Regular Exercise

An Effective Means to Reduce Oxidative Stress in Old Rats

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ABSTRACT: A healthy diet and regular exercise are among the major factors that influence quality of life (QOL) in old age. Exercise is believed to be beneficial to improve QOL, retarding age-related decline of physiological functions and preventing age-related diseases. Regular physical exercise can possibly improve age-related functional decline and delay onset of age-related diseases by attenuating potentially harmful oxidative damage and suppressing inflammatory processes even in older age.

KEYWORDS: exercise; oxidative; stress; damage; old; rats; NF-κB

In recent years, the average life span in industrialized countries has increased remarkably, while the quality of life (QOL) and activities of daily living of elderly people are not necessarily satisfactory either for themselves or for their families and society. A healthy diet and regular exercise are among the major factors that influence QOL in old age. Exercise is believed to be beneficial to improve QOL, retarding age-related decline of physiological functions and preventing age-related diseases.

While moderate exercise is obviously healthful, it is often claimed to induce oxidative stress due to an excessive oxygen uptake to meet the high demand of adenosine triphosphate (ATP) that can result in elevated reactive oxygen species (ROS) formation in mitochondria. Other enzymatic systems including xanthine oxidase and NADPH dehydrogenase can also be involved in increasing ROS generation.

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Increased utilization of oxygen in mitochondria and in other enzymatic processes would enhance the generation of superoxide anion and hence hydrogen peroxide due to Mn- or Cu,Zn-superoxide dismutase (SOD) activity. As a result of excessive ROS generation, proteins, nucleic acids, and membrane phospholipids may be oxidatively modified, possibly leading to deleterious consequences. Oxidative modification of proteins can influence a variety of cellular functions of many tissues. In fact, a bout of treadmill exercise increased protein oxidation as measured by the carbonyl content in the skeletal muscle[1] as well as in the lung[2] in rat. While a bout of exercise of sedentary animals is likely to cause increased harmful oxidative modifications of proteins, moderate regular exercise can be beneficial by reducing the damage. We have tested this hypothesis using middle-aged and old rats in two different protocols of regular exercise.

EFFECTS OF REGULAR EXERCISE ON COGNITIVE FUNCTIONS AND PROTEIN CARBONYLS IN THE BRAIN OF AGING RATS

Regular swimming exercise for 9 weeks improved cognitive functions in young (4 weeks) and middle-aged (14 months) Wistar rats with a parallel decrease in protein carbonyls of the brain.[3] This finding is consistent with the reports by others that age-related decrease in the cognitive function parallels the decrease in protein carbonyls in the brain of animals treated with a spin-trap compound, *N-tert*-butyl-α-phenylnitrone (PBN).[4] Beneficial effects of the moderate regular exercise and the PBN treatment appear to be brought about at least in part by upregulation of the activity of proteasome that is believed to be responsible for the degradation of oxidatively or otherwise modified proteins,[3,4] in addition to an increase in the activities of antioxidant enzymes.[5] Upregulation of proteasome activity by the exercise was observed also in the skeletal muscle.[6]

EFFECTS OF REGULAR EXERCISE ON OXIDATIVE STATUS AND TRANSCRIPTION FACTORS RELATED TO INFLAMMATION IN AGING RAT LIVER

Middle-aged (18-month-old) and old (28-month-old) male F344 rats were subjected to regular treadmill exercise for 8 weeks. Training intensity was set at about 75% of VO_{2max} for individual age groups. In both groups, maximal oxygen uptake increased by about 40%.[7] The body weight was reduced by about 10% as compared with sedentary groups. We studied the oxidative status of the liver of the animals.[8] ROS level as measured with dichlorodihydrofluorescein diacetate was significantly higher in the old sedentary groups than in middle-aged counterparts. The regular exercise tended to attenuate the increase, although not significantly, compared with the sedentary controls. Redox status evaluated by glutathione (GSH) showed more than twofold increase in exercised groups, together with decrease in the oxidized form (GSSG). It thus appears that the cellular milieu is shifted to a less oxidative state, suggesting a preventive or reversal effect of the exercise regimen even at old ages. We have also investigated nuclear factor κB (NF-κB) activity. NF-κB is a very important redox-sensitive transcription factor that regulates various inflammatory and im-

mune responses. It forms a complex with the inhibitory protein I-κB, thereby being retained as an inactive form in the cytoplasm. Upon stimulation by oxidative or other stresses, I-κB is phosphorylated and then degraded by proteasome, releasing NF-κB that can move into the nucleus for transcriptional activation of inflammatory protein genes or inactivation of anti-inflammatory protein genes. Binding activity of NF-κB in nuclear extracts to oligonucleotide with the responsive element (electrophoretic mobility shift assay, EMSA) increased with age as expected from the increased oxidative stress mentioned above. Increase in the amount of NF-κB was verified by increase in the amount of p50 and p65 subunits as detected by Western blot. The amount of I-κB in the cytoplasm was higher in the middle-aged animals than in the old. These findings confirm previous reports that NF-κB is activated by increased degradation of I-κB with advancing age.[9] The regular exercise may thus prevent or reverse the age-related changes that promote inflammatory processes.

Glucocorticoids (GC) have anti-inflammatory activities and are used to suppress inflammation in chronic diseases such as asthma and rheumatoid arthritis. GC inhibit gene expression of proinflammatory cytokines including various interleukins and tumor necrosis factor α as well as enzymes or receptors responsible for inflammatory processes such as inducible nitric oxide synthase and cyclooxygenase-2. They also activate gene expression of anti-inflammatory proteins such as lipocortin-1 and β_2-adrenoreceptor. GC receptor (GR) is a transcription factor that influences directly or

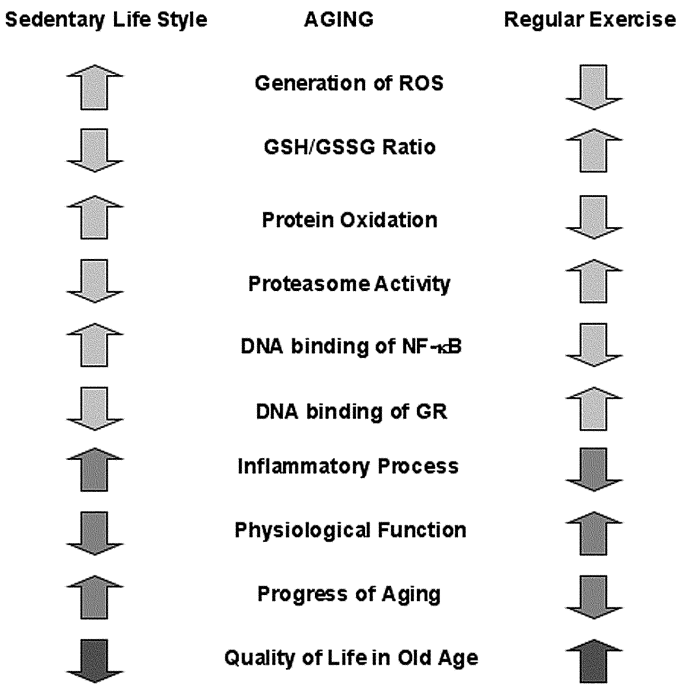

FIGURE 1. Regular exercise can attenuate oxidative status and inflammatory processes, leading to improved quality of life in old age.

indirectly gene expression of the inflammation-related proteins.[10] We showed that activity of GR measured by EMSA is significantly decreased in the liver of aged animals, but 8 weeks of regular exercise was able to reverse the change (Abe *et al*., in preparation). No significant difference in the amount of GR protein was detected between young adult and old animals, suggesting that the quality rather than the quantity of GR is altered with age. Serum level of GC was significantly higher in the exercised old animals than in the sedentary animals. In view of the anti-inflammatory activities of GC, these observations also support the view that regular exercise has a beneficial effect by reducing inflammation. It is interesting to note that GR can directly interact with NF-κB.[11] It is likely therefore that transcription factors GR and NF-κB synergistically downregulate the expression of inflammation-related genes.

Age-related increase in 8-hydroxy-2′-deoxyguanosine in the nuclear DNA of the skeletal muscle was significantly reduced by the regular exercise with increased DNA repair activity.[7] Although protein carbonyls did not change significantly with age or the exercise, the muscle proteins of exercised old animals were more resistant to oxidative challenge *in vitro* than those of the sedentary counterparts, suggesting increased antioxidative activities in the former.

Thus, regular physical exercise can possibly improve age-related functional decline and delay onset of age-related diseases by attenuating potentially harmful oxidative damage and suppressing inflammatory processes even at old ages (FIG. 1).

REFERENCES

1. REZNICK, A.Z., E. WITT, M. MATSUMOTO & L. PACKER. 1992. Vitamin E inhibits protein oxidation in skeletal muscle of resting and exercised rats. Biochem. Biophys. Res. Commun. **189:** 801–806.
2. RADÁK, Z., A. NAKAMURA, H. NAKAMOTO, *et al*. 1998. A period of anaerobic exercise increases the accumulation of reactive carbonyl derivatives in the lungs of rats. Pflüg. Arch. **435:** 439–441.
3. RADÁK, Z., T. KANEKO, S. TAHARA, *et al*. 2001. Regular exercise improves cognitive function and decreases oxidative damage in rat brain. Neurochem. Int. **38:** 17–23.
4. CARNEY, J.M., P.E. STARKE-REED, C.N. OLIVER, *et al*. 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 the spin-trapping compound *N-tert*-butyl-alpha-phenylnitrone. Proc. Natl. Acad. Sci. USA **88:** 3633–3636.
5. POWERS, S.K., D. CRISWELL, J. LAWLER, *et al*. 1994. Influence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. Am. J. Physiol. **266:** R375–R380.
6. RADÁK, Z., T. KANEKO, S. TAHARA, *et al*. 1999. The effect of exercise training on oxidative damage of lipids, proteins, and DNA in rat skeletal muscle: evidence for beneficial outcomes. Free Radical Biol. Med. **27:** 69–74.
7. RADÁK, Z., H. NAITO, T. KANEKO, *et al*. 2002. Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle. Pflüg. Arch. **445:** 273–278.
8. RADÁK, Z., H.Y. CHUNG, H. NAITO, *et al.* 2004. Age-associated increase in oxidative stress and nuclear factor kappaB activation are attenuated in rat liver by regular exercise. FASAB J. **18:** 749–750.
9. CHUNG, H.Y., H.J. KIM, J.W. KIM & B.P. YU. 2001. The inflammation hypothesis of aging: molecular modulation by calorie restriction. Ann. N.Y. Acad. Sci. **928:** 327–335.
10. ADCOCK, I.M. 2000. Molecular mechanisms of glucocorticosteroid actions. Pulm. Pharmacol. Ther. **13:** 115–126.
11. RAY, A. & K.E. PREFONTAINE. 1994. Physical association and functional antagonism between the p65 subunit of transcription factor NF-κB and the glucocorticoid receptor. Proc. Natl. Acad. Sci. USA **91:** 752–756.