



Regular exercise reduces 8-oxodG in the nuclear and mitochondrial DNA and modulates the DNA repair activity in the liver of old rats

Hideko Nakamoto ^a, Takao Kaneko ^b, Shoichi Tahara ^b, Eri Hayashi ^a, Hisashi Naito ^c, Zsolt Radak ^d, Sataro Goto ^{a,*}

^a Department of Biochemistry, Faculty of Pharmaceutical Sciences, Toho University, Miyama 2-2-1, Funabashi, Chiba 274-8510, Japan

^b Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

^c Department of Sports Physiology, School of Sports Sciences, Juntendo University, Japan

^d Laboratory of Exercise Physiology, School of Sport Science, Semmelweis University, Budapest, Hungary

Received 7 September 2006; received in revised form 8 November 2006; accepted 14 November 2006

Abstract

Exercise is often said to increase the generation of reactive oxygen species that are potentially harmful. On the other hand, regular exercise has various health benefits even late in life. The specific aim of this study was to explore effects of regular exercise on oxidative status of DNA in aged animals. We report that 2 months of regular treadmill running of aged rats (21 month old) significantly reduced 8-oxodG content to the level of young adult animals (11 month old) in both nuclear and mitochondrial DNA of the liver. The mitochondrial DNA showed 10-fold higher content of the oxidative lesion than the nuclear DNA. The levels in old animals were 2- and 1.5-fold higher than that in young adults for the nucleus and mitochondria, respectively. The activity of the repair enzyme OGG1 was upregulated significantly in the nucleus but not in mitochondria by the exercise. To our knowledge, this is the first report demonstrating that regular exercise can reduce significantly oxidative damage to both the nuclear and mitochondrial DNA. We suggest that the apparent beneficial outcomes in reducing the DNA damage by regular exercise can be interpreted in terms of hormetic effect by moderate oxidative stress and potential adaptation to stronger stresses.

© 2006 Elsevier Inc. All rights reserved.

Keywords: Aging; Liver; Regular exercise; DNA oxidation; 8-oxodG; DNA repair; OGG1; Nucleus; Mitochondria

1. Introduction

Reactive oxygen species (ROS) unavoidably generated in normal energy metabolism in mitochondria as well as in physiological enzymatic processes have been implicated in aging and age-related diseases since the seminal paper of Denham Harman (Harman, 1956). Nucleic acids, proteins and lipids are subjected to modifications by ROS in aerobic organisms, potentially leading to functional impairment of these vital molecules and thus decreasing cellular activities with advancing age if not repaired (Beckman and Ames,

1998; Martin et al., 1996). To cope with such detrimental consequences cells are equipped with multiple antioxidants and antioxidative enzymes as the primary and secondary defense, respectively (Finkel and Holbrook, 2000). In addition, oxidative damage, if it occurred, can be repaired or the damaged molecules replaced by intact ones as the tertiary defense mechanism (Davies, 1986). Nevertheless, the damage can accumulate with time due to incomplete defenses, leading to increased vulnerability and to eventual death of an organism. Investigators have, therefore, tested numerous natural and synthetic antioxidative chemicals in an attempt to retard aging and attenuate age-related oxidative diseases, but with limited success except for improving some pathological conditions. For example, vitamins C and E,

* Corresponding author. Tel./fax: +81 47 472 1531.
E-mail address: goto@phar.toho-u.ac.jp (S. Goto).

well-appreciated antioxidants, have failed to reduce oxidative stress and other detrimental consequences in apparently healthy individuals (Herbert et al., 2006; Miller et al., 2005; Vivekananthan et al., 2003) while deficiency of these vitamins obviously induces oxidative stress and other disorders. This is, however, not unexpected in view of the fact that cellular redox systems are in a delicate balance that may be disturbed by an uncontrolled intake of such chemicals (Bailey et al., 2006; Galati et al., 2006). On the other hand, antioxidant enzymes may be induced or activated in reducing oxidative damage by other means such as dietary or caloric restriction (Nagai et al., 2000; Sanz et al., 2005) and exercise (Judge et al., 2005; Radák et al., 1999, 2001, 2004). We have reported that regular swimming exercise can attenuate oxidative damage to proteins and/or DNA in the skeletal muscle (Radák et al., 1999) and brain (Radák et al., 2001) in rats. Oxidative stress was reduced in the liver as well by regular treadmill exercise training in rats (Radák et al., 2004). These findings illustrate beneficial mechanisms of the exercise, if intensity were appropriate, in reducing oxidative stress rather than increasing it, contrary to what is generally believed. We have proposed that a moderate level of regular exercise can be a form of hormesis in that while excessive exercise to an unprepared body is likely harmful due to massive generation of ROS that exceeds the antioxidative capacity of cells, moderate exercise can be beneficial inducing an adaptive response to cope with higher oxidative and possibly other stresses that may be encountered later (Radák et al., 2005). The notion of hormesis that was described originally in radiobiology has now been extended to a variety of medical and biological domains including toxicology and gerontology (Calabrese, 2004, 2006; Rattan, 2004, 2005).

Oxidative modifications of DNA bases in the nuclei and mitochondria have been suggested as a mechanism of biological aging (Ames et al., 1993; Hamilton et al., 2001; Harman, 1972) and age-related diseases (Cooke et al., 2003; Lombard et al., 2005), particularly cancer (Jackson and Loeb, 2001; Loft and Poulsen, 1996). We have been interested in beneficial the effects of regular exercise, focusing on possible attenuation of oxidative stress in rodent (Goto et al., 2004) as well as in human (Radák et al., 2003). The specific aim of the present study was to examine whether or not exercise of regular treadmill running can reduce oxidative DNA damage in nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) of old rat livers.

2. Materials and methods

2.1. Animals and exercise protocol

Male specific pathogen free (SPF) F344/Du rats were purchased from the Tokyo Metropolitan Institute of Gerontology at the age of 11 and 21 month old. They were kept under clean conventional conditions in our animal facility of Faculty of Pharmaceutical Sciences, Toho University (Radák et al., 2004) until they were killed for the

excision of tissues 1 week, for the younger adult animals, or 8 weeks, for the older animals, after the regular exercise period. Sixteen older animals were familiarized with walking on a motor-driven treadmill for 5 days at 5–7 m/min, once a day for 10–20 min. Five adult animals were similarly handled for 5 days. After this acclimation period, 8 old animals each were randomly assigned to either a sedentary control or an exercise trained group. The animals of training group ran 30–40 min per day at 8–10 m/min with an 8% slope for the first 2 weeks and then for gradually increasing times at higher speed up to 90 min per day at 14–15 m/min in the last 3 weeks. During the last 3 weeks the slope was decreased to 5% because the motivation of running appeared to be reduced. A soft brush but not electric shock was sometimes used manually to stimulate the animals to run. They ran 5 times a week regularly for 8 weeks altogether. The body weight was measured once a week. The sedentary control animals walked for 5 min on the treadmill at the same frequency as the trained animals. The animals were killed by heart puncture under phenobarbital anesthetization. The excised livers were cut into 1 g portions, frozen in liquid nitrogen and stored at -80°C until use. The experiments were approved by the Committee for Laboratory Animals of our Faculty.

2.2. Isolation of nuclear DNA

One gram of the liver was homogenized in 6 vol. of a buffer containing 0.25 M sucrose, 50 mM Tris-HCl (pH 7.4) and 1 mM EDTA with a Teflon/glass homogenizer. The homogenate was layered on 5 ml of a cushion containing 0.35 M sucrose, 50 mM Tris-HCl (pH 7.4) and 1 mM EDTA in centrifuge tubes and centrifuged at 700g for 10 min at 4°C . The fractions around the interface between the applied homogenate and the lower cushion were saved as crude mitochondria preparations (see next section). The nuclear pellets were suspended and nDNA extraction was performed as described previously in an argon atmosphere to prevent artificial oxidation of the DNA (Kaneko et al., 2004). Briefly, the pellet suspensions equivalent to 150 mg of the tissue were centrifuged and the resultant pellets were suspended in a buffer containing 1% Triton X-100, 0.3 M sucrose, 0.1% Desferal, 5 mM MgCl_2 and 10 mM Tris-HCl (pH 8.0). The pellets obtained after centrifugation were treated with proteinase K solution and the mixtures were centrifuged. The pellets containing DNA were treated with NaI and isopropyl alcohol. The crude DNA obtained by centrifugation was washed with isopropyl alcohol and ethanol. The final DNA pellets were dissolved in $0.01 \times \text{SSC}$, 1 mM EDTA and 10 mM Tris-HCl (pH 8.0) and the mixtures were incubated with RNase T1 and RNase A in the argon atmosphere. The solutions were treated with a chloroform and isoamyl alcohol mixture and centrifuged. The DNA in the aqueous phase was precipitated with 7 M NaI and isopropyl alcohol. The final DNA pellets were dissolved in distilled water treated with Chedex 100. The purity (absorption ratio at 260 and

280 nm) and concentration (absorption at 260 nm) of the DNA were determined by a UV spectrophotometer.

2.3. Isolation of mitochondrial DNA

The crude mitochondrial suspensions prepared as above were centrifuged at 7000g for 10 min at 4 °C. The pellets were washed twice with the buffer and then with saline. The final pellets were suspended in 50 µl of DNA Extraction Solution I (Wako Pure Chemicals Co., Japan) and then with an equal mixture of 50 µl of DNA Extraction Solution II (A) and II (B) following the manufacturer's instructions. After standing on ice for 5 min, 75 µl of cold Solution III was added. The mixture was centrifuged at 12000g for 5 min at 4 °C. Three hundred microliters of NaI and isopropanol was added and centrifuged at 14000g for 5 min. The pellet was washed twice with the Washing Solution by centrifugation at 14000g for 5 min at 4 °C. The DNA pellets were dissolved in 300 µl of 0.01 × SSC containing 0.1 mM EDTA buffer (pH 8.0). The solution was incubated with RNase T1 (2.5 U) and RNase A (50 ng) for 20 min at 37 °C in the argon atmosphere. The 300 µl mixture of chloroform and isoamyl alcohol (24:1, v/v) was then added, the air being replaced by argon, followed by centrifugation at 14000g for 10 min at 4 °C. The resultant aqueous layer was added with 300 µl of 7 M NaI and 500 µl of isopropyl alcohol. The mixture was allowed to stand for 10 min at –20 °C in argon and then centrifuged at 14000g for 10 min at 4 °C. The DNA pellets were washed by the mixture of 40% isopropyl alcohol and 70% ethanol, and then dissolved in water treated with Chelex 100. The purity and concentration of the DNA was assessed as in the nDNA.

2.4. Measurement of 8-oxodG in DNA

Oxidative damage to DNA was determined by measuring 8-oxo 2'-deoxyguanosine (8-oxodG) for the nuclei and mitochondria as described (Kaneko et al., 2004). Briefly, the DNA was digested with nuclease P1 in the atmosphere of argon. The digests were then treated with alkaline phosphatase also under argon gas. After filtering through an Ultrafree-MC filter (Millipore Co., USA), the reaction mixture was applied to a HPLC system with a Symmetry C18 column (Waters Co., USA) that was attached to a Coulochem II 5200 electrochemical detector (ESA, USA) (Kaneko et al., 2004). The content of 8-oxodG was expressed as the molar ratio of 8-oxodG to dG that was measured by the absorption at 260 nm in the same chromatographic separation.

2.5. Assay for OGG1 activity

The assay was carried out essentially according the method described (Cardozo-Pelaez et al., 2000; Radák et al., 1999, 2002). Five hundred milligrams of the liver was homogenized with a glass Teflon homogenizer in 5 ml

of buffer A containing 0.25 M sucrose, 1 mM EDTA and 50 mM Tris/HCl (pH 7.5). The homogenates were centrifuged at 80g for 10 min at 4 °C. The supernatants were layered on 5 ml of 0.35 M sucrose/1 mM EDTA and 50 mM Tris/HCl (pH 7.4) and centrifuged at 700g for 10 min at 4 °C. The pellet was saved as a nuclear fraction and the supernatant above the border of the two layers was saved as a crude mitochondrial suspension. The nuclear pellet was washed with buffer A twice by centrifuging at 700g for 10 min at 4 °C. The final pellet was mixed with 5 ml of buffer EB containing 20 mM Tris/HCl (pH 8.0), 1 mM EDTA, 1 mM DTT, 0.5 mM spermine, 1 mM spermidine, protease inhibitor mixture and 50% glycerol. The mixture was centrifuged at 1000g for 10 min at 4 °C. This washing procedure was repeated twice. The pellets were treated with buffer EB containing 0.5% NP40 and centrifuged at 1000g for 10 min at 4 °C to obtain the final pellets. The nuclear extract was prepared by treating the final pellets with 0.5 ml of buffer EB containing 0.25 M KCl for 30 min followed by centrifugation at 14 000g for 30 min at 4 °C. The crude mitochondrial suspensions were centrifuged at 7000g for 10 min at 4 °C and the pellets were washed twice with buffer A. The final pellets were suspended in 50 mM Tris–HCl (pH 7.4) buffer. The protein concentration was determined by Bradford method using BSA as a standard.

The single stranded 24 mer oligonucleotide containing an 8-oxoguanine (Trevigen, USA) was labeled with [γ ³²P]ATP as described (Cardozo-Pelaez et al., 2000) and then hybridized with non-radioactive complimentary oligonucleotide for 3 min at 95 °C as indicated by the manufacturer. For the nicking assay, 5 µl of the enzyme extracts was incubated with 5 µl of the double stranded labeled substrate solution and 20 µl 1 × REC buffer containing 10 mM HEPES–KCl (pH 7.4)/100 mM KCl/10 mM EDTA/0.1 mg/ml BSA) at 30 °C for 15–60 min. The reaction mixture was mixed with 30 µl of chloroform and centrifuged. The supernatant was mixed with an equal volume of loading buffer (90% formamide/10 mM NaOH/tracking dye) and electrophoresed on 20% denaturing polyacrylamide gel containing 7 M urea. The radioactive signals were measured by the Storm/Image-Quant system (Molecular Dynamics, USA) after being visualized on an Imaging Plate (Fuji Film, Japan).

2.6. Statistical analysis

The statistical significance between groups studied for individual animals was evaluated as means ± standard deviation (SD) using two-way analysis of variance (ANOVA), followed by Scheffe's post-hoc test. Significance level was set at $p < 0.05$.

3. Results

3.1. Body weight change

Body weights of old rats in the sedentary control and exercise trained groups at 21 months of age were

419 ± 22 g and 403 ± 18 g, respectively, when the animals were assigned randomly to each group soon after the acclimation period. The body weight of the exercise group was steadily decreasing while that of the sedentary group remained statistically unchanged. At the end of the exercise period the body weight of the control and exercise groups was 425 ± 29 g and 361 ± 21 g at 23 month old, respectively. The body weight of the exercised group was reduced to the level statistically indistinguishable from that of the younger adult animals of 11 months of age (372 ± 20 g). The weights of major tissues such as the liver, kidney, lung, heart, brain and gastrocnemius muscle were not changed significantly by the exercise regimen (data not shown), suggesting that the fat tissue was reduced in the exercised group.

3.2. Effect of age and exercise on 8-oxodG in the liver DNA

To see the effect of regular exercise on DNA oxidation in the liver of aged rats we first studied age-related changes of the modification in the nuclei and mitochondria by separating 8-oxodG from dG in the enzymatic digests of the respective DNA on HPLC followed by electrochemical detection. The 8-oxodG content of both the nDNA and mtDNA was found significantly increased with age, being about 2- and 1.5-fold higher, respectively, in the liver of old sedentary rats compared with younger adult animals (Fig. 1A and B). It is noted that the extent of the DNA oxidation in the mitochondria was about 10-fold higher than that in the nucleus. Eight weeks of the regular treadmill running (30–90 min per day, 5 days a week) resulted in significant reduction of the 8-oxodG content in both the nuclei and mitochondria (Fig. 1A and B). After the exercise training, the levels of the DNA oxidation of the old rats were close to those of the young adult animals in both the nucleus and mitochondria.

3.3. Effect of age and exercise on OGG1 activity in the liver

The reduction of the oxidative modification of DNA by the exercise may be explained by the increased repair of the DNA or theoretically by increased turnover or biogenesis of mitochondria together with upregulation of cellular antioxidative activities. The possibility of increased mitochondrial biogenesis is discussed later (see Section 4). The major repair enzyme for 8-oxoguanine is the 8-oxoguanine DNA glycosylase (OGG1) that catalyses the removal of 8-oxoguanine from DNA and also hydrolysis of the phosphodiester bond at the apurinic site (AP lyase activity), thus generating a cleavage product. We studied the repair activity using a radioactively labeled synthetic double stranded oligonucleotide that contains an 8-oxoguanine in place of a G as a substrate (Fig. 2A). Fig. 2B and C show the time course of the cleavage reaction of the substrate by the nuclear and mitochondrial extracts. The incubation with *Escherichia coli* Formamidopyrimidine-DNA glycosylase Fpg (Trevigen, USA) a homologue of mammalian OGG1, was included as a positive control of the reaction. The reactions were linear up to at least 60 min. Each extract from different groups of rats was incubated with the substrate for 15 or 30 min. The Image Quant patterns for the OGG1 activity of individual animals are shown in Fig. 3A and B for the nuclei and mitochondria, respectively. The enzyme activity is summarized as percentage cleavage of the substrate per unit amount of protein in Fig. 3C and D. The nuclear activity was decreased slightly but significantly in the old compared with the young adult animals. Of particular note is that it was upregulated significantly after 8 weeks of the regular exercise. The activity of the mitochondrial OGG1 did not change significantly with age but, interestingly, it was decreased significantly by the exercise in old animals.

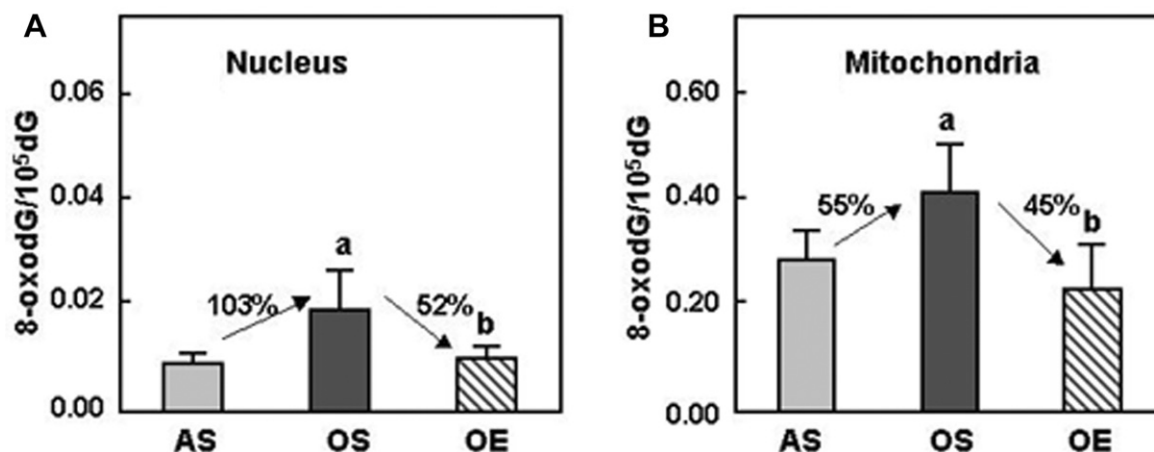


Fig. 1. Effects of age and regular exercise on 8-oxodG in nuclear and mitochondrial DNA of rat livers. The content of 8-oxodG per dG in the nuclear (A) and mitochondrial (B) DNA is shown for the young adult sedentary control (AS), old sedentary control (OS) and old exercised (OE) rats. AS, 11-month-old rats; OS, 23-month-old sedentary rats; OE: 23-month-old rats exercised regularly for 8 weeks. Means ± SD for the nucleus ($n = 5-8$) and mitochondria ($n = 4-5$). (a) $p < 0.01$ (AS versus OS) and (b) $p < 0.01$ (OS versus OE).

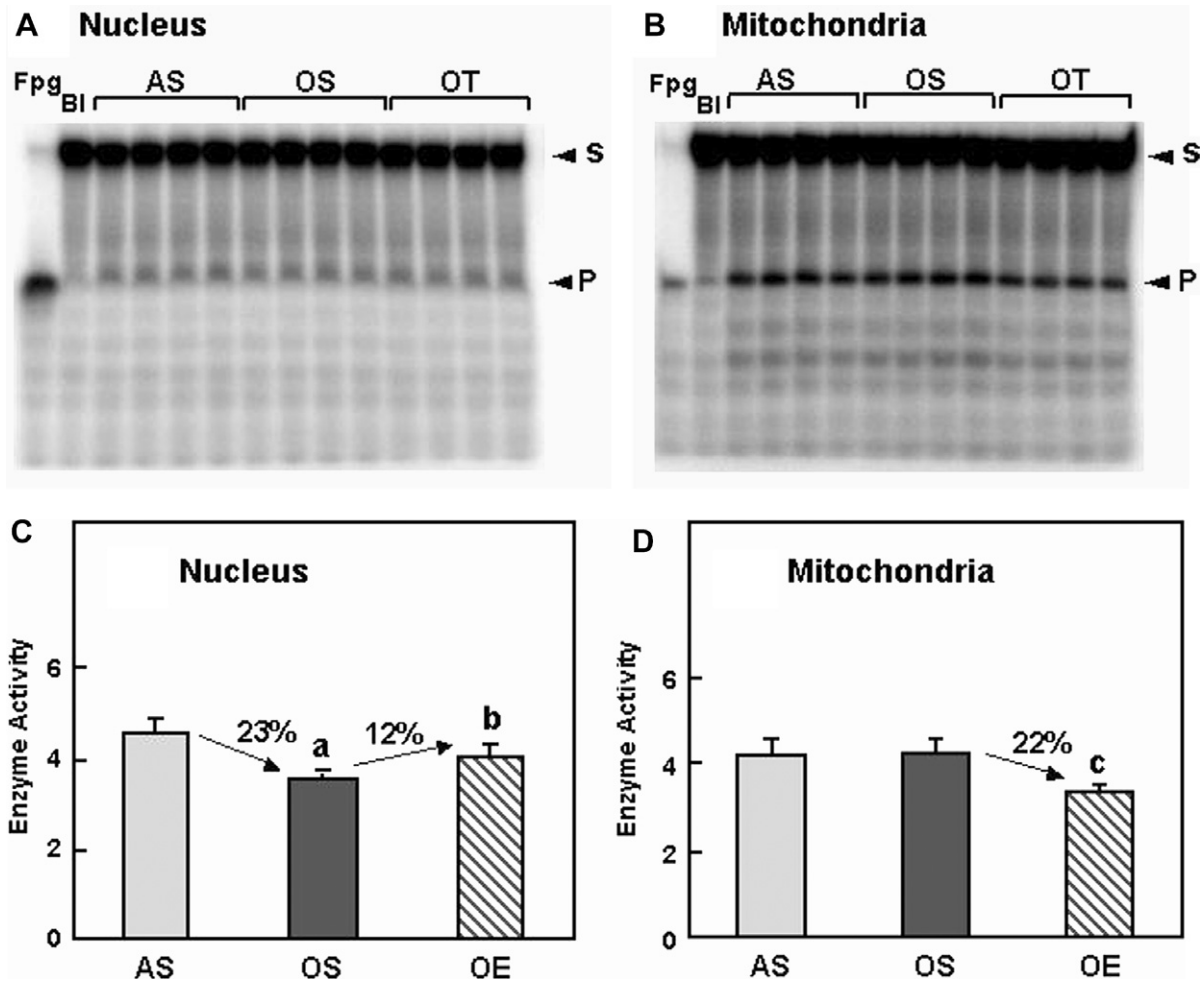


Fig. 3. Effects of age and regular exercise on nuclear and mitochondrial OGG1 activity in rat livers. The enzyme extracts of the nucleus and mitochondria of young sedentary adult, old sedentary and old exercised animals were incubated with the substrate. The results of the product analysis are shown in (A) for the nucleus and (B) for mitochondria. The percentage cleavage was calculated as 100% being the sum of the signal intensity of each lane. The enzyme activities summarized in (C) and (D) for the nucleus and mitochondria, respectively, are expressed as % degradation/5 μ g protein. AS, 11-month-old sedentary rats; OS, 23-month-old sedentary rats; OE, 23-month-old rats exercised regularly for 8 weeks. Means \pm SD ($n = 4$). (a) $p < 0.01$ (AS versus OS), (b) $p < 0.05$ (OS versus OE) and (c) $p < 0.01$ (OS versus OE).

incidence is increased significantly after 2 years of age in rodents (Weindruch, 1992).

Exercise increases oxygen consumption and ROS generation and, therefore, can enhance oxidative damage to nucleic acids, proteins and lipids in cells (Davies et al., 1982; Lovlin et al., 1987; Poulsen et al., 1996). On the other hand, it has been well recognized that regular physical activity has health benefits such as reducing risk and progression of cardiovascular diseases, type 2 diabetes mellitus, cancer and neurodegenerative diseases (Larson et al., 2006; Shephard and Fitcher, 1997; Singh, 2002). Paradoxically, these diseases are suggested to be induced and exacerbated by ROS. We have reported that regular swimming exercise reduced oxidative damage to brain proteins (Radák et al., 2001) and skeletal muscle DNA (Radák et al., 1999) in rats. It has also been demonstrated that regular treadmill running attenuates oxidative stress in the liver of old rats as shown by reduced activation of the transcription factor NF- κ B and increase in GSH (Radák

et al., 2004). Thus, regular exercise can reduce oxidative stress in tissues other than the skeletal and cardiac muscles that are expected to be more easily affected by training. In the present study we showed that the 8-oxodG content of nDNA and mtDNA in the liver of old rats was significantly reduced to the levels of young adult animals after 8 weeks of regular treadmill running. To our knowledge, this is the first report showing that regular exercise can reduce oxidative damage to the mtDNA. The percentage reduction from the level of sedentary old animals was 52% and 45% for nDNA and mtDNA, respectively. It is noteworthy that regular exercise is effective in reducing the oxidative damage to DNA particularly at old age. Conceivably, the reduction may be explained by increased biogenesis of mitochondria or replication of mtDNA due to the exercise training because newly generated intact DNA would dilute the damaged molecules so that the 8-oxodG content per unmodified dG would apparently decrease. Exercise training is in fact reported to induce mitochondrial biogenesis

(Hood, 2001), but this would not explain the percentage reduction since the amount of mtDNA recovered from the liver did not differ significantly among groups studied in our experiment, i.e. the difference between the sedentary and exercised groups being less than 1% (data not shown). The other obvious possibility to account for the reduced oxidative damage is upregulation of the DNA repair activity.

The DNA containing 8-oxoG is repaired mostly by base excision repair (BER) that operates in both nDNA and mtDNA, unlike nucleotide excision repair that functions only in the nucleus (Slupphaug et al., 2003). The initial process of BER is catalyzed by DNA glycosylases that specifically recognize oxidized bases. The OGG1 is the major 8-oxoguanine DNA glycosylase in mammals since cellular extracts from *ogg1* $-/-$ animals does not show appreciable activity for a substrate containing 8-oxoG (Klungland et al., 1999). The OGG1 gene encodes four different isoforms of the enzyme (Takao et al., 1998) including two major isoforms, i.e. OGG1- α for the nDNA and OGG1- β for mtDNA, generated via alternative splicing of the primary transcript (Nishioka et al., 1999). We have measured the OGG-1 activity for the nuclear and mitochondrial extracts using an oligodeoxyribonucleotide substrate containing a single 8-oxoG. The activity of OGG1 in nuclear extracts was significantly lower in the old rats compared with young animals but there was no significant difference between young and old in the mitochondrial enzyme activity (Fig. 3C and D). Contrary to our findings, it is reported that the OGG1 activity does not change with age in the nucleus in mouse and rat livers while the activity of the mitochondrial isoform is higher in old animals (de Souza-Pinto et al., 2001; Souza-Pinto et al., 1999). The reason for the discrepancy may not be simple to explain but the difference in the species and/or the age of animals compared is a possibility, i.e. we compared 11- and 23-month-old rats while they studied rats 6, 12 and 23 months of age (Souza-Pinto et al., 1999) and mice 6, 14 and 23 months of age (de Souza-Pinto et al., 2001). The import of mtDNA repair enzyme into the matrix is reported impaired in aged mouse livers (Szczesny et al., 2003) and the nuclear accumulation of the enzyme caused by oxidative stress is delayed (Szczesny et al., 2004). It might, therefore, be possible that changes of such intracellular localization of the enzyme could have influenced apparent difference of the activity change if combined with the difference in the preparation of the organelles: i.e. they used Percoll gradient centrifugation while we did not.

Interestingly, the reduced repair activity in the liver nucleus of old animals was upregulated by the regular exercise, suggesting that the reduced oxidative damage to the nDNA is at least partly due to the increased repair. Consistent with this finding, we have found that the nuclear OGG1 activity was upregulated by regular exercise in the skeletal muscle (Radák et al., 2002), but not in the brain (Ogonovszky et al., 2005). To account for the reduced oxidative stress of DNA, it is also conceivable that antioxidant

enzymes are upregulated. Ji (1993) reported, however, that both cytosolic and mitochondrial GSH peroxidase activities are decreased significantly in the liver by chronic training on a treadmill for 12 weeks while the activities of other antioxidant enzymes such as SOD and catalase remain unchanged. Thus, the contribution of antioxidant enzymes in reducing the oxidative damage may be low if any. The increase in the content of GSH must have contributed to the reduced oxidation of DNA as we have reported for the liver previously (Radák et al., 2004). Significantly, the mitochondrial OGG1 activity was downregulated by the regular exercise rather than increased as in the nucleus despite significant reduction of the DNA oxidation. The reason for the apparent discrepancy is not clear. It is possible, however, that the activity had been upregulated in early stages of the training but reduced later at the time when the oxidative damage was reduced and the activity was measured. The report that mitochondrial GSH peroxidase is downregulated in chronically trained animals (Ji, 1993) is consistent with our present finding that the mitochondrial OGG1 activity is downregulated by the regular exercise. It is, therefore, possible that reduced activities of these enzymes are adaptive responses to reduced oxidative damage.

In conclusion, we have demonstrated that regular exercise can lower the oxidative damage to DNA in the nucleus and mitochondria of old rat livers to a level of adult animals, suggesting a mechanism for regular exercise in retarding aging and possibly delaying onset of age-related diseases such as cancer (Blair et al., 1989; Sawada et al., 2003). It should be stressed that such beneficial effects of regular exercise may be systemic as shown in this and our previous studies for the liver and brain (Radák et al., 2001, 2004), and not necessarily restricted to the skeletal and cardiac muscles that are apparently more easily affected by exercise. It should also be emphasized that regular exercise has apparently beneficial effects even initiated late in life. The mechanism of how the physical exercise can influence the oxidative status of the liver is not clear but it may be caused by direct changes in the oxygen metabolism in the tissue that includes mitochondrial respiration and enzymatic reactions catalyzed by enzymes such as NADPH oxidase and xanthine oxidase, although the changes, if any, is expected to be less than in the skeletal and cardiac muscles. Alternatively, it could be an indirect effect due to increased oxygen consumption or elevated level of ROS in the muscles. In either case, it seems that the oxidative status in the liver is influenced by exercise (Ji, 1993; Itoh et al., 1998; Radák et al., 2004; Seo et al., 2006). We and others recently proposed that beneficial effects of regular exercise are due to moderate increase in ROS generation, resulting in adaptation to stronger oxidative stress that may be encountered in future (Arumugam et al., 2006; Ji et al., 2006; Judge et al., 2005; Radák et al., 2005; Vollaard et al., 2005). In this regard, Poulsen et al. (1999) have suggested a hypothesis that the relationship between exercise and health is U-shaped in

general, in that too much exercise to unprepared tissues is harmful while it can be beneficial particularly if moderate in the relationship between oxidative modification of DNA and intensity of exercise. Thus, regular exercise that could induce mild oxidative stress with increased consumption of oxygen should be reevaluated as an anti-aging practice.

References

- Ames, B.N., Shigenaga, M.K., Hagen, T.M., 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA* 90, 7915–7922.
- Arumugam, T.V., Gleichmann, M., Tang, S.C., Mattson, M.P., 2006. Hormesis/preconditioning mechanisms, the nervous system and aging. *Ageing Res. Rev.* 5, 165–178.
- Bailey, D.M., Raman, S., McEneny, J., Young, I.S., Parham, K.L., Hullin, D.A., Davies, B., McKeeman, G., McCord, J.M., Lewis, M.H., 2006. Vitamin C prophylaxis promotes oxidative lipid damage during surgical ischemia-reperfusion. *Free Radic. Biol. Med.* 40, 591–600.
- Balaban, R.S., Nemoto, S., Finkel, T., 2005. Mitochondria, oxidants, and aging. *Cell* 120, 483–495.
- Beckman, K.B., Ames, B.N., 1998. The free radical theory of aging matures. *Physiol. Rev.* 78, 547–581.
- Blair, S.N., Kohl 3rd, H.W., Paffenbarger Jr., R.S., Clark, D.G., Cooper, K.H., Gibbons, L.W., 1989. Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA* 262, 2395–2401.
- Calabrese, E.J., 2004. Hormesis: a revolution in toxicology, risk assessment and medicine. *EMBO Rep.* 5, S37–S40.
- Calabrese, E.J., 2006. The failure of dose–response models to predict low dose effects: a major challenge for biomedical, toxicological and aging research. *Biogerontology* 7, 119–122.
- Cardozo-Pelaez, F., Brooks, P.J., Stedeford, T., Song, S., Sanchez-Ramos, J., 2000. DNA damage, repair, and antioxidant systems in brain regions: a correlative study. *Free Radic. Biol. Med.* 28, 779–785.
- Cheng, K.C., Cahill, D.S., Kasai, H., Nishimura, S., Loeb, L.A., 1992. 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and A→C substitutions. *J. Biol. Chem.* 267, 166–172.
- Cooke, M.S., Evans, M.D., Dizdaroglu, M., Lunec, J., 2003. Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J.* 17, 1195–1214.
- Davies, K.J., Quintanilha, A.T., Brooks, G.A., Packer, L., 1982. Free radicals and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun.* 107, 1198–1205.
- Davies, K.J., 1986. Intracellular proteolytic systems may function as secondary antioxidant defenses: an hypothesis. *J. Free Radic. Biol. Med.* 2, 155–173.
- de Souza-Pinto, N.C., Hogue, B.A., Bohr, V.A., 2001. DNA repair and aging in mouse liver: 8-oxodG glycosylase activity increase in mitochondrial but not in nuclear extracts. *Free Radic. Biol. Med.* 30, 916–923.
- Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. *Nature* 408, 239–247.
- Galati, G., Lin, A., Sultan, A.M., O'Brien, P.J., 2006. Cellular and in vivo hepatotoxicity caused by green tea phenolic acids and catechins. *Free Radic. Biol. Med.* 40, 570–580.
- Ghosh, R., Mitchell, D.L., 1999. Effect of oxidative DNA damage in promoter elements on transcription factor binding. *Nucleic Acids Res.* 27, 3213–3218.
- Goto, S., Radák, Z., Nyakas, C., Chung, H.Y., Naito, H., Takahashi, R., Nakamoto, H., Abe, R., 2004. Regular exercise: an effective means to reduce oxidative stress in old rats. *Ann. N.Y. Acad. Sci.* 1019, 471–474.
- Hamilton, M.L., Van Remmen, H., Drake, J.A., Yang, H., Guo, Z.M., Kewitt, K., Walter, C.A., Richardson, A., 2001. Does oxidative damage to DNA increase with age? *Proc. Natl. Acad. Sci. USA* 98, 10469–10474.
- Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 2, 298–300.
- Harman, D., 1972. The biological clock: the mitochondria? *J. Am. Geriatr. Soc.* 20, 145–147.
- Herbert, K.E., Fletcher, S., Chauhan, D., Ladapo, A., Nirwan, J., Munson, S., Mistry, P., 2006. Dietary supplementation with different vitamin C doses: no effect on oxidative DNA damage in healthy people. *Eur. J. Nutr.* 45, 97–104.
- Hood, D.A., 2001. Invited review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. *J. Appl. Physiol.* 90, 1137–1157.
- Itoh, H., Ohkuwa, T., Yamamoto, T., Sato, Y., Miyamura, M., Naoi, M., 1998. Effects of endurance physical training on hydroxyl radical generation in rat tissues. *Life Sci.* 63, 1921–1929.
- Jackson, A.L., Loeb, L.A., 2001. The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. *Mutat. Res.* 477, 7–21.
- Ji, L.L., 1993. Antioxidant enzyme response to exercise and aging. *Med. Sci. Sports Exerc.* 25, 225–231.
- Ji, L.L., Gomez-Cabrera, M.C., Vina, J., 2006. Exercise and hormesis: activation of cellular antioxidant signaling pathway. *Ann. N.Y. Acad. Sci.* 1067, 425–435.
- Judge, S., Jang, Y.M., Smith, A., Selman, C., Phillips, T., Speakman, J.R., Hagen, T., Leeuwenburgh, C., 2005. Exercise by lifelong voluntary wheel running reduces subsarcolemmal and interfibrillar mitochondrial hydrogen peroxide production in the heart. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R1564–R1572.
- Kaneko, T., Tahara, S., Takabayashi, F., Harada, N., 2004. Suppression of 8-oxo-2'-deoxyguanosine formation and carcinogenesis induced by *N*-nitrosobis(2-oxopropyl)amine in hamsters by esculetin and esculin. *Free Radic. Res.* 38, 839–846.
- Klungland, A., Rosewell, I., Hollenbach, S., Larsen, E., Daly, G., Epe, B., Seeburg, E., Lindahl, T., Barnes, D.E., 1999. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc. Natl. Acad. Sci. USA* 96, 13300–13305.
- Larson, E.B., Wang, L., Bowen, J.D., McCormick, W.C., Teri, L., Crane, P., Kukull, W., 2006. Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. *Ann. Intern. Med.* 144, 73–81.
- Loft, S., Poulsen, H.E., 1996. Cancer risk and oxidative DNA damage in man. *J. Mol. Med.* 74, 297–312.
- Lombard, D.B., Chua, K.F., Mostoslavsky, R., Franco, S., Gostissa, M., Alt, F.W., 2005. DNA repair, genome stability, and aging. *Cell* 120, 497–512.
- Lovlin, R., Cottle, W., Pyke, I., Kavanagh, M., Belcastro, A.N., 1987. Are indices of free radical damage related to exercise intensity. *Eur. J. Appl. Physiol. Occup. Physiol.* 56, 313–316.
- Martin, G.M., Austad, S.N., Johnson, T.E., 1996. Genetic analysis of ageing: role of oxidative damage and environmental stresses. *Nat. Genet.* 13, 25–34.
- Mecocci, P., MacGarvey, U., Kaufman, A.E., Koontz, D., Shoffner, J.M., Wallace, D.C., Beal, M.F., 1993. Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. *Ann. Neurol.* 34, 609–616.
- Miller 3rd, E.R., Pastor-Barriuso, R., Dalal, D., Riemersma, R.A., Appel, L.J., Guallar, E., 2005. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann. Intern. Med.* 142, 37–46.
- Nagai, M., Takahashi, R., Goto, S., 2000. Dietary restriction initiated late in life can reduce mitochondrial protein carbonyls in rat livers: Western blot studies. *Biogerontology* 1, 321–328.
- Nishioka, K., Ohtsubo, T., Oda, H., Fujiwara, T., Kang, D., Sugimachi, K., Nakabeppu, Y., 1999. Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs. *Mol. Biol. Cell.* 10, 1637–1652.
- Ogonovszky, H., Berkes, I., Kumagai, S., Kaneko, T., Tahara, S., Goto, S., Radák, Z., 2005. The effects of moderate-, strenuous- and

- over-training on oxidative stress markers, DNA repair, and memory, in rat brain. *Neurochem. Int.* 46, 635–640.
- Poulsen, H.E., Loft, S., Vistisen, K., 1996. Extreme exercise and oxidative DNA modification. *J. Sports Sci.* 14, 343–346.
- Poulsen, H.E., Weimann, A., Loft, S., 1999. Methods to detect DNA damage by free radicals: relation to exercise. *Proc. Nutr. Soc.* 58, 1007–1014.
- Radák, Z., Kaneko, T., Tahara, S., Nakamoto, H., Ohno, H., Sasvari, M., Nyakas, C., Goto, S., 1999. The effect of exercise training on oxidative damage of lipids, proteins, and DNA in rat skeletal muscle: evidence for beneficial outcomes. *Free Radic. Biol. Med.* 27, 69–74.
- Radák, Z., Kaneko, T., Tahara, S., Nakamoto, H., Pucso, J., Sasvari, M., Nyakas, C., Goto, S., 2001. Regular exercise improves cognitive function and decreases oxidative damage in rat brain. *Neurochem. Int.* 38, 17–23.
- Radák, Z., Naito, H., Kaneko, T., Tahara, S., Nakamoto, H., Takahashi, R., Cardozo-Pelaez, F., Goto, S., 2002. Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle. *Pflugers Arch.* 445, 273–278.
- Radák, Z., Apor, P., Pucso, J., Berkes, I., Ogonovszky, H., Pavlik, G., Nakamoto, H., Goto, S., 2003. Marathon running alters the DNA base excision repair in human skeletal muscles. *Life Sci.* 72, 1627–1633.
- Radák, Z., Chung, H.Y., Naito, H., Takahashi, R., Jung, K.J., Kim, H.J., Goto, S., 2004. Age-associated increase in oxidative stress and nuclear factor κ B activation are attenuated in rat liver by regular exercise. *FASEB J.* 18, 749–750.
- Radák, Z., Chung, H.Y., Goto, S., 2005. Exercise and hormesis: oxidative stress-related adaptation for successful aging. *Biogerontology* 6, 71–75.
- Rattan, S.I., 2004. Aging intervention, prevention, and therapy through hormesis. *J. Gerontol. A Biol. Sci. Med. Sci.* 59, 705–709.
- Rattan, S.I., 2005. Anti-ageing strategies: prevention or therapy? Slowing ageing from within. *EMBO Rep. Spec.*, S25–S29.
- Sanz, A., Caro, P., Ibanez, J., Gomez, J., Gredilla, R., Barja, G., 2005. Dietary restriction at old age lowers mitochondrial oxygen radical production and leak at complex I and oxidative DNA damage in rat brain. *J. Bioenerg. Biomembr.* 37, 83–90.
- Sastre, J., Pallardo, F.V., Vina, J., 2003. The role of mitochondrial oxidative stress in aging. *Free Radic. Biol. Med.* 35, 1–8.
- Sawada, S.S., Muto, T., Tanaka, H., Lee, I.M., Paffenbarger Jr., R.S., Shindo, M., Blair, S.N., 2003. Cardiorespiratory fitness and cancer mortality in Japanese men: a prospective study. *Med. Sci. Sports Exerc.* 35, 1546–1550.
- Seo, A.Y., Hofer, T., Sung, B., Judge, S., Chung, H.Y., Leeuwenburgh, C., 2006. Hepatic oxidative stress during aging: effects of 8% long-term calorie restriction and lifelong exercise. *Antioxid. Redox Signal.* 8, 529–538.
- Shephard, R.J., Fitcher, R., 1997. Physical activity and cancer: how may protection be maximized? *Crit. Rev. Oncog.* 8, 219–272.
- Singh, M.A., 2002. Exercise comes of age: rationale and recommendations for a geriatric exercise prescription. *J. Gerontol. A Biol. Sci. Med. Sci.* 57, M262–M282.
- Slupphaug, G., Kavli, B., Krokan, H.E., 2003. The interacting pathways for prevention and repair of oxidative DNA damage. *Mutat. Res.* 531, 231–251.
- Souza-Pinto, N.C., Croteau, D.L., Hudson, E.K., Hansford, R.G., Bohr, V.A., 1999. Age-associated increase in 8-oxo-deoxyguanosine glycosylase/AP lyase activity in rat mitochondria. *Nucleic Acids Res.* 27, 1935–1942.
- Szczesny, B., Hazra, T.K., Papaconstantinou, J., Mitra, S., Boldogh, I., 2003. Age-dependent deficiency in import of mitochondrial DNA glycosylases required for repair of oxidatively damaged bases. *Proc. Natl. Acad. Sci. USA* 100, 10670–10675.
- Szczesny, B., Bhakat, K.K., Mitra, S., Boldogh, I., 2004. Age-dependent modulation of DNA repair enzymes by covalent modification and subcellular distribution. *Mech. Ageing Dev.* 125, 755–765.
- Takao, M., Aburatani, H., Kobayashi, K., Yasui, A., 1998. Mitochondrial targeting of human DNA glycosylases for repair of oxidative DNA damage. *Nucleic Acids Res.* 26, 2917–2922.
- van Remmen, H., Ikeno, Y., Hamilton, M., Pahlavani, M., Wolf, N., Thorpe, S.R., Alderson, N.L., Baynes, J.W., Epstein, C.J., Huang, T.T., Nelson, J., Strong, R., Richardson, A., 2003. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol. Genomics* 16, 29–37.
- Vivekananthan, D.P., Penn, M.S., Sapp, S.K., Hsu, A., Topol, E.J., 2003. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet* 361, 2017–2023.
- Vollaard, N.B., Shearman, J.P., Cooper, C.E., 2005. Exercise-induced oxidative stress: myths, realities and physiological relevance. *Sports Med.* 35, 1045–1062.
- Wang, J., Xiong, S., Xie, C., Markesbery, W.R., Lovell, M.A., 2005. Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. *J. Neurochem.* 93, 953–962.
- Weindruch, R., 1992. Effect of caloric restriction on age-associated cancers. *Exp. Gerontol.* 27, 575–581.