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Habitual exercise induced resistance to oxidative stress

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Abstract

We investigated whether habitual exercise (HE) modulates levels of oxidative DNA damage and responsiveness to oxidative stress induced by renal carcinogen Fe-nitrilotriacetic acid (Fe-NTA). During a ten week protocol, two groups of rats either remained sedentary or underwent swimming for 15–60 min per day, 5 days per week, with or without a weight equivalent to 5% of their body weight. Then we injected Fe-NTA and sacrificed the rats 1 h after the injection. We determined the activity of superoxide dismutase (SOD) in diaphragm and kidney, evaluated levels of 8-hydroxydeoxyguanosine (8OHdG), catalase, and glutathione peroxidase, and assayed OGG1 protein levels in kidney. SOD activity in the diaphragm and kidney was increased in HE rats. By itself, HE had no effect on the level of 8OHdG, but it did significantly suppress induction of 8OHdG by Fe-NTA, and the amount of suppression correlated with intensity of exercise. These results suggest that HE induces resistance to oxidative stress and, at least at the initiation stage, inhibits carcinogenesis.

Keywords: *Exercise, Fe-nitrilotriacetic acid, 8-hydroxydeoxyguanosine, OGG1, superoxide dismutase, swimming*

Abbreviations: *dG, deoxyguanosine; Fe-NTA, Fe-nitrilotriacetic acid; GSHPx, glutathione peroxidase; HE, habitual exercise; 8OHdG, 8-hydroxydeoxyguanosine; hMTH, human MutT homolog; OGG1, 8-oxoguanine-DNA glycosylase 1; ROS, reactive oxygen species; SOD, superoxide dismutase*

Introduction

Exercise, especially habitual aerobic exercise is considered essential to promote and maintain health [1]. By increasing energy consumption and decreasing body weight, exercise reduces obesity, a well-known risk factor for cancer [2]. On the other hand, exercise also increases oxygen uptake [3], of which as much as 2% may possibly be converted to reactive oxygen species (ROS) [4,5]. ROS are thought to be strongly implicated in carcinogenesis [6,7]. Thus, exercise may be a double-edged sword when wielded in the fight against cancer. Numerous studies have investigated whether exercise increases oxidative damage, especially oxidative DNA damage [8–11]. While

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exhausting exercise is reported to increase DNA damage [9,10] moderate aerobic exercise is reported not to increase, and may even decrease, oxidative DNA damage [8,11]. Some findings have indicated that exercise increases the expression of superoxide dismutase (SOD), a scavenger of ROS [12,13], and human MutT homolog (hMTH), a damaged nucleotide sanitization enzyme [14]. These findings suggest that moderate aerobic exercise is unlikely to be harmful and is probably beneficial for the prevention of ROS-related cancer. However, no reports have yet investigated whether exercise lessens the effects of oxidative stress, one of the major causes of cancer [6,7].

We investigated whether habitual exercise (HE) changes the levels of oxidative DNA damage in the kidney and, at the same time, whether it modulates responsiveness against oxidative stress induced by Fe-nitrilotriacetic acid (Fe-NTA), a potent renal carcinogen [15,16].

Materials and methods

Animals and exercise protocols

All procedures in the animal experiments were performed in accordance with the guidelines of the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, published by the Physiological Society of Japan. This study was approval by the Animal Committee of the National Institute of Fitness and Sports. Thirty-five five-week-old male Wistar rats were obtained from CLEA Japan (Japan). The rats were kept under controlled conditions that included maintaining room temperature at 22 ± 1°C and a diurnal cycle of 12 h darkness and 12 h light. Food and water were provided *ad libitum*. After being allowed a week to adapt to their new surroundings, the rats were randomly allocated to two groups: habitual exercise (HE; $n = 19$), and sedentary (Control; $n = 16$). As shown in Table I, rats in the HE group were subjected to swimming. In blue–gray plastic garbage containers filled with water, maintained at 33°C to an average water depth of 60 cm, they swam in groups of two or three. The duration of swimming was initially 15 min, gradually increasing towards a full 60 min for all HE rats by week 7. Up to week 6, all HE rats were able to swim for the current target period of 45 min with a weight equivalent to 5% of body weight attached at the front of the chest. When the time was increased to 60 min, 9 out of 19 rats could not complete the session with the weight attached. Thereafter, 10 rats continued to swim with the weight (hard HE group) and 9 rats without the weight (soft HE group). Control group rats were not subjected to any swimming.

Fe-NTA treatment

Ten weeks into the protocol, 4 days after the final day of swimming, in each group, half of the rats intraperitoneally received Fe-NTA (15 mg Fe/ kg body weight) as described by Toyokuni et al. [15], and the remaining rats were similarly injected with an equivalent volume of saline. One hour after the injection, the rats were sacrificed, and their organs removed, frozen in liquid nitrogen, and stored at − 80°C until analysis.

Determination of 8-hydroxydeoxyguanosine (8OHdG)

Kidney was homogenized with 10 volumes of Dulbecco's phosphate buffered saline (w/v), then 550 μl samples of homogenate were transferred to tubes and centrifuged at 3800*g* for 2 min. The pellets were assayed for 8OHdG determination. DNA extraction and digestion were done under anaerobic conditions as described by Nakajima et al. [17]. 8OHdG and deoxyguanosine (dG) were separated with HPLC and evaluated by electrochemical detection and UV detection using previously described methods [18]. The presence of 8OHdG was quantified as the ratio of 8OHdG per 10^5 dG.

Table I. Exercise protocol.

Week	Duration of swimming	Attached weight 5% of body weight	Frequency times/week
1	none	none	None
2	15 min	none to +	5
3	15 to 30 min	+	5
4	30 min	+	5
5 to 6	45 min	+	5
7 to 11	60 min	+ or −	5

After being allowed a week to adapt to new surroundings, a group of five- week- old rats started a daily regimen of HE. The duration of swimming was gradually increased from 15 to 60 min. All of rats could swim for 45 min with a weight, but 9 out of 19 rats could not complete 60 min swimming with a weight. Consequently, from week 7 to 11 these 9 rats swam without a weight. Ten rats swam with a weight throughout the HE experiment.

Determination of SOD activity

Tissue homogenates were prepared as described by Oh-ishi et al. [19]. Briefly, diaphragm or kidney was homogenized with 9 volumes of a buffer containing 0.25 mol/l sucrose, 10 mmol/l Tris-HCl pH 7.4 and 0.1 mmol/l EDTA (w/v). After centrifugation at 770*g* for 15 min, SOD activity in the supernatants was measured using WST SOD assay kits (Dojindo, Japan). SOD activity in the samples was calculated by comparison with known activities of purified SOD (Wako pure chemical, Japan).

Determination of catalase and glutathione peroxidase (GSHPx) activities

Kidney was homogenized with 4 volumes of 100 mmol/l Tris-HCl pH 7.4 (w/v), then the homogenates were centrifuged at 510*g* for 10 min. Enzyme activity in the supernatants were measured as previously described [20].

Determination of 8-oxoguanine-DNA glycosylase 1 (OGG1)

The presence of OGG1 protein in kidney tissue was evaluated by immunoblotting. Tissue homogenates were prepared using the method described by Potts et al. [21]. To get intense and clear signals, the samples were not heated prior to electrophoresis. As previously described, homogenate samples containing 150 μg protein were subjected, under reducing conditions, to 12.5% sodium dodecyl sulfate—polyacrylamide gel electrophoresis and then transferred onto PVDF membranes (Immobilon-P, Millipore, Bedford, MA) at 370 mA for 35 min [22]. Prior to immunoblotting, the proteins on the PVDF membranes were stained with 1% Ponceau S in 5% acetic acid at room temperature for 10 min. After de-staining with Buffer A (0.35 mol/l NaCl, 10 mmol/l Tris-HCl pH 8.0, 0.05% Tween 20), the membranes were blocked, for 60 min at room temperature, with buffer A containing 3% bovine serum albumin and then incubated overnight at 4°C with a 500-fold dilution of polyclonal antibody against helix-hairpin-helix PDV motif (aa260–aa271) of mouse OGG1 [23] with Can Get Signal (Toyobo, Japan). The membranes were washed three times with buffer A and then incubated for 60 min with a 2000-fold dilution of horseradish-peroxidase-conjugated anti-rabbit IgG (Amersham Biosciences, Buckinghamshire, UK) with Can Get Signal. The membranes were washed for 1 min with buffer A, evenly coated using the ECL system (Amersham Biosciences, UK) and then immediately exposed to Hyperfilm™ ECL (Amersham Biosciences, UK). The protein levels of rat OGG1 were determined using Science Lab 2003 Multi Gauge software Ver 2.2 (FUJIFILM, Japan). OGG1 protein levels were standardized against a calibration sample prepared from a kidney from one of the control rats, and this sample was used in every electrophoresis. The OGG1 protein band intensity value of each sample was divided by that of the standard sample.

Protein determination

Protein concentrations in tissue homogenates were evaluated with Bio Rad protein assay solution, and the protein concentrations in samples were calculated in relation to known concentrations of bovine serum albumin.

Statistical analyses

The differences between samples were analyzed by one-way ANOVA followed by Fisher's least significant difference test or student *t*-test: *p* values of less than 0.05 were considered significant. Data were presented as mean ± standard error (SE).

Results

Effects of HE on body weight

As Figure 1 shows, HE significantly suppressed increase in body weight. A difference in body weight between HE and control groups was apparent even after the first week. Although the difference was not significant, animals in the hard HE group rats were lighter than those in the soft HE group. Comparing the weight of the hard and soft HE groups, *p* values were 0.10 at week 9, and 0.08 at weeks 10 and 11.

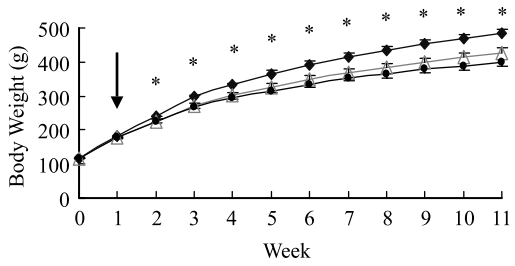

Figure 1. Changes in body weight during the experiment. Body weight was measured weekly and data is presented as means ± SE. Arrow indicates the point when HE was started. ◆, control group which did not swim throughout the experiment; Δ, soft HE group, which swam without a weight after week 7; •, hard HE group, which swam with a weight throughout the eaperiment. Deashes indicate SE. $*p < 0.05$ when compared to control group.

8OHdG levels in kidney

As Figure 2 shows, HE did not increase 8OHdG levels in saline-injected rats. Although the difference was not significant ($p = 0.11$), the HE group showed lower 8OHdG levels than the control group. No significant difference was found in 8OHdG levels between hard and soft HE groups, i.e. 8OHdG levels in the hard group was $0.47 \pm 0.08/10^5$dG ($n = 5$) and in the soft group, $0.47 \pm 0.03/10^5$dG ($n = 4$).

8OHdG levels in kidney after Fe-NTA injection

As Figure 2 shows, Fe-NTA significantly increased 8OHdG in both HE and control groups. However, the resultant 8OHdG levels in the HE groups were significantly lower than in the control group. Furthermore, 8OHdG values correlated inversely with intensity of exercise (Figure 3, $r = 0.48$, $p < 0.05$). In the hard HE group the level of 8OHdG was significantly lower than in the control group, however the difference between the soft HE group and the control group did not reach significance.

Activities of antioxidant enzymes

As shown in Figure 4, without significance differences between the hard and soft groups, HE group diaphragm and kidney samples showed greater SOD activity. SOD activities in diaphragm of hard and soft HE groups were 10.7 ± 0.9 units/mg protein ($n = 5$) and 10.0 ± 0.7 units/mg protein ($n = 4$), and those in kidney were 20.5 ± 3.2 units/mg protein (n = 5) and 24.7 ± 1.1 units/mg protein (n = 4), respectively. There was no evidence that HE increased the activity of either catalase or GSHPx activities (Figure 5).

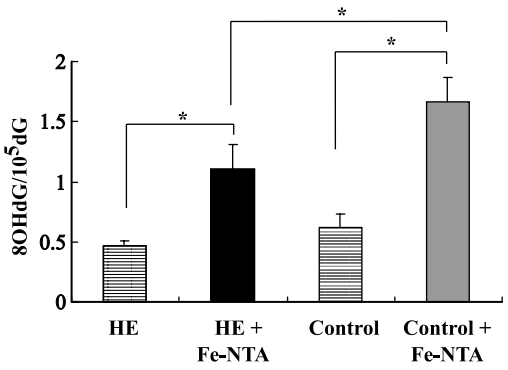

Figure 2. Effects of HE on 8OHdG levels in kidney. As described in Materials and methods, control ($n = 8$) and HE ($n = 9$) subgroups were injected with saline, and control +Fe-NTA ($n = 8$) and HE +Fe-NTA ($n = 10$) subgroups were injected with Fe-NTA. Rats were sacrificed 1 h after the final injection. 8OHdG was evaluated as described in Materials and methods. Dashes indicate SE. $*p < 0.05$.

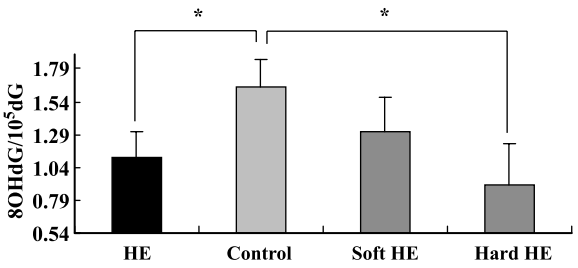

Figure 3. Effects of intensity of exercise on 8OHdG induction by Fe-NTA. The HE bar on the left indicates the mean for all rats injected with Fe-NTA after 10 weeks' swimming ($n = 10$). Control bar indicates the mean for 8 rats injected with Fe-NTA after 10 sedentary weeks. Soft HE and Hard HE bars indicate the means for 5 rats injected with Fe-NTA. Soft HE rats swam without a weight after week 7 and hard HE rats swam with a weight throughout the experiment. 8OHdG was evaluated as described in Materials and methods. The value $0.54/10^5$dG was the average 8OHdG level of rats injected with saline. Dashes indicate SE. $*p < 0.05$.

OGG1 protein levels in kidney.

Immunoblots of OGG1 protein in kidney samples revealed two major protein bands: one was calculated to be 46 kDa (# in Figure 6A); and the other to be 38 kDa (→ in Figure 6A). We assumed that OGG1 was indicated by the 38 kDa protein bands, which indicated that OGG1 protein levels were significantly lower in HE group samples (Figure 6A and C). As Figure 6B shows, the amounts of protein in the HE and control lanes were similar. No significant differences in OGG1 protein levels were observed between the hard (0.67 ± 0.06, $n = 5$) and soft (0.60 ± 0.06, $n = 4$) HE groups.

Discussion

Because HE is able to modulate the responsiveness to oxidative stress caused by higher ROS production in

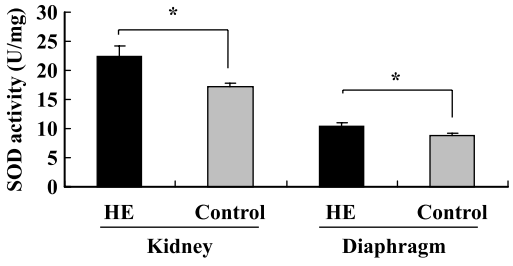

Figure 4. Effects of HE on SOD activities in kidney and diaphragm. As described in Materials and methods, SOD activity in kidney was evaluated in samples from sedentary (control, $n = 8$) and HE rats ($n = 9$) that had been injected with saline. SOD activity in diaphragm was determined for control ($n = 16$) and HE ($n = 19$) rats that had been injected with saline or Fe-NTA. Dashes indicate SE. $*p < 0.05$.

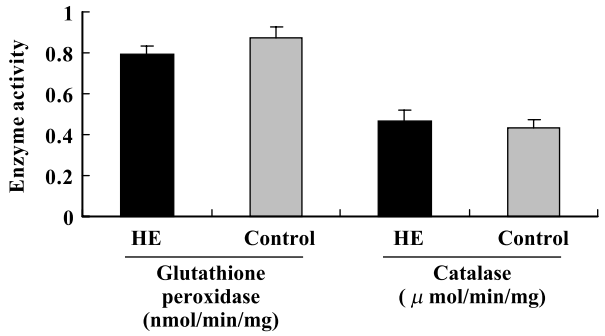

Figure 5. Effects of HE on catalase or glutathione peroxidase (GSHPx) activity in kidney. For Control ($n = 8$) and HE ($n = 9$) rats that had been injected with saline, the activity of catalase or GSHPx was evaluated as described in Materials and methods. Bars indicate SE.

the body, we investigated whether HE has any effect on ROS-related carcinogenesis. We originally wanted to test carcinogens that increase ROS production and induce cancer in lung tissue, where ROS production is most likely to increase during aerobic exercise. We could not, however, find any good candidate carcinogen for a lung-model experiment. We did discover Fe-NTA, a ROS-related carcinogen that reliably induces oxidative stress and cancer in kidney [15,16] and so designed an experiment to evaluate oxidative stress in kidney.

We measured 8OHdG levels in kidney and found that HE did not increase 8OHdG. We then investigated the effects of Fe-NTA and found that, similarly to previous reports [15,16], Fe-NTA increased 8OHdG in kidney. However, the 8OHdG level in the HE/Fe-NTA group was significantly lower than in the control/Fe-NTA group. When we analyzed the effects of intensity of exercise on 8OHdG induction, we found that lowest 8OHdG values in the hard HE group, significantly lower than in the control group. In the soft HE group, the 8OHdG level was between those in the hard HE and control groups, but not significantly lower than control values. This finding suggests that it was exercise, not just water immersion that had an effect on 8OHdG induction by Fe-NTA: amount of suppression correlated with intensity of exercise.

We then investigated why HE suppressed the 8OHdG induction by Fe-NTA. Because HE is reported to increase SOD activity in diaphragm [12,19], we evaluated SOD activities in diaphragm as well as in kidney; we found that HE increased SOD activities both in diaphragm and kidney. Increased SOD activity in the diaphragm indicates that the exercise loads were sufficient. It was not necessary to investigate precisely which SOD species were induced by HE because we were able to detect an overall increase in SOD activity in the HE group. We also tested catalase and GSHPx activity, but did not find any increase due to HE. The findings are in good agreement with that of Gunduz et al. in which they found that long-term daily swimming increased SOD activity but not catalase or GSHPx activity in kidney [24]. Other research suggests that, because the kidneys of Fe-NTA-injected rats become swollen and congestive/hyperemic, after injection of Fe-NTA, the activities of antioxidant enzymes in kidney are decreased [25,26]: consequently, we present data only from saline injected rats.

We also investigated whether HE increased 8OHdG repair activity. Because 8OHdG is mostly removed by OGG1 [27,28], we evaluated OGG1 protein levels in kidney samples. Fe-NTA injection is reported to modulate OGG1 activity in kidney [16], thus we investigated OGG1 protein levels in saline injected rats. OGG1 protein levels were lower in the HE group, a result that agrees well with our previous finding that HE decreases the expression of OGG1 [29]. We speculate that the mechanisms which bring about the lower levels of OGG1 that are associated with HE involve an increase in SOD, which may reduce ROS and decrease the generation of 8OHdG. This assumption is supported by the tendency for 8OHdG values in HE rats to be lower than those in control rats. These lower levels of 8OHdG in HE rats might have decreased the need for OGG1, because cells did not have to repair 8OHdG as efficiently as

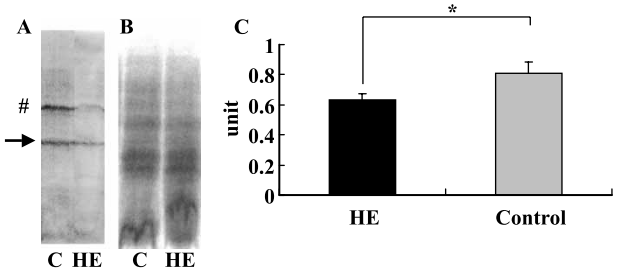

Figure 6. Effects of HE on OGG1 protein levels in kidney. (A) Immunoblots of OGG1. Samples (150 μg protein), from control or HE rats that had been injected with saline, were subjected to electrophoresis and then transferred on to membranes. OGG1 was detected with anti-OGG1 antibody and visualized as described in Materials and methods. Two major protein bands were detected: The molecular weight of the upper band (#) was 46 kDa, and the lower band (→) was 38 kDa. The right arrow (→) indicates OGG1. C lane, sample prepared from a control rat; HE lane, sample prepared from a hard HE rat. (B) Protein staining of the membrane. Proteins on the blotted membrane were stained with Ponceau S as described in Materials and methods. Lanes C and HE are the same lanes as those shown in Figure 6A. (C) OGG1 protein levels evaluated by immunoblotting. The unit indicates the ratio of OGG1 protein band intensity in samples against a standard sample, which was subjected to every electrophoresis. Dashes indicate SE. $*p < 0.05$.

control rats. This could plausibly account for the lower levels of OGG1 in HE rats. Our previous finding that HE decreased OGG1 levels [29] supports this speculation. In another previous finding, in contrast to OGG1, hMTH increased in an HE group [14]: as a result 8OHdG generation may have been decreased even more, resulting in a further decrease in OGG1.

In conclusion our findings suggest that HE suppresses induction of 8OHdG by Fe-NTA, mostly due to the efficient removal of superoxide by SOD, which suppresses Fe reduction. Associated with the intensity of HE in our protocol, we found no increase in oxidative DNA damage. Our results suggest that HE induces resistance to oxidative stress and suppresses the initiation step of carcinogenesis due to ROS. At the same time HE suppresses increases in body weight. These findings indicate that HE is beneficial for cancer prevention.

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References

[1] Centers for Disease Control, National Center for Chronic Disease Prevention and Health Promotion Physical activity and good nutrition: Essential elements to prevent chronic diseases and obesity. Nutr Clin Care 2003;6:135–138.

[2] Stein CJ, Colditz GA. Modifiable risk factors for cancer. Br J Cancer 2004;90:299–303.

[3] Sen CK. Antioxidants in exercise nutrition. Sports Med 2001;31:891–908.

[4] Inoue M, Sato EF, Nishikawa M, Park AM, Kira Y, Imada I, Utsumi K. Mitochondrial generation of reactive oxygen species and its role in aerobic life. Curr Med Chem 2003;10:2495–2505.

[5] Wickens AP. Ageing and the free radical theory. Respir Physiol 2001;128:379–391.

[6] Halliwell B. Oxygen and nitrogen are pro-carcinogens. Damage to DNA by reactive oxygen, chlorine and nitrogen species: Measurement, mechanism and the effects of nutrition. Mutat Res 1999;443:37–52.

[7] Kasai H. Chemistry-based studies on oxidative DNA damage: Formation, repair, and mutagenesis. Free Radic Biol Med 2002;33:450–456.

[8] Inoue T, Mu Z, Sumikawa K, Adachi K, Okochi T. Effect of physical exercise on the content of 8-hydroxydeoxyguanosine in nuclear DNA prepared from human lymphocytes. Jpn J Cancer Res 1993;84:720–725.

[9] Poulsen HE, Weimann A, Loft S. Methods to detect DNA damage by free radicals: Relation to exercise. Proc Nutr Soc 1999;58:1007–1014.

[10] Tsai K, Hsu TG, Hsu KM, Cheng H, Liu TY, Hsu CF, Kong CW, Oxidative DNA damage in human peripheral leukocytes induced by massive aerobic exercise. Free Radic Biol Med 2001;31:1465–1472.

[11] Kasai H, Iwamoto-Tanaka N, Miyamoto T, Kawanami K, Kawanami S, Kido R, Ikeda M. Life style and urinary 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage: Effects of exercise, working conditions, meat intake, body mass index, and smoking. Jpn J Cancer Res 2001;92:9–15.

[12] Powers SK, Criswell D, Lawler J, Martin D, Ji LL, Herb RA, Dudley G. Regional training-induced alterations in diaphragmatic oxidative and antioxidant enzymes. Respir Physiol 1994;95:227–237.

[13] Yamashita N, Hoshida S, Otsu K, Asahi M, Kuzuya T, Hori M. Exercise provides direct biphasic cardioprotection via manganese superoxide dismutase activation. J Exp Med 1999;189:1699–1706.

[14] Sato Y, Nanri H, Ohta M, Kasai H, Ikeda M. Increase of human MTH1 and decrease of 8-hydroxydeoxyguanosine in leukocyte DNA by acute and chronic exercise in healthy male subjects. Biochem Biophys Res Commun 2003;305:333–338.

[15] Toyokuni S, Mori T, Hiai H, Dizdaroglu M. Treatment of Wistar rats with a renal carcinogen, ferric nitrilotriacetate, causes DNA-protein cross-linking between thymine and tyrosine in their renal chromatin. Int J Cancer 1995;62:309–313.

[16] Yamaguchi R, Hirano T, Asami S, Chung MH, Sugita A, Kasai H. Increased 8-hydroxyguanine levels in DNA and its repair activity in rat kidney after administration of a renal carcinogen, ferric nitrilotriacetate. Carcinogenesis 1996;17:2419–2422.

[17] Nakajima M, Takeuchi T, Morimoto K. Determination of 8-hydroxydeoxy-guanosine in human cells uncer oxygen-free conditions. Carcinogenesis 1996;17:787–791.

[18] Takeuchi T, Nakajima M, Ohta Y, Mure K, Takeshita T, Morimoto K. Evaluation of 8-hydroxydeoxyguanosine, a typical oxidative DNA damage, in human leukocytes. Carcinogenesis 1994;15:1519–1523.

[19] Oh-ishi S, Toshinai K, Kizaki T, Haga S, Fukuda K, Nagata N, Ohno H. Effects of aging and/or training on antioxidant enzyme system in diaphragm of mice. Respir Physiol 1996;105:195–202.

[20] Takeuchi T, Nakajima M, Morimoto K. Establishment of a human system that generates O_2^- and induces 8-hydroxydeoxyguanosine, typical of oxidative DNA damage, by a tumor promotor. Cancer Res 1994;54:5837–5840.

[21] Potts RJ, Watkin RD, Hart BA. Cadmium exposure down-regulates 8-oxoguanine DNA glycosylase expression in rat lung and alveolar epithelial cells. Toxicology 2003;184:189–202.

[22] Komatsu M, Sumizawa T, Mutoh M, Chen ZS, Terada K, Furukawa T, Yang XL, Gao H, Miura N, Sugiyama T, Akiyama S. Copper-transporting P-type adenosine triphosphatase (ATP7B) is associated with cisplatin resistance. Cancer Res 2000;60:1312–1316.

[23] Hirano T, Kudo H, Doi Y, Nishino T, Fujimoto S, Tsurudome Y, Ootsuyama Y, Kasai H. Detection of a smaller, 32-kDa 8-oxoguanine DNA glycosylase 1 in 3′-methyl-4-dimethylamino-azobenzene-treated mouse liver. Cancer Sci 2004;95:118–122.

[24] Gunduz F, Senturk UK, Kuru O, Aktekin B, Aktekin MR. The effect of one year's swimming exercise on oxidant stress and antioxidant capacity in aged rats. Physiol Res 2004;53:171–176.

[25] Iqbal M, Athar M. Attenuation of iron-nitrilotriacetate (Fe-NTA)-mediated renal oxidative stress, toxicity and hyperproliferative response by the prophylactic treatment of rats with garlic oil. Food Chem Toxicol 1998;36:485–495.

[26] Singh D, Chander V, Chopra K. Protective effect of naringin, a bioflavonoid on ferric nitrilotriacetate-induced oxidative renal damage in rat kidney. Toxicology 2004;201:1–8.

[27] Nishimura S. Involvement of mammalian OGG1(MMH) in excision of the 8-hydroxyguanine residue in DNA. Free Radic Biol Med 2002;32:813–821.

[28] Shinmura K, Yokota J. The OGG1 gene encodes a repair enzyme for oxidatively damaged DNA and is involved in human carcinogenesis. Antioxid Redox Signal 2001;3:597–609.

[29] Asami S, Hirano T, Yamaguchi R, Itoh H, Kasai H. Reduction of 8-hydroxyguanine in human leukocyte DNA by physical exercise. Free Radic Res 1998;29:581–584.