Tergitol-type NP-40, 10% glycerol, 1 mg/mL leupeptin, 0.5 mM sodium vanadate, 100 mg/mL AEBSF [4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride], and 1 mM PMSF (phenylmethylsulfonyl fluoride). Specimens were then centrifuged at 14,000 rpm for 3 min at 4°C, and the supernatant collected and stored at -20°C.

**Experimental procedures**

**DNA oxidative damage assay**

8-OHdG is an important biomarker for the measurement of oxidative DNA damage. The concentration of 8-OHdG was determined by sandwich enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Chongqing Biospes, Chongqing, China). According to the kit method chosen, the plate was coated with purified anti-8-OHdG antibody, the sample added, and any 8-OHdG in the sample would then connect to the anti-8-OHdG antibody. Horseradish peroxidase (HRP)-conjugated anti-8-OHdG antibody was added to detect the antibody, and it connected to 8-OHdG attached to that antibody. TMB (3,3',5,5'-tetramethylbenzidine) was then added as a substrate, which was converted by the HRP into a blue color that then changed to yellow after adding the acid stop solution. The absorbance of each microplate at 450 nm was recorded. All assay procedures were carried out according to the manufacturer’s instructions and guidelines. The concentration of 8-OHdG in the hippocampal tissue was calculated, and reported in nanograms per milligram of protein.

**Measurement of TAC**

The ferric reducing antioxidant power (FRAP) assay is a method for determining the TAC. According to this method, a blue ferrous complex is formed by the reduction of the colorable ferric tripyridyl triazine complex in the presence of an antioxidant. The absorbance of samples was determined at 593 nm using an ultraviolet-visible spectrophotometer (model 6505, JENWAY, UK). After comparing the absorbance of each sample with the standard curve, the quantity of antioxidant power was calculated and the TAC was reported in nanomoles per milligram of protein.

**Statistical analysis**

The Kolmogorov-Smirnov test indicated that the data conformed with a normal distribution of data, and so ANOVA was applied. Furthermore, in cases of significant differences in any of the indicators, Tukey’s post-hoc test was used to evaluate differences between the different groups with a significance cutoff of \( p \leq 0.05 \).

**RESULTS**

The body weights of the Wistar rats in the different groups at the beginning and end of the regular treadmill exercise and

| Parameter               | Group            |
|-------------------------|------------------|
|                         | Baseline | Sham | Lead    | Exercise+lead    |
| 8-OHdG (ng/mg protein)  | 22.6±7.3*   | 24.1±8.5*   | 52.3±16.6    | 37.5±12.3*   |
| TAC (nmol/mg protein)   | 265.40±0.38*  | 248.50±0.62* | 114.30±0.76 | 193.80±0.29* |

Data are mean±SD values.

*Significant difference compared to lead group \( (p<0.05) \), †Significant difference compared to lead group \( (p<0.01) \).
also their wet brain weights are listed in Table 1. Table 2 lists the 8-OHdG levels and TAC values of the study groups. These tables indicate that the intraperitoneal injection of 20 mg of lead acetate solution per kilogram of body weight three times weekly for 8 weeks can lead to a significant increase in the hippocampal DNA oxidative-damage marker (8-OHdG) and a decrease in the TAC value in the rat brain in comparison with baseline and sham groups of the same age \( p < 0.01 \). Moreover, 8-OHdG decreased significantly and the TAC increased significantly for the hippocampal tissue in the exercise+lead group relative to the lead group \( p < 0.05 \). Additionally, there were no significant exercise-induced or lead-induced changes in the brain weight in the study groups \( p = 0.0704 \).

**DISCUSSION**

The present study is one of the first to investigate the effects of 8 weeks of regular treadmill exercise on the level of a DNA oxidative-damage marker (8-OHdG) and the TAC value in rat hippocampal tissue exposed to lead acetate. It was found that the intraperitoneal injection of lead acetate led to a significant reduction in TAC, whereas implementing 8 weeks of regular exercise concurrent with lead injection contributed to a significant improvement in TAC values in the exercise+lead group relative to the lead group. Our previous studies have indicated that antioxidant supplements and exercise can suppress the negative effects of exposure to contaminants such as lead so as to protect the central nervous system against lead-induced oxidative stress and its deleterious effects.\(^3\)^\(^4\)

Lead is an environmental toxin found at various concentrations in the air, water reservoirs, soil, and food, and exposure to it in industrial cities is associated with body toxicity and is accompanied by osteoporosis, cataracts, and hematologic, glandular, gastrointestinal, reproductive, cardiovascular, neurological, and cognitive disorders and other numerous problems in adults, and especially in children and adolescents.\(^1\)^\(^2\)^\(^12\)^\(^13\)

The hypothesis that childhood lead exposure is a risk factor for problems with the nervous system has received considerable attention in recent decades. Aykin-Burns et al.\(^14\) reported that exposure to lead can contribute to oxidative stress, and that younger rats are more susceptible to this and its consequences, which could be due to the gastrointestinal absorption of lead being greater in younger rats.

Since the subjects in the present study were 50-day-old young rats, the reduction in TAC may be attributed to the effects of lead and the greater vulnerability of their developing nervous systems. Likewise, there are reports on delayed effects of exposure to air pollutants on the nervous system.\(^12\)

For example, Ling et al.\(^15\) reported that Parkinson’s disease can be at least partly associated with a combination of changes in environmental factors during the prenatal period among brain cells and subsequent aging-related changes. On the other hand, some researchers have reported that exposure to pollutants in the embryonic and elderly periods does not impact the production capacity of interferon gamma. However, both primary and secondary exposure to pollutants can lead to complete failure of the cytokine cycle.\(^16\) White et al.\(^2\) showed that any stressful stimulus causes the adrenal cortex to produce glucocorticoids, particularly cortisol, via the hypothalamic-pituitary-adrenal (HPA) axis. Glucocorticoids act via type I and type II receptors. In the central nervous system, type I receptors or mineralocorticoids are primarily located in the septo-hippocampal system, whereas type II receptors are located throughout the brain. The HPA axis regulates the secretion of glucocorticoids during exposure to stressors. Glucocorticoids play critical roles in all body organs, and so failure or dysfunction in this axis has negative consequences for most body systems.\(^1\)^ In the present study, a significant increment in 8-OHdG and reduction in TAC were found in the lead group after injecting lead. Moreover, the intraperitoneal placement of the needle did not induce oxidative stress in the animals, since the alterations in the 8-OHdG level and the TAC did not differ significantly between the sham group that received intraperitoneal injections of ethyl oleate for 8 weeks and the baseline group that received no injections (Table 2). There have been some reports that chronic exposure to lead can cause damage to serotonin transporters and long-term reductions in the expression of neurotrophic factors that may have negative consequences on neurogenesis in the hippocampus.\(^17\) Meanwhile, the available data show that exercise can exert positive effects on the antioxidant status.\(^4\)

The molecular mechanisms underlying the beneficial effects of exercise are not completely understood, but several hypotheses have been proposed. Regular exercise is accompanied by increased neurogenesis\(^6\)^\(^8\)^\(^14\) and trophic effects,\(^6\) whereas intense exercise can reverse these effects.\(^19\) Regulation of the cellular oxidative status is another potential mechanism. Free radicals in either small or large quantities can damage cellular functions. Small amounts of free radicals lead to inadequate gene expression for oxidation-reduction homeostasis and ultimately increased vulnerability. On the other hand, large amounts of free radicals that exceed the cell compatibility tolerance can result in oxidative damage, apoptosis, and necrosis. Exercise helps to maintain the level of free radicals between these two limits, by affecting free-radical production in the brain via the calcium-dependent pathways involved in neuronal activity. In addition, cytokinase as well as oxidative and mitochondrial enzymes are important producers of free radicals during exercise. Although exercise can
stimulate the production of free radicals, which is probably harmful to cellular function, regular exercise reportedly reduces aging indicators, including oxidative stress, and in turn improves the cellular antioxidant system, increases cellular resistance to oxidative stress, and thereby decreases oxidative damage to cells. However, the effects of exercise on oxidative damage or the antioxidative condition of the brain are contradictory, and this indicates that there is a complex relationship between physical activity and brain oxidative status. For example, it was reported that exercise increases levels of lipid peroxidation in the rat brain, while regular exercise can reduce oxidative damage to proteins.\(^{/1,2}\)

Our previous studies demonstrated that treadmill exercise can significantly increase the level of brain-derived neurotrophic factor in the hippocampus and the plasma level of TAC in rats exposed to lead acetate.\(^{/2,3}\) Exercise significantly decreases the plasma level of malondialdehyde, which is a marker of lipid peroxidation, and it can decrease neurotoxicity and alleviate oxidative stress in rats exposed to lead acetate. However, acute endurance exercise did not invoke these beneficial effects.

Despite the destructive effects of chronic exposure to air pollutants such as lead on the nervous system that have been reported by researchers, the possible preventative role of exercise among people living in industrial cities in facing nervous-system-associated problems needs to be investigated further. In addition to the need for more investigations into interactions between lead and the effects of different types of exercise on neurogenesis processes, further studies are needed into the possible biochemical mechanisms underlying exercise-induced neuronal protection, as well as the benefits of antioxidant substances alone or in combination with exercise.

This study has demonstrated the toxic effects of lead on hippocampal neurodegeneration, and that performing regular exercise can suppress these toxic effects in Wistar rats, thus improving hippocampal neurogenesis through reducing oxidative stress.

**Conflicts of Interest**

The authors have no financial conflicts of interest.

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