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Single bout of exercise eliminates the immobilization-induced oxidative stress in rat brain

Zsolt Radak [a,*], Maria Sasvari [b], Csaba Nyakas [b], Takao Kaneko [c], Shoichi Tahara [c], Hideki Ohno [d], Sataro Goto [e]

[a] *Laboratory of Exercises Physiology, School of Sport Science, Semmelwies University, HUPE Alkotas u. 44, H-1123 Budapest, Hungary*
[b] *Central Laboratory Hungary, School of Health Science, Semmelweis University, Budapest, Hungary*
[c] *Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan*
[d] *Department of Hygiene, School of Medicine, Kyorin University, Tokyo, Japan*
[e] *Department of Biochemistry, School of Pharmaceutical Science, Toho University, Funabashi, Japan*

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Abstract

We were interested in the effects of immobilization (IM), a single bout of exercise (E) and immobilization followed by exercise (EIM) on memory and oxidative damage of macromolecules in hippocampus of rat brain. Eight hours of IM resulted in impairment of passive avoidance test (memory retrieval deficit) and increased latency to start locomotion in an open-field test. Two hours of swimming did not significantly alter the memory retrieval deficit and latency, while the EIM group had longer latency and similar memory than control and E groups. The oxidative damage of lipids, proteins and nuclear DNA increased significantly in IM group and no increase was observed in E and EIM animals. The activity of proteasome was not altered in any groups. The activity of glutamine synthetase (GS) was decreased in IM group ($P < 0.05$), this down regulation was not observed in E and EIM groups. These data suggest that oxidative damage of macromolecules is associated with impaired cognitive function. Single bout of exercise after immobilization eliminates the oxidative damage of macromolecules and normalizes memory function, probably by its ability to restore the activity level of GS and eliminate the consequences of immobilization-induced prolonged efflux of glutamate.

1. Introduction

Reactive oxygen species (ROS) are generated by a variety of physiological and pathological conditions and despite their vital importance to normal cell function including proliferation, growth, signalling and apoptosis (Sen and Packer, 1996; Reid, 1997) they cause continuous damage to lipids, proteins and DNA (Ames et al., 1993; Beckman and Ames, 1997; Goto and Nakamura, 1997). The physiological ROS concentration might be dependent on cell types, age, and even the history of oxidative stress exposure could be a modulating factor (Radak and Goto, 1998). A single bout of physical exercise has been shown to induce formation of ROS and nitrogen species and the related oxidative damage (Davies et al., 1982; Radak et al., 1999a). On the other hand, regular training is known to increase the resistance against ROS induced lipid peroxidation (Alessio and Goldfarb, 1988), and to decrease the accumulation of oxidative protein and DNA damage (Leeuwenburgh et al., 1998; Radak et al., 1999b). In addition, the activity of poteasome complex increases due to exercise training, which means that the repair of oxidative damage in proteins is also up-regulated (Radak et al., 1999b).

The accumulation of reactive carbonyl derivatives (RCD) in the brain has been linked to age-related loss of cognitive function (Carney et al., 1991; Forster et al., 1996) and this decrease in the accumulation has been associated with improved cognitive performance in aged gerbils (Carney et al., 1991; Butterfield et al., 1997). This relationship could to be valid in the age-related decline in cognitive function. We have attempted to show that regular exercise-induced modification is

* Corresponding author. Tel.: +36-309-707811; fax: +36-13566337.
E-mail address: radak@mail.hupe.hu (Z. Radak).

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also linked to the accumulation of oxidative damage in rat brain, and this may be age-related (Radak et al., 2001). Therefore, we used the model in which Liu et al. (1996) showed that 8 h of immobilization resulted in increases in lipid peroxidation, RCD and 8-hydroxydexyguanosine ((8-OHdG) DNA adduct) in regions of the brain of rats. Whether, the immobilization-induced increase in oxidative damage of macromolecules is reflected in cognitive function of young rats is unknown. Since the effect of a single bout of exercise on cognitive function and oxidative damage of lipids, proteins, and DNA is not known we were puzzled by the possible outcome. Therefore, we examined the effects of a single bout of swimming following immobilization to obtain more information about the exercise and immobilization induced stress on rat brain. Glutamine synthetase activity can be readily reduced by oxidative stress in the brain (Carney et al., 1991; Stadtman, 1992) and inactivation of GS might promote oxidative damage of neurons via the decreased uptake of glutamate. Therefore, we also evaluated the possibility that alteration of the activity of GS is associated with oxidative damage in the brain.

2. Methods

2.1. Animals

Twenty four male Wistar rats (6 week old) were used in the study and were cared for according to the 'Guiding Principles for the Care and Use of Animals' based upon the Helsinki Declaration, 1964. The study was approved by the local Animal Welfare Committee. Six rats were randomly assigned to each of four groups; control (C), immobilized (IM), exercised (E), and exercised after immobilization (EIM). All rats including control were lightly anaesthetized with ether and then IM and EIM groups were immobilized during their physically active daily period by taping down all four limbs for 8 h at room temperature as described by Liu et al. (1996), by tapping the limbs of the animals. The exercised rats were exposed to swimming for 2 h. The water temperature was set at 32°C. Swimming was selected because exercise-stimulating electric shock could be avoided. Upon the completion of the immobilization, a single bout of swimming and/or immobilization followed by swimming, 2 h resting period was given and then the animals were tested by open-field and passive avoidance tests. The animals subjected to behavioral test were randomized. Two hour resting was chosen to eliminate the possible physical disability due to immobilization. Right after the behavioral tests, all rats were decapitated and the brains were removed and the hippocampus was immediately dissected and frozen in liquid nitrogen.

2.2. Orientation response to novelty

Novelty-induced orientation response was evaluated 2 h after termination of immobilization or the swimming episodes. The rats were positioned into the center of an open-field box consisting of cylindrical arena of 80 cm diameter, divided into 20 sectors by concentric and radial lines, and surrounded by a 35 cm high wall. The diameter of the central sector of circle shape, in which the rats were placed, was 16 cm. The latency time to leave this circle with all four paws was measured (s) and served as the behavioral reaction to orientation in a novel environment (Nyakas et al., 1991).

2.3. Retention of passive avoidance learning response

The retention of passive avoidance learning behaviour was investigated in a one-trial step-through paradigm (Ader et al., 1972). The apparatus consisted of two compartments. One being dark and the other a well-lit white area ($20 \times 25 \times 25$ cm^3). A small sliding door separated the two compartments. Training started two days before the immobilization and swimming exposures. On day 1 of training, 3 min adaptation was allowed in the dark compartment which was followed by a single trial by placing the rat into the illuminated white compartment and allowing it to enter the dark chamber. On day 2 after the third trial a strong electric foot shock (0.6 mA, 3 s) was delivered to the dark box via the stainless steel bars, which served as the floor. On day 3, immediately after the open-field test, the latency of entering into the dark compartment was recorded, and this measure was used to rank order the animals for statistical analysis and served to express the retention of the learned avoidance response. At the given experimental conditions this test might be consider as a mixed anxiety/memory test, thus the results could represent both affective and cognitive processes.

2.4. Assay

For the estimation of the lipid peroxidation the thiobarbituric acid reactive substances (TBARS) were measured (Ohkawa et al., 1979). The measurement of RCD was done by spectrophotometer as described previously (Levine et al., 1994; Nakamura and Goto, 1996; Radak et al., 1997). The isolation of nuclear DNA and the measurement of 8-OHdG was 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 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 h to digest the DNA nucleotides. Then, 5 μl of 1 M Tris–HCl (pH 7.4) and 0.65 units of alkaline phosphatase were added and the mix-

ture was incubated at 37°C for 1 h to hydrolyze the nucleotides to nucleosides. Nucleosides in samples were analyzed by HPLC/ECD system that consists 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. dG was calculated from the absorbance at 260 nm using UV detector. 8-OHdG was measured simultaneously by ECD. The amount of 8-OHdG in the sample was expressed relative to the concentration of dG.

The proteasome complex have at least five distinct protease activity (Hough et al., 1987; Cardozo and Michaud, 1993) and among these, two types of peptidase activities were measured as described previously (Hayashi and Goto, 1998). These activities were determined fluorometrically by measuring the release of 7-amino-4-methyl-coumarion from the peptides succinyl–Leu–Leu–Val–Tyr–MCA (SUC–LLVY–MCA) and butyloxycarbonyl –Leu–Arg–Arg–MCA (BOC–LRR–MCA) for chymotrypsynlike and trypsin-like activities, respectively. The activity of GS was measured as we described earlier (Radak et al., 1998).

2.5. Statistical analysis

The results of the cognitive tests were evaluated by the Mann–Whitney test, while other measures of statistical significance was assessed using ANOVA, followed by Scheffe's posthoc test. The significance level was set at $P < 0.05$.

3. Results

Eight hours of immobilization resulted in emotional stress as measured by latency to start the locomotion in the open-field test (Fig. 1). Immobilization was associated with a decline in the performance of passive avoidance tests, as evaluated by the latency time to enter the compartment receiving the electrical stimulus ($P < 0.05$). This decrease in cognitive function was not seen in the exercised groups. A single bout of swimming attenuated the cognitive performance thus decreasing the effects of immobilization (Fig. 2).

An increase in lipid peroxidation, carbonylated protein and 8-OHdG concentrations was measured in the hippocampus of IM group (Fig. 3). No significant changes occurred in the other groups. The activity of proteasome complex in the brain was not altered in any group (Fig. 4). The activity of GS in brain decreased significantly due to immobilization stress ($P < 0.05$), but the activity was restored to control level in groups having exercise (Fig. 5).

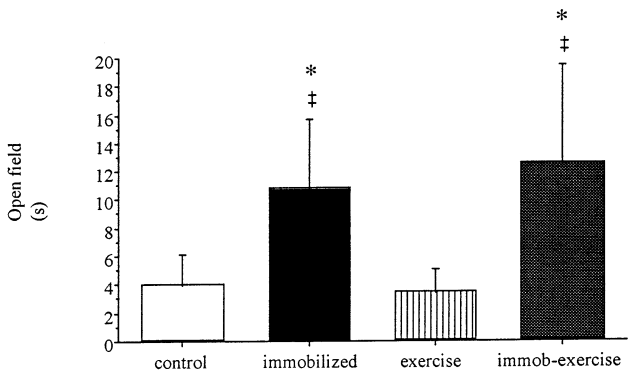

Fig. 1. Eight hour of immobilization and immobilization followed by 2 h of swimming resulted in increased latency time to start the locomotion in open-field test. Single bout of swimming did not alter the latency time. Values are means SD $*P < 0.05$ vs. control, ‡ $P < 0.05$ vs. exercise.

4. Discussion

The data from the present study support the observation of Liu et al. (1996) that limb immobilization causes oxidative stress in the brain. This oxidative brain damage might have functional consequences, because it is associated with decreased cognitive performance, measured by impaired memory. The differences in the result of open-field and passive avoidance tests might indicate that behavioral and cognitive (memory) processes are not equally effected by immobilization. The current study also revealed that a single bout of exercise did not result in decreased cognitive performance, at least as indicated by the used tests. Moreover, a single bout of exercise restored the oxidative stress and related decline in cognitive performance caused by limb immobilization. Thus, the findings of the present study might indicate a causative link between oxidative brain damage and certain cognitive performances. This is in accordance with the data of Carney et al. (1991) and Forster et al. (1996), which suggest that there exists an aging associated decline in cognitive performance asso-

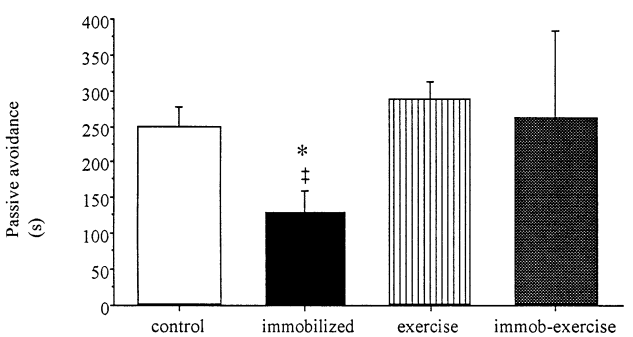

Fig. 2. Immobilization impaired memory function, which was assessed by the decreased time to enter the punished compartment, measured by passive avoidance test. No significant changes were observed in other groups. Values are means SD $*P < 0.05$.

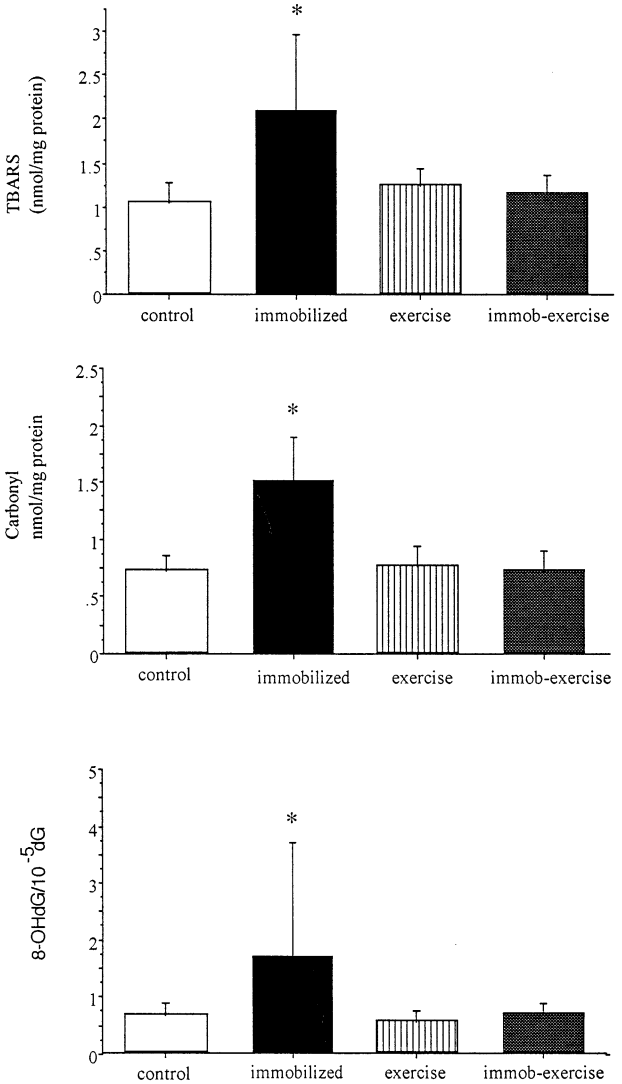

Fig. 3. The accumulation of lipid peroxidation (TBARS), oxidative modification of proteins (RCD) and nuclear DNA damage (8-OHdG) was increased significantly in hippocampus of IM rats. No apparent changes were present in other groups. Values are means SD * $P < 0.05$.

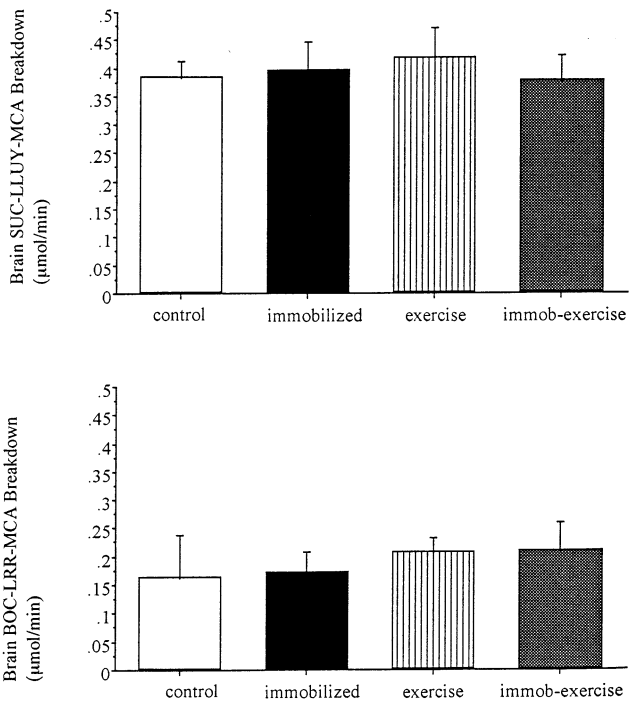

Fig. 4. The activity of proteasome complex did not change as a result of immobilization and single bout of exercise. Values are means SD.

ciated with increases in RCD level. The decrease in oxidatively modified protein concentration has eliminated the age-associated decline in cognitive performance in gerbils (Carney et al., 1991). A recent study from our laboratories also showed that the level of cognitive performance might be linked to the rate of RCD accumulation, while lipid and DNA damage up to a certain level do not alter cognitive function (Radak et al., 2001). Moreover, it appears that the decline in cognitive function due to oxidative damage of the hippocampus might be valid in young animals as well. Therefore, it seems that free radical induced damage of the hippocampus might be one of the factors, which results in impairment of cognitive function.

The beneficial effects of exhaustive exercise has not been suggested as a modulator since it was shown to induce ROS production in liver and skeletal muscle (Davies et al., 1982) and radical species can exert damaging effects distant from their site of generation (Yokoyama et al., 1990). However, the observation that the immobilization-induced increase in oxidative damage markers of macromolecules was not present after single bout of swimming indicates that the free radicals generation and their effects were counterbalanced by the protective systems. The oxidative damage due to immobilization has been shown to cause efflux of glutamate (Lowy et al., 1995), and glutamate is bound to the glutamate receptor of *N*-methyl-D-aspartate receptors

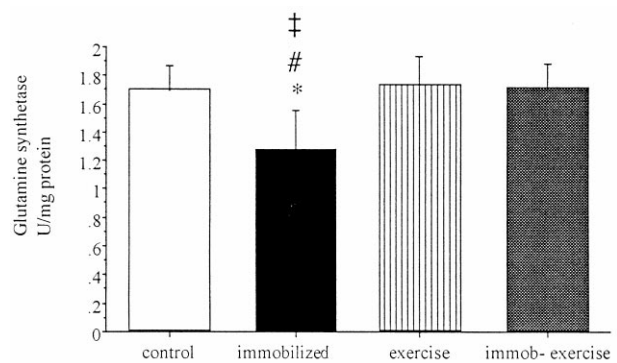

Fig. 5. The activity of glutamine synthetase decreased by 8 h of immobilization and this decline was not present in exercised groups. Values are means SD *$P < 0.05$ vs. control, ‡ $P < 0.05$ vs. exercise.

causing massive increases in intracellular Ca^{2+} concentration through the opened Ca^{2+}-channels (Choi, 1992; Hertz et al., 1999; Kandel and O'Dell, 1992; Mawatari et al., 1996). Then Ca^{2+} can initiate free radical generation via xanthine dehydrogenase/oxidase system, NOS and mitochondria (Mawatari et al., 1996). In addition, a stress-induced elevation of glucocorticoids likely potentiates the accumulation of glutamate (Stein-Behrens et al., 1994). Therefore, an increase in glutamate transport would attenuate the oxidative stress caused by immobilization. We suggest that immobilization-induced oxidative stress does not end by the termination of immobilization. If exercise is done right after the immobilization, it restores the GS activity to the normal level, and thus increase the glutamate uptake (Butterfield et al., 1997; Jabaudon et al., 1999). Ammonia concentration also increases after a single bout of exercise and brain plays an important role in the detoxication of ammonia by glutamine synthesis, leading to decreases in brain glutamate levels (Guezennec et al., 1998; Korf, 1996). Exercise increases the glial transport, which involves glutamate uptake (Kandel and O'Dell, 1992) and also increases the circulating adenosine concentration, a known antioxidant, which thereby can attenuate the oxidative stress. Therefore, it appears that exercise has a beneficial effect on the elimination of the immobilization caused glutamate efflux.

The finding of the present study suggest that limb immobilization induces oxidative damage to the hippocampus, and this damage is associated with or results in impairment of cognitive function. Therefore, the link between oxidative stress mediated changes in cognitive function is not age-associated phenomenon. Single bout of exercise might have a capability to increase the glutamate uptake and thus eliminate the biochemical and cognitive stresses caused by immobilization.

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References

Ader, R., Weijmen, J.A.W.M., Moleman, P., 1972. Retention of passive avoidance response as a function of the intensity and duration of electric shock. Psychol. Sci. 26, 125–128.

Alessio, H.M., Goldfarb, H., 1988. Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. J. Appl. Physiol. 64, 1333–1336.

Ames, B.N., Shinegawa, M.K., Hagen, T.M., 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA 90, 7915–7922.

Beckman, B., Ames, B.N., 1997. Oxidative decay of DNA. J. Biol. Chem. 272, 19633–19636.

Butterfield, D.A., Howard, B.J., Yaltin, S., Allen, K.J.M., Carney, J.M., 1997. Free radical oxidation of brain proteins in accelerated senescence and its modulation by *N-tert*-butyl-alpha-phenylnitrone. Proc. Natl. Acad. Sci. USA 94, 674–678.

Cardozo, M.C., Michaud, C., 1993. Evidence for the presence of five distinct proteolytic components cleaving bonds on the carboxyl side of branched chain and small neutral amino acids. Biochemistry 32, 1563–1572.

Carney, J.M., Starke-Reed, P.E., Oliver, C.N., Landum, R.W., Cheng, M.S., Wu, J.F., Floyd, R.A., 1991. Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of spin-trapping compound N-*tert*-butyl-alpha phenylnitrone. Proc. Natl. Acad. Sci. USA 88, 3633–3636.

Choi, D.W., 1992. Bench to beside: the glutamate connection. Science 258, 241–243.

Davies, J.K.A., Quintanilha, A.T., Brooks, G.A., Packer, L., 1982. Free radicals and tissue damage produced by exercise. Biochem. Biophys. Res. Commun. 107, 1198–1205.

Forster, M., Dubey, A., Dawson, K.M., Stutts, W.A., Lal, H., Sohal, R.S., 1996. Age-related losses of cognitive function and motor skills in mice are associated with oxidative damage in the brain. Proc. Natl. Acad. Sci. USA 93, 4765–4769.

Goto, S., Nakamura, A., 1997. Age-associated, oxidatively modified proteins: a critical evaluation. Age 20, 81–89.

Guezennec, C.Y., Abdelmalki, A., Serrurier, B., Merino, D., Bigard, X., Berthelot, M., Pierard, C., Peres, M., 1998. Effects of prolonged exercise on brain ammonia and amino acids. Int. J. Sports Med. 19, 323–327.

Hayashi, T., Goto, S., 1998. Age-related changes in the 20S and 26S proteasome activities in the liver of male F344 rats. Mech. Ageing Develop. 102, 55–66.

Hough, R., Pratt, G., Rechsteiner, M., 1987. Purification of two high molecular weight proteases from rabbit reticulocyte lysate. J. Biol. Chem. 262, 8303–8313.

Hertz, R., Dringen, R., Schousboe, A., Robinson, S.R., 1999. Astrocytes: glutamate producers for neurons. J. Neurosci. Res. 57, 417–428.

Jabaudon, D., Shimamoto, K., Yasuda-Kamatani, Y., Scanziani, M., Gahwiler, B.H., Gerber, U., 1999. Inhibition of uptake unmasks rapid extracellular turnover of glutamate of nonvesicular origin. Proc. Natl. Acad. Sci. USA 96, 8733–8738.

Kandel, E.R., O'Dell, T.J., 1992. Are adult learning mechanisms also used for development? Science 258, 243–245.

Korf, J., 1996. Intracerebral trafficking of lactate in vivo stress, exercise, electroconvulsive shock and ischemia as studied with microdialysis. Develop. Neurosci. 18, 405–414.

Mawatari, K., Yasui, Y., Takadera, T., Kato, S., 1996. Reactive oxygen species involved in the glutamate toxicity of C6 glioma cells via xc antiporter system. Neoroscience 73, 201–208.

Leeuwenburgh, C., Hansen, P., Shaish, A., Holoszy, J.O., Heinrcke, J.W., 1998. Markers of protein oxidation by hydroxyl radical and reactive nitrogen species in tissues of aging rats. Am. J. Physiol. 274, R453–R461.

Levine, R.L., Williams, J.A., Stadtman, E.R., Shacter, E., 1994. Carbonyl assay for determination of oxidatively modified proteins. Meth. Enzymol. 37, 346–357.

Liu, J., Wang, X., Shigenaga, M.K., Yeo, H.C., Mori, A., Ames, J.N., 1996. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. FASEB J. 10, 1532–1538.

Lowy, M.T., Wittenberg, L., Yamamoto, B.K., 1995. Effect of acute stress on hippocampal glutamate levels and spectrin proteolysis in young and aged rats. J. Neurochem. 65, 268–274.

Kaneko, T., Tahara, S., 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 Radical Biol. Med. 23, 76–81.

Nakamura, A., Goto, S., 1996. Analysis of protein carbonyls with 2,4-dinitrophenylhydrazine and its antibodies by immunoblot in two-dimensional gel electrophoresis. J. Biochem. Tokyo 119, 768–774.

Nyakas, C., Markel, E., Schuurman, T., Luiten, P.G.M., 1991. Impaired learning and abnormal open-field behaviours of rats after early postnatal anoxia and the beneficial effects of calcium antagonist nimodipine. Eur. J. Neurosci. 3, 168–174.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Ann. Biochem. 95, 351–358.

Radak, Z., Asano, K., Lee, C., Ohno, H., Nakamura, A., Nakamoto, H., Goto, S., 1997. High altitude increases reactive carbonyl derivatives but not lipid peroxidation in skeletal muscle of rats. Free Radical Biol. Med. 22, 1109–1114.

Radak, Z., Asano, K., Nakamura, A., Nakamoto, H., Ohno, H., Goto, S., 1998. A period of anaerobic interval exercise increases accumulation of reactive carbonyl derivatives in lungs of rats. Pflugers Arch. Eur. J. Physiol. 435, 439–441.

Radak, Z., Goto, S., 1998. The effects of exercise, ageing and caloric restriction on protein oxidation and DNA damage in skeletal muscle. In: Reznick, A.Z., Packer, L., Sen, C.K., Holloszy, J.O., Jackson, M.J. (Eds.), Oxidative Stress in Skeletal Muscle. Basel, Birkhauser, pp. 89–103.

Radak, Z., Pucsok, J., Mecseki, S., Csont, T., Ferdinandy, P., 1999a. Muscle soreness-induced reduction of force generation is accompanied by increased nitric oxide content and DNA damage in human skeletal muscle. Free Radical Biol. Med. 26, 1059–1063.

Radak, Z., Kaneko, T., Tahara, T., Nakamoto, H., Ohno, H., Sasvari, M., Nyakas, C., Goto, S., 1999b. The effect of exercise training on oxidative damage of lipids, proteins, and DNA in rats skeletal muscle: evidence for beneficial outcomes. Free Radical Biol. Med. 27, 69–74.

Radak, Z., Kanek, T., Tahara, S., Nakamoto, H., Pucsok, 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.

Reid, M.B., 1997. Reactive oxygen and nitric oxide in skeletal muscle. News Physiol. Sci. 11, 114–119.

Sen, C.K., Packer, L., 1996. Antioxidant and redox regulation of gene transcription. FASEB J. 10, 709–720.

Stadtman, E.R., 1992. Protein oxidation and aging. Science 257, 1220–1224.

Stein-Behrens, B.A., Lin, W.J., Sapolsky, R.M., 1994. Physiological elevation of glucocorticoids potentiate glutamate accumulation in the hippocampus. J. Neurochem. 63, 596–602.

Yokoyama, Y., Beckman, J.S., Beckman, T.K., Wheat, J., Cash, T.G., Freeman, B.A., Park, D.A., 1990. Circulating xanthine oxidase: potential mediator of ischemic injury. Am. J. Physiol. 158, G564–G570.