

The Effects of Moderate, Strenuous, and Overtraining on Oxidative Stress Markers and DNA Repair in Rat Liver

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Mots-clés: *exercice, dommage par oxydation, adaptation, OGG1*

Abstract/Résumé

Physical exercise above a certain load has been suggested as being a cause of oxidative stress. We have tested whether training with moderate (MT), strenuous (ST), or over (OT) load can cause alterations in the activities of antioxidant enzymes, lipid peroxidation, protein oxidation, DNA damage, or activity of 8-oxoG-DNA glycosylase (OGG1) in rat liver. The levels of corticosterone decreased in all exercising groups but the differences were not significant. Adrenocorticotrophin hormone (ACTH) levels decreased, not significantly, in MT and OT compared to C. Activity levels of antioxidant enzymes did not change significantly in the liver. The levels of reactive carbonyl derivative (RCD) content decreased in the liver of exercising animals, and the differences reached significance between control and moderately trained groups. The changes in the levels of lipid peroxidation (LIPOX) were not significant, but were lower in the exercised groups. The 8-hydroxydeoxyguanosine (8-

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OHdG) levels increased in the OT group, and the activity of OGG1 measured from crude cell extracts tended to increase in MT and ST. The findings of this study imply that overtraining induces oxidative damage to nuclear DNA, but not to liver lipids and proteins.

L'exercice physique semble causer un stress par oxydation au-dessus d'une charge donnée. Nous avons testé l'effet d'un entraînement au moyen d'une charge modérée (MT), d'une charge intense (ST), et d'une surcharge (OT) sur les activités des enzymes antioxydantes, la peroxydation lipidique et protéinique, les dommages à l'ADN, et l'activité de la 8-oxo-G-DNA glycosylase (OGG1) dans le foie d'un rat. Les taux de corticostérone ont diminué chez tous les groupes soumis à l'exercice, mais les différences ne sont pas significatives. La concentration de corticotrophine (ACTH) a diminué dans les groupes MT et OT, mais de façon non significative comparativement au groupe de contrôle. L'activité des enzymes antioxydantes n'a pas changé significativement dans le foie. La concentration des dérivés du carbonyle réactif (RCD) a diminué dans le foie des animaux soumis à l'effort; les différences entre les groupes de contrôle et les groupes modérément entraînés sont significatives. Quoique moins importantes chez les groupes entraînés, les modifications au niveau de la peroxydation lipidique (LIPOX) ne sont pas significatives. Les taux de 8-hydroxy-désoxyguanosine (8-OHdG) ont augmenté dans le groupe OT et l'activité de l'OGG1 mesurée dans des extraits cellulaires bruts a tendance à augmenter avec une MT et une ST. D'après ces observations, une surcharge à l'entraînement cause des dommages par oxydation à l'ADN du noyau, mais pas aux lipides et protéines hépatiques.

Introduction

The generation of reactive oxygen species (ROS) is a necessary consequence of aerobic metabolism. Interestingly, both higher oxygen demand and hypoxia are known to increase the formation of ROS (Bresgen et al., 2003; Ji, 1993; Ji and Fu, 1992; Moller et al., 2001). Physical exercise, especially a single bout of exercise, due to the limited adaptation of intensity and/or duration, could generate increased levels of ROS and result in oxidative damage to macromolecules (Davies et al., 1982; Radak et al., 2001). On the other hand, regular exercise has been shown to protect against oxidative stress-associated diseases (Carnethon et al., 2003; Duncan et al., 1997) and decrease the levels of lipid peroxidation (Alessio and Goldfarb, 1988; Venditti and Meo, 1997), oxidative protein, and DNA damage (Radak et al., 1999; 2002). Liver is one of the most sensitive organs for exercise-induced oxidative stress, and therefore adaptation (Radak et al., 1999; 2001). The oxidative stress related effects on liver remained to be studied.

There is little information as to whether regular exercise above a certain intensity or duration could be harmful. It would be of interest to identify the optimum exercise loading that would enhance certain physiological functions, have a significant preventive effect against diseases, and cause no accumulation of oxidative damage. According to the current understanding of adaptation to physical exercise, until an overtraining syndrome becomes apparent, regular exercise has beneficial effects. With overtraining, however, which is still poorly understood, the homeostatic balance involving a wide range of hormonal, metabolic, and immunologic changes is altered. Tiidus (1998) has suggested that ROS play a role and might be an associative or causative factor of overtraining.

Therefore we tested the hypothesis that regular exercise with different loadings, including a load aimed at achieving overtraining, can cause oxidative damage to lipids and proteins. We further were interested in the effect of exercise loading on the activity of antioxidant and DNA molecules in liver, using the rat model.

Methods

ANIMALS AND EXERCISE

The protocol of the study was reviewed and approved by the local ethics committee. A total of 24 Wistar rats, age 20 months, were assigned to the following groups: control (C); moderately trained (MT, 1 hr swimming/day, 5 days a week for 8 weeks); strenuously trained (ST, swimming duration increased by 30 min each week until reaching 4.5 hrs for the last week); and overtrained (OT, 1 hr swimming/day, 5 days a week for 6 weeks, at which point the duration was abruptly increased to 4.5 hrs for the remaining 2 weeks). One day after the last exercise session, blood was collected from the rats to enable a determination of ACTH and corticosterone levels, which were used as markers of overtraining. Liver was excised, washed, and frozen in liquid nitrogen and stored at -80°C until analyses.

BIOCHEMICAL ASSAYS

Plasma corticosterone was measured from **10 μL** of unextracted plasma with an RIA using a specific antiserum developed in our institute from rabbits against the corticosterone-3-carboxymethyloxim-BSA. The corticosterone antibody cross-reactivity with other naturally occurring adrenal steroids was 0.05%, except for desoxycorticosterone (1.5%) and progesterone (2.3%). The final dilution of the antibody was 1:40,000. Incubation time was 24 hrs at **4 $^{\circ}\text{C}$** , and a second antibody (anti-rabbit from goat) and 6% polyethylene glycol solution were used for separation. A calibration curve was prepared from corticosterone (Calbiochem) and ranged from 0.27 to 40 pmol/tube. The intra- and interassay CVs were 12.3 and 15.33%, respectively (Zelena et al., 1999).

The ACTH antibody (No. 8514), directed against the midportion of the h-ACTH1-39 molecule, had been raised in rabbit in the Institute of Experimental Medicine, Hungarian Academy of Sciences (Budapest) (Zelena et al., 2003).

The activity of superoxide dismutase (SOD) was measured by the method of Mishra and Fridovich (1972). The content of glutathione (GSH) was measured by the method of Tietze (1969). Catalase activity was measured as described by Beers and Seizer (1952). The activity of glutathione peroxidase (GPX) was assayed as described by Sedlak and Lindsay (1968). The level of lipid peroxidation (LIPOX) was assessed by the determination of thiobarbituric acid reactive substance (TBARS) concentration (Mihara and Uchiyama, 1978). The oxidative modification of amino acid residues was measured by the accumulation of reactive carbonyl derivatives (RCD) as described by Radak et al. (1997).

Isolation of nuclear DNA and the measurement of 8-hydroxydeoxyguanosine (8-OHdG) were carried out as described by Kaneko et al. (1997). In brief, after the isolation of DNA, the aqueous solution containing 50 μg DNA was adjusted to 45 μL , and 5 μL of 200 mM sodium acetate buffer (pH 4.8) and 5 μg of nuclease P1

were added. After a purge with a nitrogen stream, the mixtures were incubated at 37 °C for 1 hr to digest the DNA to nucleotides. Then, 5 µl of 1-M Tris-HCl (pH 7.4) and 0.65 units of alkaline phosphatase were added and the mixture was incubated at 37 °C for 1 hr to hydrolyze the nucleotides to nucleosides. Nucleosides in samples were analyzed by an HPLC/ECD system consisting of a Pegasil ODS column connected to a Shimadzu LC-10 pump (Tokyo, Japan) coupled to an ECD (ESA Coulechem II 5200; Bedford, MA). The solvent system used was a mixture of 6% methanol, 12.5 mM citric acid, 30 mM sodium hydroxide, 25 mM sodium acetate, and 10 mM acetic acid. The flow rate was 1.4 ml/min. The quantities of dG and 8-OHdG were determined from the absorbance at 260 nm using an UV detector and simultaneously by ECD, respectively. The amount of 8-OHdG in the sample was expressed relative to the concentration of dG

EXCISION ASSAYS

Samples were homogenized in buffer containing 20 mM of Tris (pH 8.0), 1 mM of EDTA, 1 mM dithiothreitol, 0.5 mM spermidine, 0.5 mM spermine, 50% glycerol, and protease inhibitors. Homogenates were rocked for 30 min after the addition of a 1/10 vol/vol 2.5 M of KCl and centrifuged at 14,000 rpm for 30 min. The supernatant was aliquoted and stored at $-80\text{ }^{\circ}\text{C}$. Protein levels were measured by the BCA method. The excision assay was carried out according to the protocol described earlier (Radak et al., 2003). In brief, 20 picomoles of synthetic probe containing either 8-oxoG (Trevigen, Gaithersburg, MD) were labeled with P^{32} at the 5' end using polynucleotide T4 kinase (**Boehringer Mannheim**, Germany). For the nicking reaction, protein extract (2 µg) was mixed with 20 µg of a reaction mixture containing 0.5 M of *N*-[2-hydroxyethyl] piperazine-*N'*-[ethanesulfonic acid], 0.1 M EDTA, 5 mM dithiothreitol, 400 mM KCl, purified BSA, and labeled probe (approx. 2,000 cpm).

The reaction was carried out at 30 °C for 5–15 min and stopped by placing the solution in ice. Then 30 µl chloroform were added, samples were centrifuged, and 15 µl was taken and mixed with loading buffer containing 90% formamide, 10 mM NaOH, and blue-orange dye. After 3 minutes of heating at 95 °C, samples were chilled and loaded into polyacrylamide gel (20%) with 7 M urea and 1 × TBE and run at 400 mV for 2 hrs. Gels were quantified using a BAS 2000 Bioimaging Analyzer (**Fuji Film Co.**, which city? Japan). Radioactive signal densities were determined using the software designed for this system.

Statistical significance was assessed using ANOVA, followed by the Scheffé post hoc test. The significance level was set at $p < 0.05$.

Results

The body masses of control and MT rats (521.4 ± 23.6 g and 512.8 ± 23.9 g, respectively) were significantly higher than those of ST and OT rats (447.86 ± 11.5 g and 432.8 ± 14.7 g, respectively). Overtraining has previously been associated with loss of body mass (Fry et al., 1991). The decreases in plasma ACTH levels in MT and OT compared to control did not reach a significant level (Figure 1). Corticosterone levels (Figure 2) decreased in all exercising groups but the changes were not significant.

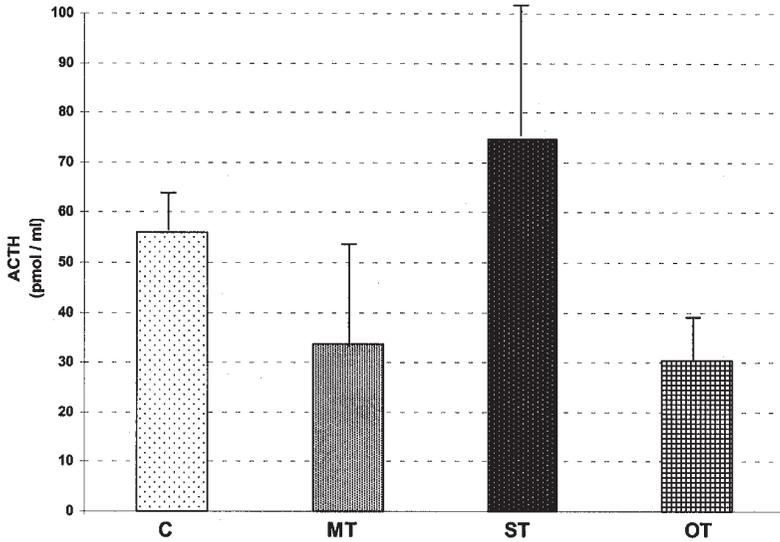


Figure 1. ACTH content was measured to assess the overtrained state. No significant differences were found among the groups. Values are means \pm SD for 6 rats per group.

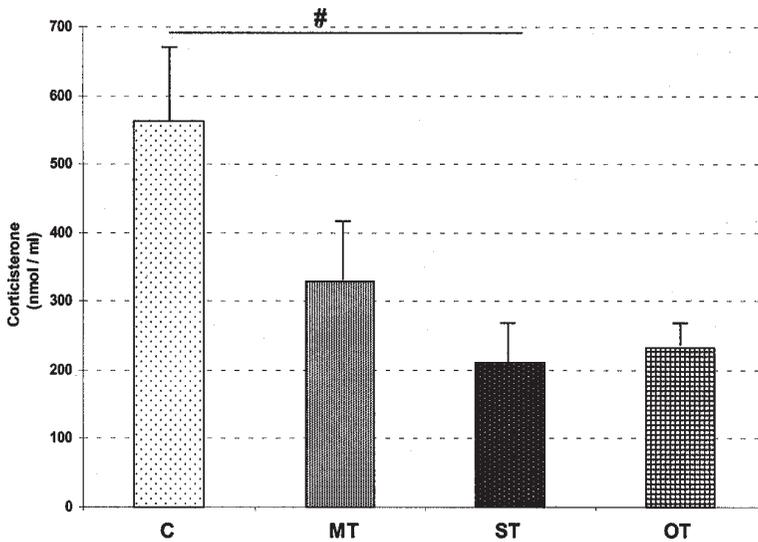


Figure 2. The corticosterone level, a potential marker of overtraining, showed a decreased concentration in exercising groups but no significant alteration was found. Values are means \pm SD for 6 rats per group.

Table 1 Activity of Antioxidants and Level of Lipid Peroxidation ($n = 6$, means \pm SD)

	Control	Moderately trained	Strenuously trained	Overtrained
GPX (U/mg protein)	14.7 \pm 0.28	14.6 \pm 0.59	14.4 \pm 0.45	15.5 \pm 0.56
GSH (mM/mg protein)	0.044 \pm 0.02	0.045 \pm 0.01	0.041 \pm 0.01	0.047 \pm 0.02
SOD (U/mg/protein)	1.216 \pm 916	1.270 \pm 793	1.133 \pm 774	1.067 \pm 625
Catalase (BU/ml hom.)	138.33 \pm 2.44	135.17 \pm 2.12	121.5 \pm 6.53	129.83 \pm 3.27
TBARS (nmol/mg protein)	0.358 \pm 0.03	0.280 \pm 0.01	0.277 \pm 0.01	0.304 \pm 0.01

The activities of antioxidant enzymes did not change significantly in the rat liver (Table 1). RCD content decreased in the liver of exercising rats, and the decrease was significantly different between the MT and control groups (Figure 3). LIPOX levels, as assessed by TBARS, decreased in all experimental groups but the changes were not significant (Table 1). The 8-OHdG levels from the nuclear DNA increased significantly in the liver of OT, but no significant alterations were observed in the other exercising groups (Figure 4). The activity of 8-oxoG repair enzyme, OGG1, measured from the crude cell extracts, tended to increase in MT and ST samples, but the alteration was not statistically significant (Figure 5).

Discussion

Regular exercise is carried out in order to evoke adaptations that include a wide range of beneficial effects. It generally results in an increased mean lifespan (Hawkins et al., 2003; Wannamethee et al., 1998) and protection against various diseases including oxidative stress related diseases (Carnethon et al. 2003; Duncan et al., 1997). One important signal of adaptation could be a decrease in the accumulation of oxidative damage, which could represent the combined efficiency of antioxidant and damage repair systems. In the present study we found that oxidative protein damage decreased in the liver of the MT group compared to control, which suggests either a decreased level of ROS production or an increased level of antioxidant and repair processes.

The repair process of oxidative protein damage is primarily executed by the proteasome enzyme complex. Interestingly, in this study the levels of RCD were not elevated by overtraining. This lack of change might be due to an overtraining-induced catabolic process. As a result, the increased degradation of oxidized proteins would overcompensate the oxidative stress, which is suspected to occur during overtraining. Surprisingly, no significant alteration was detected in the activity of antioxidant enzymes. Generally, regular exercise is believed to result in enhanced activity of antioxidant enzymes and enhanced protection against ROS (Hollander et al., 2000; Ji, 1995, 1999; Sen, 1995; Sen et al., 1992; Somani and Husain, 1996). Ji (1993) has pointed out that exercise training has little effect on the anti-

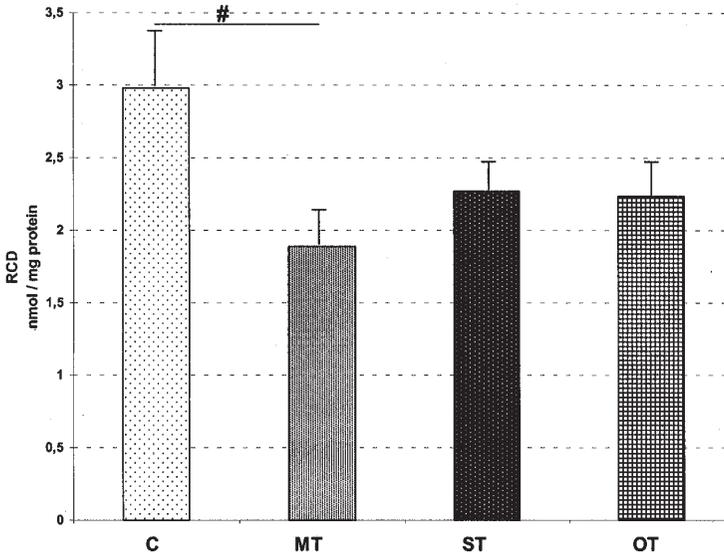


Figure 3. Reactive carbonyl derivatives, marker of oxidative protein damage, decreased in all exercising groups, and there were significant differences between control and moderately trained groups. Values are means \pm SD for 6 rats per group. #Significantly different from control, $p < 0.05$.

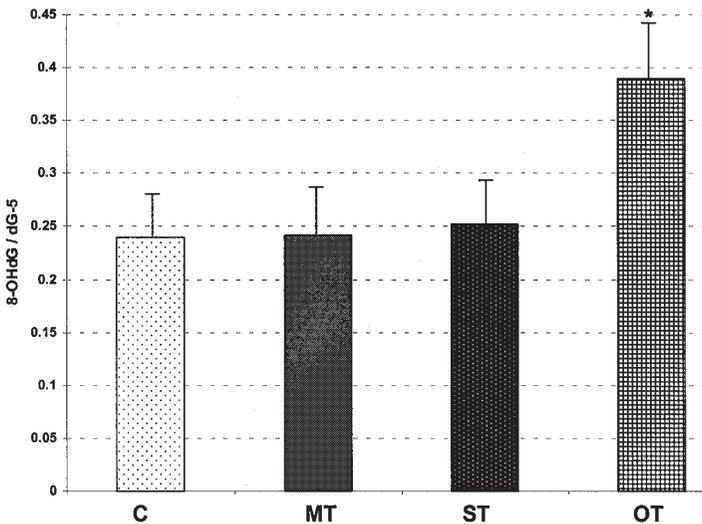


Figure 4. Oxidative damage of nuclear DNA was measured by 8-OHdG. The liver of overtrained rats contained significantly more damage than in the control group. Values are means \pm SD for 6 rats per group. *Significantly different from control, $p < 0.05$.

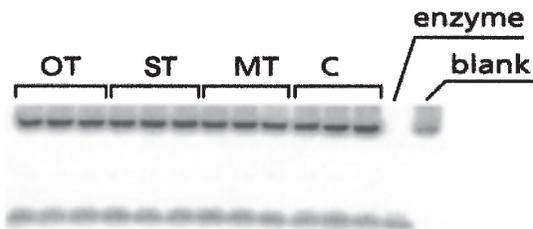


Figure 5. The repair of OHdG was measured by the activity of OGG1. Graph shows representative data of 3 animals per group. The activity tended to increase in MT and ST samples compared to control.

oxidant enzyme status of liver. However, we are uncertain why the applied moderate, strenuous, and overtraining did not change the activity of antioxidant enzymes, as liver seems to be sensitive to oxidative stress.

We **did not observe increases in DNA damage** in brain and skeletal muscle (data are not shown), which implies that the antioxidant system should be very **inducible**. Our recent data suggest that regular exercise reduces the formation of ROS in liver (Radak et al., 2004), but this cannot be the case for OT, since **we measured an increase in DNA damage**. One plausible explanation is that the maladaptation, which probably occurs during overtraining, disrupts the liver's regulating system. Indeed, sugar balance, which is primarily controlled by the liver, is known to be severely affected by overtraining (Petibois et al., 2003). Hence it cannot be excluded that the altered homeostasis of liver is responsible, at least in part, for the lack of an increase in antioxidant enzymes.

To date, very little is known about the characteristics of the enzymes involved in the repair of oxidative damage during exercise. Recent articles have revealed that exercise can increase the activity of some base excision-repair enzymes in both human and animal models (Radak et al., 2002; 2003). The mRNA content of human MutT homologue, which repairs 8-hydroxydeoxyguanosine, has also been observed to be elevated in the leukocyte DNA in trained but not in sedentary subjects (Sato et al., 2003). A small increase in OGG1 activity has been attributed to exercise training when measured from 2 μ g of crude cell proteins. A greater increase in OGG1 activity has been suggested in nuclear proteins. OGG1 is able to **prevent** a variety of DNA damages, but its main substrate is 8-oxoG, which is known to have carcinogenic effects. Therefore, the small increase measured over an extended time period could have very significant consequences.

It must be emphasized that although our aim was to reach an overtrained state in at least one group, it is uncertain whether we succeeded. The changes in body mass suggest that the OT group suffered the most severe challenge of exercise training. On the other hand, the corticosterone levels, which were used as potential markers of overtraining, do not completely confirm that OT reached an overtrained state. Unfortunately, according to Fry et al. (1991), there is still no exact marker of overtraining. It appears that protection and repair of DNA in the liver are not efficient enough to prevent the increase in 8-OHdG. However, our hypothesis, that exercise above a certain load causes massive oxidative stress, is

not fully confirmed by our present findings. Further studies are needed to examine the link between exercise load and the antioxidant/repair systems.

References

- Alessio, H.M., and Goldfarb, A.H. (1988). Lipid peroxidation and scavenger enzymes during exercise: Adaptive response to training. **J. Appl. Physiol.** 64: 1333-1336.
- Beers, R.F., and Seizer, I.W. (1952). Spectrophotometry for measuring the breakdown of hydrogen peroxide by catalase. **J. Biol. Chem.** 195: 133-140.
- Bresgen, N., Karlhuber, G., Krizbai, I., Bauer, H., Bauer, H.C., and Eckl, P.M. (2003). Oxidative stress in cultured cerebral endothelial cells induces chromosomal aberrations, micronuclei, and apoptosis. **J. Neurosci. Res.** 72: 327-333.
- Carnethon, M.R., Gidding, S.S., Nehgme, R., Sidney, S., Jacobs, D.R., Jr., and Liu, K. (2003). Cardiorespiratory fitness in young adulthood and the development of cardiovascular disease risk factors. **JAMA** 290: 3092-3100.
- Davies, K.J., Quintanilha, A.T., Brooks, G.A., and Packer, L. (1982). Free radicals and tissue damage produced by exercise. **Biochem. Biophys. Res. Commun.** 107: 1198-1205.
- Duncan, K., Harris, S., and Ardies, C.M. (1997). Running exercise may reduce risk for lung and liver cancer by inducing activity of antioxidant and phase II enzymes. **Cancer Lett.** 116: 151-158.
- Fry, R.W., Morton, A.R., and Keast, D. (1991). Overtraining in athletes: An update. **Sports Med.** 12: 32-65.
- Hawkins, S.A., Wiswell, R.A., and Marcell, T.J. (2003). Exercise and the master athlete—A model of successful aging? **J. Gerontol. A. Biol. Sci. Med. Sci.** 58: 1009-1011.
- Hollander, J., Bejma, J., Ookawara, T., Ohno, H., and Ji, L.L. (2000). Superoxide dismutase gene expression in skeletal muscle. Fiber-specific effect of age. **Mech. Ageing Dev.** 116: 33-45.
- Ji, L.L. (1993). Antioxidant enzyme response to exercise and aging. **Med. Sci. Sports Exerc.** 25: 225-231.
- Ji, L.L. (1995). Exercise and oxidative stress: Role of the cellular antioxidant system. In: J.O. Holloszy (Ed.), **Exerc. Sport Sci. Rev.** pp. 135-166.
- Ji, L.L. (1999). Antioxidants and oxidative stress in exercise. **Proc. Soc. Exp. Biol. Med.** 222: 283-292.
- Ji, L.L., and Fu, R. (1992). Responses of glutathione system and antioxidant enzymes to exhaustive exercise and hydroperoxide. **J. Appl. Physiol.** 72: 549-554.
- Kaneko, T., Tahara, S., and Matsuo, M. (1997). Retarding effect of dietary restriction on the accumulation of 8-hydroxy-2-deoxyguanosine in organs of Fisher 344 rats during aging. **Free Radic. Biol. Med.** 23: 76-81.
- Mihara, M., and Uchiyama, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. **Ann. Biochem.** 86: 271-278.
- Mishra, H.P., and Fridovich, I. (1972). The role of superoxide anion in the antioxidantation of epinephrine and simple assay for superoxide dismutase. **J. Biol. Chem.** 247: 3170-3175.
- Moller, P., Steffen, L., Carsten, L., and Niels, V.O. (2001). Acute hypoxia and hypoxic exercise induce DNA strand breaks and oxidative DNA damage in humans. **FASEB J.** 15: 1181-1186.
- Petibois, C., Cazorla, G., Poortmans, J.R., and Deleris, G. (2003). Biochemical aspects of overtraining in endurance sports: The metabolism alteration process syndrome. **Sports Med.** 33: 83-94.

- Radak, Z., Apor, P., Pucso, J., Berkes, I., Ogonovszky, H., Pavlik, G., Nakamoto, H., and Goto, S. (2003). Marathon running alters the DNA base excision repair in human skeletal muscle. **Life Sci.** 72: 1627-1633.
- Radak, Z., Asano, K., Lee, K.C., Ohno, H., Nakamura, A., Nakamoto, H., and Goto, S. (1997). High altitude training increases reactive carbonyl derivatives but not lipid peroxidation in skeletal muscle of rats. **Free Rad. Biol. Med.** 22: 1109-1114.
- Radak, Z., Chung, Y.H., Naito, H., Takahashi, R., Jung, K.J., Kim, H.J., and Goto, S. (2004). Age-associated increase in oxidative stress and nuclear factor kB activation are attenuated in rat liver by regular exercise. **FASEB J.** 18: 749-750.
- Radak, Z., Kaneko, T., Tahara, S., Nakamoto, H., Ohno, H., Sasvari, M., Nyakas, C., and 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 Rad. Biol. Med.** 27: 69-74.
- Radak, Z., Naito, H., Kaneko, T., Tahara, S., Nakamoto, H., Takahashi, R., Cardozo-Pelaez, F., and 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.
- Radak, Z., Taylor, A.W., Ohno, H., and Goto, S. (2001). Adaptation to exercise induced oxidative stress: From muscle to brain. **Exerc. Immunol. Rev.** 7: 90-107.
- Sato, Y., Nanri, H., Ohta, M., Kasai, H., and Ikeda, M. (2003). 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.** 305: 333-338.
- Sedlak, S.J., and Lindsay, R.H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. **Ann. Biochem.** 25: 192-205.
- Sen, C.K. (1995). Oxidants and antioxidants in exercise. **J. Appl. Physiol.** 79: 675-686.
- Sen, C.K., Marin, E., Kretzschmar, M., and Hanninen, O. (1992). Skeletal muscle and liver glutathione homeostasis in response to training, exercise, and immobilization. **J. Appl. Physiol.** 73: 1265-1272.
- Somani, S.M., and Husain, K. (1996). Exercise training alters kinetics of antioxidant enzymes in rat tissues. **Biochem. Mol. Biol. Int.** 38: 587-595.
- Tietze, F. (1969). Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. **Ann. Biochem.** 27: 502-522.
- Tiidus, P.M. (1998). Radical species in inflammation and overtraining. **Can. J. Physiol. Pharmacol.** 76: 533-538.
- Venditti, P., and Di Meo, S. (1997). Effect of training on antioxidant capacity, tissue damage, and endurance of adult male rats. **Int. J. Sports Med.** 18: 497-502.
- Wannamethee, S.G., Shaper, A.G., and Walker, M. (1998). Changes in physical activity, mortality, and incidence of coronary heart disease in older men. **Lancet** 351: 1603-1608.
- Zelena, D., Kiem, D.T., Barna, I., and Makara, G.B. (1999). Alfa2-Adrenoceptor subtypes regulate ACTH and endorphin secretion during stress in the rat. **Psychoneuroendocrinology** 24: 333-343.
- Zelena, D., Mergl, Z., Foldes, A., Kovacs, K.J., Toth, Z., and Makara, G.B. (2003). Role of hypothalamic inputs in maintaining pituitary-adrenal responsiveness in repeated restraint. **Am. J. Physiol. Endocrinol. Metab.** 285: E1110-E1117.