Pflugers Arch - Eur J Physiol (2002) 445:273–278
DOI 10.1007/s00424-002-0918-6

ORIGINAL ARTICLE

Zsolt Radák · Hisashi Naito · Takao Kaneko · Shunichi Tahara · Hideko Nakamoto · Ryoya Takahashi · Fernando Cardozo-Pelaez · Sataro Goto

Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle

Received: 21 February 2002 / Revised: 1 June 2002 / Accepted: 15 July 2002 / Published online: 13 September 2002

Abstract Regular physical exercise retards a number of age-associated disorders, in spite of the paradox that free radical generation is significantly enhanced with exercise. Eight weeks of treadmill running resulted in nearly a 40% increase in maximal oxygen uptake in both middle-aged (20-month-old) and aged (30-month-old) rats. The age-associated increase in 8-hydroxy-2'-deoxyguanosine (8-OHdG) content was significantly attenuated in gastrocnemius muscle by exercise. The 8-OHdG repair, as measured by the excision of ^{32}P-labeled damaged oligonucleotide, increased in muscle of exercising animals. The reactive carbonyl derivatives (RCD) of proteins did not increase with aging. However, when the muscle homogenate was exposed to a mixture of 1 mM iron sulfate and 50 mM ascorbic acid, the muscle of old control animals accumulated more RCD than that of the trained or adult groups. The chymotrypsin-like activity of proteasome complex increased in muscle of old trained rats. We suggest that regular exercise-induced adaptation attenuates the age-associated increase in 8-OHdG levels, and increases the activity of DNA repair and resistance against oxidative stress in proteins.

Keywords Aging · Base excision repair · DNA repair · Exercise · Oxidative stress · Proteasome

Z. Radák (✉)
Laboratory of Exercise Physiology, School of Sport Sciences, Semmelweis University, Alkotas u. 44, H-1123, Budapest, Hungary
e-mail: radak@mail.hupe.hu
Tel.: +36-309-707811
Fax: +36-13566-337

H. Naito
Department of Exercise Physiology, School of Sports Sciences, Juntendo University, Inba, Japan

T. Kaneko · S. Tahara
Tokyo Metropolitan Institute of Gerontology, Itabashi, Tokyo, Japan

H. Nakamoto · R. Takahashi · S. Goto
Department of Biochemistry, Faculty of Pharmaceutical Sciences, Toho University, Funabashi, Japan

F. Cardozo-Pelaez
Department of Pharmaceutical Sciences, Center for Environmental Health Sciences, Missoula, USA

Introduction

Regular exercise results in adaptation, which involves a wide range of general and specific changes in different organs, such as an improved cardiovascular system, better cognitive processing, and increased muscular mass and strength [2, 8, 23]. These changes must be the result of certain cellular alterations. However, the cellular and molecular mechanisms underlining these changes are poorly understood. It is well known that exercise increases the formation of reactive oxygen and nitrogen species (RONS) [26]. It is also clear that moderate regular exercise is likely to cause adaptations to antioxidant and oxidative damage repair systems [29]. Therefore, it appears that oxidative stress-induced adaptations might play an important role in the beneficial effects of regular exercise.

The incidence of diseases, such as certain types of cancers, rheumatic inflammation, diabetes II, and muscular dystrophy, is increased as a function of age [3]. One of the most accepted theories of aging claims that aging is associated with increased formation of RONS, decreased capacity of antioxidant and repair systems, and increased accumulation of oxidative damage in macromolecules [1, 5, 9, 16, 22].

Skeletal muscle is the tissue with the largest mass in the body, and consists of postmitotic cells, which are more prone to accumulate oxidative damage [29]. Aging is associated with sarcopenia (loss of muscle mass) and dysfunction in motor coordination. The exercise-induced adaptation is well described in skeletal muscle. Therefore, the current investigation was stimulated by the hypothesis

that adaptation induced by regular exercise results in decreased accumulation of oxidative damage of DNA and proteins. We further suggest that exercise training attenuates the age-associated decrease in protein and DNA repair. In addition, we propose that aged skeletal muscle will be more prone to exogenous oxidative stress than middle-aged muscle and exercise training could increase the resistance against oxidative stress.

Materials and methods

Animals

This experiment followed the guidelines published by the Council of the Physiological Society of Japan. Adult (18-month-old) and old (28-month-old) specific pathogen-free male F344/DuCrj rats were individually housed in a climate-controlled laboratory animal facility (23±1°C, 50±5% relative humidity, and 12-h:12-h light-dark photoperiod) and were fed standard rat chow and water ad libitum. The animals had a mean life span of 29 months in our animal facility [33].

Exercise training protocol

All animals were familiarized with walking on a motor-driven treadmill (5%, 6–8 m/min, 10 min/day) for 5 days. At the end of this period, animals from each age group were weight matched and randomly assigned to either a sedentary control or an endurance exercise trained group. Hence, four experimental groups were formed: (1) adult sedentary control; (2) adult exercise trained; (3) old sedentary control; and (4) old exercise trained. The treadmill-training program was intended to exercise both adult and old animals at the same relative exercise intensity (i.e., percentage maximal oxygen consumption, $\%\dot{V}O_{2max}$) for the two age groups during the 8-week training period. Electric shock was rarely used to motivate the animals to run. Both adult and old sedentary control animals also ran once a week for 10 min on a 15% grade at speeds of 8 m/min for adults and 6 m/min for old rats to familiarize them with handling and treadmill running.

Measurement of maximal oxygen consumption

At the end of the seventh week of training, $\dot{V}O_{2max}$ was measured on all animals by the use of a flow-through open-circuit system. Briefly, animals were placed in a sealed treadmill chamber that allowed a unidirectional flow of gas and the progressive exercise test began on a 15% grade at the initial speed used for training (adult 10 m/min and old 7.5 m/min). The treadmill speed was increased 5 m/min for adult and 2.5 m/min for old animals every 3 min until the animals were unable to maintain the required running speed within 21 min. $\dot{V}O_{2max}$ was defined as the highest $\dot{V}O_2$ obtained during the exercise test. Ambient air was pumped through the chamber at a flow rate of 5.5 l/min. Gas was sampled (500 ml/min) from a small mixing chamber located at the back of the treadmill and analyzed for CO_2 and O_2 concentrations via electronic gas analyzers (Minato MG-360, Tokyo, Japan). The gas analyzers were calibrated immediately before and after each test using standardized gases.

Seventy-two hours after the $\dot{V}O_{2max}$ measurement, animals were anesthetized with pentobarbital sodium (25 mg/kg), the gastrocnemius muscles were quickly removed, weighed and frozen in liquid nitrogen. Samples were then stored at –80°C until analyzed.

Assays

Isolation of nuclear DNA and the measurement of 8-OHdG were carried out as described by Kaneko et al. [20]. 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 h 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 h to hydrolyze the nucleotides to nucleosides. Nucleosides in samples were analyzed by an 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, Mass., USA). 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 assay

The obtained samples were homogenized with buffer containing 20 mM Tris (pH 8.0), 1 mM 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 of 2.5 M KCl and centrifuged at 10,000 *g* (14,000 rpm) for 30 min. The supernatant was divided into aliquots and stored at –80°C. The protein levels were measured by the BCA method. The assay was carried out according to the protocol described by Cardozo-Pelaez et al. [7]. In brief, 20 pmol of synthetic probe containing 8-OHdG (Trevigen, Gaithersburg, Md., USA) was labeled with ^{32}P at the 5' end using polynucleotide T4 kinase (Boeringer Mannheim, Germany). For the nicking reaction, protein extract (2–4 μg) was mixed with 20 μg of a reaction mixture containing 0.5 M of HEPES, 0.1 M EDTA, 5 mM of dithiothreitol, 400 mM KCl, purified BSA and labeled probe (approximately 2000 cpm). The reaction was carried out at 30°C for 5–15 min and stopped by placing the solution on ice. Then 30 μl chloroform was added and samples were centrifuged and 15 μl taken and mixed with loading buffer containing 90% formamide, 10 mM NaOH, and blue-orange dye. After 3 min 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 h. Gels were quantified using BAS 2000 Bioimaging Analyzer (Fuji Film, Japan). Radioactive signal densities were determined using the software designed for this system. The activity to repair 8-OHdG was determined and expressed as a percentage of substrate cleaved [7].

Proteasome activity and content

The proteasome complex has at least five distinct protease activities [25] and, among these, two types of peptidase activities were measured as described previously [15]. These activities were determined fluorometrically by measuring the release of 7-amino-4-methyl-coumarin from the peptides succinyl-Leu-Leu-Val-Tyr-MCA (SUC-LLVY-MCA) for chymotrypsin-like activity at 380 nm excitation and 440 nm emission, respectively. Benzyloxycarbonyl Leu-Leu Glu-β-naphthylamide (Z-LLE-NA) was used as a substrate for peptidylglutamyl-peptide hydrolyzing (PGPH) activities to measure the released β-naphthylamine at 335 nm excitation and 410 nm emission. Proteasome co-exists in the 20S and 26S forms with the former known to be readily stimulated by sodium dodecyl sulfate (SDS) and mainly responsible for the degradation of oxidatively modified proteins [19]. Therefore, we used SDS stimulation as described by Hayashi and Goto [17]. Antiserum against a rat proteasome α subunit RC2 was generated and used in

Western blots to determine the relative protein content as described [17].

Protein carbonyls

The measurement of RCD was done by spectrophotometer and Western blot as described previously [24, 27]. In brief, proteins precipitated with trichloroacetic acid were suspended and incubated in a solution containing 10 mM 2,4-dinitrophenylhydrazine (DNPH) and 2 N HCl for 1 h at 15°C. The resulting protein hydrazones were pelleted in a centrifuge at 11,000 *g* for 5 min, the pellets were washed three times with ethanol-ethyl acetate (1:1) and then once with ice-cold acetone. The final precipitates were dissolved in 1 ml buffer containing 8 M urea and 5% 2-mercaptoethanol. The protein content was re-measured following the RCD spectrophotometric measurement and in some cases the same samples were further used for Western blot. Duplicate polyacrylamide gel electrophoresis of derivatized proteins was carried out in 12% polyacrylamide gels containing 0.1% SDS. After electrophoresis, the proteins were transferred to nitrocellulose membranes. Then the membranes were soaked in phosphate-buffered saline containing 3% skim milk, 0.05% Tween, and 0.05% sodium azide and then treated with anti-DNPH antibody. After washing in buffer without antibodies the membranes were treated with ^{125}I-Protein A. Finally, the radioactive signals were quantified by BAS 2000 Bioimaging Analyzer (Fuji Film, Tokyo, Japan). Half of the duplicated supernatant of each sample was challenged by freshly made 1 mM iron sulfate and 50 mM sodium ascorbate for 5 min, and then treated by 20% TCA, as described earlier [24]. Massive oxidative challenge causes very significant oxidative damage, and the possible difference in resistance could be overwhelming and not measurable due to the significant presence of RONS. Therefore, a very moderate oxidative challenge was used.

Statistical analysis

Statistical significance was assessed using ANOVA, followed by Scheffe's posthoc test. The significance level was set at $P<0.05$.

Results

Body weights, $\dot{V}O_{2max}$, and training intensity

Exercise training decreased body weight significantly ($P<0.05$). Endurance training resulted in a 39% ($P<0.05$) increase in $\dot{V}O_{2max}$ in both adult and old animals indicating that the applied load was sufficient to cause adaptation. The increase in $\dot{V}O_{2max}$ was very similar for adult and aged rats (Table 1), which suggests the work load was relatively similar.

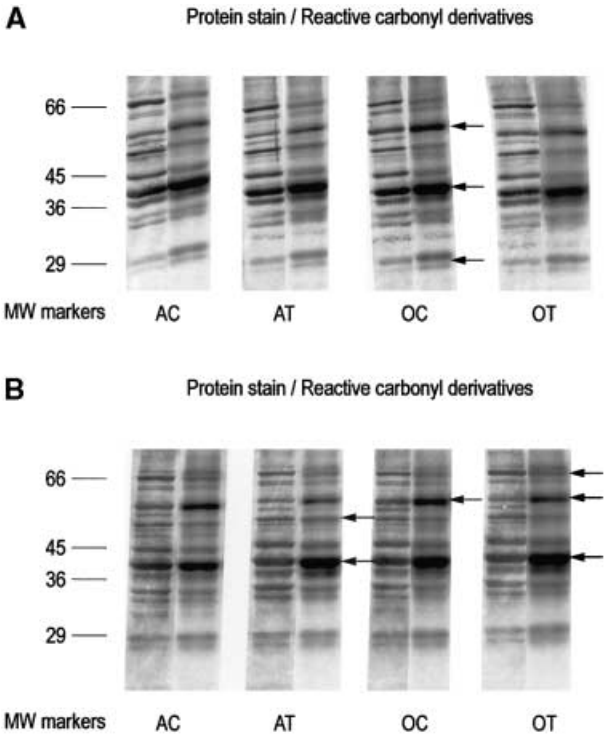

Fig. 1 Western blot data revealed a significant ($P<0.05$) protein-specific increase in reactive carbonyl derivatives in muscle supernatant treated with iron sulfate and ascorbic acid mixture (**B**) compared with native samples (**A**). Aging did not increase the reactive carbonyl derivatives (*RCD*) level. However, exercise training attenuated the exogenous oxidative stress-induced increase. Each group is displayed by one representative sample which contains a Coomassie blue panel and Western blot panel. *Arrows* indicate those proteins in which carbonyl content has changed compared to control level, due to aging, exercise or iron sulfate and ascorbic acid mixture treatment. The changes were evaluated by the Fuji BAS system as explained in Materials and methods. The immunoblot measurement was carried out on three samples and representative data are displayed. (*AC* adult control, *AT* adult trained, *OC* old control, *OT* old trained)

Assays

Neither aging nor exercise training caused significant increases in the accumulation of RCD in skeletal muscle (Figs. 1, 2). However, exposure of the supernatant to iron sulfate and ascorbic acid mixture resulted in moderate increases in RCD content and the increase reached significance for aged skeletal muscle ($P<0.05$). As seen on the Western blots, the increases occurred in a protein-specific manner, which suggests that some proteins are more readily carbonylated than others. Exercise training

Table 1 Body mass and maximal O_2 consumption ($\dot{V}O_{2max}$). Values are means ±SD (n=6–8)

Group	Post-body mass (g)	$\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1})
Adult control	406.1±24.5	50.5±5.2
Adult trained	352.9±20.0*	70.0±5.6*
Old control	372.9±28.1	49.6±7.0
Old trained	332.0±14.0*	68.7±4.9*

*Significantly different ($P<0.05$) from age-matched control group

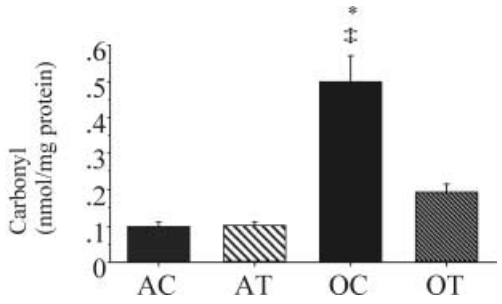

Fig. 2 An oxidative challenge with an iron sulfate and ascorbic acid mixture increased the RCD content significantly in the skeletal muscle of old untrained rats, and exercise training eliminated this increase. The graph shows the difference in carbonyl content, measured by spectrophotometer, in native and treated samples to quantify the immunoblot data. Values are means ±SD (n=6). $^{*}P$<0.05 vs AC, $^{‡}P$<0.05 vs. OT

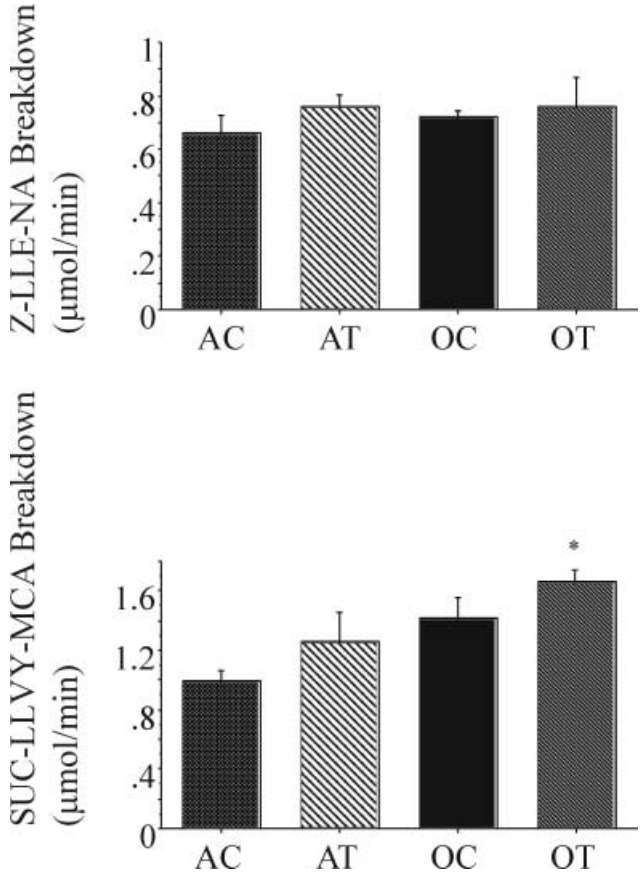

Fig. 3 The peptidylglutamyl-peptide hydrolyzing activity (breakdown of Z-LLE-NA) did not change significantly with aging or exercise. However, the chymotrypsin-like activity (SUC-LLVY-MCA) increased significantly in skeletal muscle of aged exercise-trained animals. Values are means ±SD (n=6). $^{*}P$<0.05 vs. AC

pre-conditioned the skeletal muscle and attenuated the increases. The SDS-stimulated activity of PGPH did not change as a function of age or exercise training. Aging did not affect the chymotrypsin-like activity of proteasome but exercise increased it in aged animals (Fig. 3). The amount of proteasome did not change significantly but tended to increase with aging (P<0.06) (data not shown).

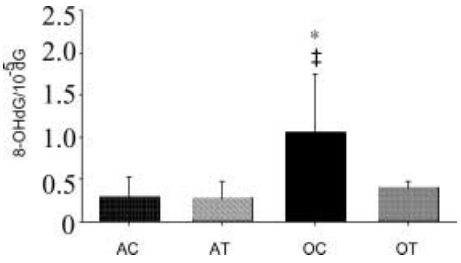

Fig. 4 The comparison of 8-hydroxy-2'-deoxyguanosine (*8-OHdG*) content in gastrocnemius muscle of adult, old, exercised and non-exercised animals shows that exercise training significantly attenuated the age-associated increase in nuclear DNA damage. Values are means ±SD (n=6). $^{*}P$<0.05 vs. AC, $^{‡}P$<0.05 vs. OT

The 8-OHdG content of nuclear DNA increased as a function of age in the skeletal muscle of control animals. Exercise training significantly attenuated this age-associated increase (Fig. 4). The activity of 8-OHdG repair enzyme tended to increase in the exercise trained group (Fig. 5). Aging did not result in alteration of 8-OHdG repair activity in the skeletal muscle of rats. The semi-quantitative activity data of 8-OHdG repair enzyme was: AC, 27%; AT, 40%; OC, 32%; OT, 42% when expressed as a percentage of substrate cleaved.

Discussion

Aging is an unavoidable process which affects each individual differently. This is not simply the result of genetic diversity, but it is also due to the effects of different environmental stressors and life-styles. Thus, proper nutrition, avoidance of smoking, and regular physical exercise can have beneficial effects on the aging process. According to Holloszy and Kohrt [18], however, regular exercise does not increase the life-span but does prevent or retard the deteriorative effects that result from being sedentary.

Aging is accompanied by the accumulation of 8-OHdG in nuclear DNA in different tissues [1]. Increased levels of DNA damage can cause the synthesis of a variety of incorrect proteins and therefore impair cellular function. In addition, an age-related accumulation of DNA damage might play a role in the increased incidence of cancer among aged people and animals [1]. Lee et al. [21] has shown that aging resulted in the induction of DNA-damage-inducible genes and increased the expression of a number of genes that are involved in the repair of protein damage in mouse gastrocnemius muscle.

One of the major findings in the present study was that regular exercise attenuates the age-associated increase in 8-OHdG in skeletal muscle of rats and, thus, underscores the importance of exercise to avoid the consequence of a sedentary lifestyle. It appears that aging does not effect the activity of 8-OHdG repair in gastrocnemius muscle, contrary to our hypothesis. However, exercise training

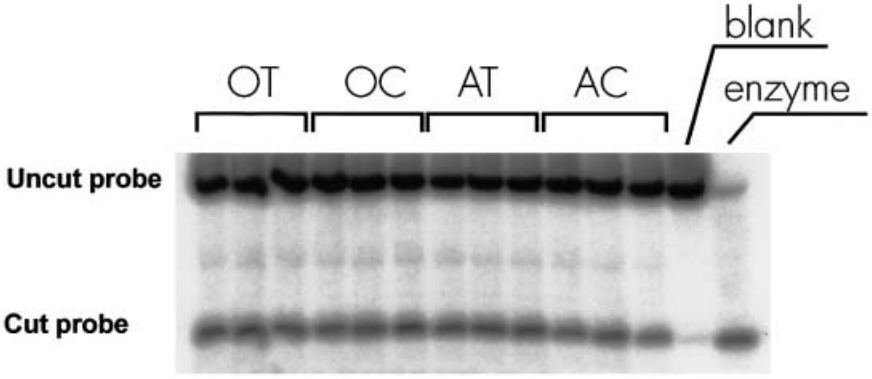

Fig. 5 For the 8-OHdG repair, oligonucleotides with the following sequence 5' GAACTAGTGOATCCCCCGGGCTGC 3' were labeled with ^{32}P, where O is 8-OHdG. Exercise training resulted in an increase, however to a limited extent, in the 8-OHdG repair in exercise trained muscle. Aging did not cause alteration. The assay was duplicated to measure the 8-OHdG repair in gastrocnemius muscle of all animals (n=6). The Fuji BAS densitometer scanning resulted in the following semi-quantitative data: AC, 27%; AT, 40%; OC, 32%; OT, 42% substrate cleaved

results in an increase in 8-OHdG repair, albeit a moderate extent. In bacteria, formamidopyrimidine-DNA glycosylase (Fpg) is the enzyme which removes 8-OHdG from the damaged DNA and the functional mammalian homologue is cloned as OGG1 [30]. The up-regulation of DNA repair is an important process because of the mutagenic potential of 8-OHdG [10] which could lead to G·C transversion to T·A [14]. A reduction of 8-OHdG levels due to regular exercise training has been reported to occur in leukocytes of human subjects and skeletal muscle of rats [4, 28]. The findings of the present study suggest that this effect could be accounted for by the exercise-induced activity of the 8-OHdG repair enzyme. It should be mentioned that the 8-OHdG level was measured from the nucleus and for the measurement of repair enzyme we used crude cell extract, because of the availability of tissue. Therefore, it cannot be excluded that the marked decrease in nuclear 8-OHdG levels might be associated with more significant alterations of the repair activity of an Fpg homolog in the nucleus. Nevertheless, the present investigation appears to be the first to examine the activity of 8-OHdG repair in an exercise study.

The hypothesis that an exercise-mediated increase of the DNA repair system is one of the mechanisms by which exercise reduces the incidence of cancer needs to be investigated.

The accumulation of RCD after light exogenous oxidative stress was more enhanced in the aged muscle, suggesting that skeletal muscle is more prone to the attack of RONS with age. Interestingly, the sensitivity to carbonylation of some proteins in skeletal muscle is enhanced by aging. According to our hypothesis it appears that exercise has the capability to reduce this vulnerability to oxidative stress. The importance of this observation is that oxidatively modified proteins are losing their physiological activity and are prone to degradation. Thus, the increased accumulation of this useless "junk" could hamper vital physiological processes. Indeed, this phenomenon occurs with aging and with diseases such as Alzheimer's, Parkinson's, and sarcopenia, which are accompanied by increased protein damage [6, 31, 32].

Exercise training tends to increase the activity of the proteasome complex, which suggest that the removal of oxidatively modified proteins is more efficient in the muscle of old trained rats [28]. This is an important adaptive response because the induction of a repair mechanism – proteasome complex is regarded as a repair enzyme [15] – could attenuate the age-associated increase in the half-life of proteins and result in a decrease in the accumulation of potentially harmful posttranslationally modified proteins [11, 12, 13]. Adaptive changes, which are due to physiological challenges such as exercise training, are rarely of a large magnitude. However, in the long term even relatively small alterations, such as we have observed in the activity of DNA and protein repair, could have significant effects.

The data from the present study suggest that, despite a possible exercise-induced increase in RONS generation, the adaptive responses of antioxidant and repair mechanisms result in a decreased accumulation of oxidative DNA damage and a reduction in the vulnerability of proteins to oxidative damage. Thus, it is conceivable that exercise training increases the resistance of aged skeletal muscle to oxidative stress, and the proteasome complex is more efficiently involved in the degradation of oxidatively modified proteins with exercise training. The prevention of age-dependent increases in 8-OHdG content in nuclear DNA by exercise training might be one mechanism by which regular training can decrease the incidence of certain cancers.

Acknowledgements The present work was supported by Japan Society for the Promotion of Science (JSPS-S01273) to Z.R. and S.G. and the Grant-in-Aid for Scientific Research (B)(12480011) to H.N.

References

1. Ames BN, Sinegawa MK, Hagen TM (1993) Oxidants, antioxidants, and degenerative diseases of aging. Proc Natl Acad Sci USA 90:7915–7922
2. Anstey K, Christensen H (2000) Education, activity, health, blood pressure and apoliprotein E as predictors of cognitive change in old age: a review. Gerentology 46:163–177
3. Arking R (1998) Biology of aging, 2nd edn. Sinauer, Sunderland, Mass. pp 1–570
4. Asami S, Hirano T, Yamaguchi R, Itoh H, Kasai H (1998) Reduction of 8-hydroxyguanine in human leukocyte DNA by physical exercise. Free Radic Res 29:581–585
5. Beckman KB, Ames BN (1998) The free radical theory of aging matures. Physiol Rev 78:547–577
6. Berlett BS, Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 272:20313–20316
7. Cardozo-Pelaez F, Brooks PJ, 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
8. Christensen H, Mackinnon A (1993) The association between mental, social, and physical activity and cognitive performance in young and old subjects. Age Ageing 22:175–182
9. Dice JF (1993) Cellular and molecular mechanism of aging. Physiol Rev 73:149–159
10. Floyd RA (1990) The role of 8-hydroxyguanine in carcinogenesis. Carcinogenesis 11:1447–1450
11. Goto S, Nakamura A (1997) Age-associated, oxidatively modified proteins: a critical evaluation. Age 20:81–89
12. Goto S, Takahashi R, Kumiyama A, Radák Z, Hayashi H, Takenouchi M, Abe R (2001) Implications of protein degradation in aging. N Y Acad Sci 928:54–64
13. Goto S, Radák Z, Takahashi R (2002) Biological implications of protein oxidation. In: Cutler R, Rodriguez H (eds) Oxidative stress and aging. World Scientific (in press)
14. Grollman AP, Moriya M (1993) Mutagenesis by 8-oxoguanine: an enemy within. Trends Genet 9:246–249
15. Grune T, Reinheckel T, Davies KJA (1997) Degradation of oxidized proteins in mammalian cells. FASEB J 11:2169–2173
16. Harman D (1956) Aging: a theory based on free radical and radiation chemistry. J Gerontol 11:298–300
17. Hayashi H, Goto S (1998) Age-related changes in the 20S and 26S proteasome activities in the liver of male F344 rats. Mech Ageing Dev 102:55–66
18. Holloszy JO, Kohrt WM (1995) Exercise. In: Masoro EJ (ed) Handbook of physiology, section 11: Aging. Oxford University Press, New York, pp 633–666
19. Hough R, Pratt G, Rechsteiner M (1987) Purification of two high molecular weight proteases from rabbit reticulocyte lysate. J Biol Chem 262:8303–8313
20. 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 Radic Biol Med 23:76–81
21. Lee CK, Klopp RG, Weindruch R, Prolla TA (1999) Gene expression profile aging and its retardation by caloric restriction. Science 285:1390–1393
22. Martin GM, Austad SN, Johnson TE (1998) Genetic analysis of ageing: role of oxidative damage and environmental stresses. Nature Genet 13:25–34
23. Miller TD, Balady GJ, Fletcher GF (1997) Exercise and its role in the prevention and rehabilitation of cardiovascular disease. Ann Behav Med 19:220-229
24. 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
25. Cardozo C, Vinitsky A, Michaud C, Orlowski M (1993) Evidence that the nature of amino acid residues in the P3 position directs substrates to distinct catalytic sites of the pituitary multicatalytic proteinase complex (proteasome). Biochemistry 33:6483–6489
26. Radák Z, Goto S (2000) Oxidative modification of proteins and DNA. In: Radák Z (ed) Free radicals in exercise and aging. Human Kinetics, Champaign, Ill. pp 177–209
27. Radák Z, Asano K, Lee KC, 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 Radic Biol Med 22:1109–1114
28. 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
29. Radák Z, Taylor AW, Ohno H, Goto S (2001) Adaptation to exercise-induced oxidative stress: from muscle to brain. Exerc Immun Rev 7:90–107
30. Rosenquist TA, Zharkov DO, Grollman AP (1997) Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. Proc Natl Acad Sci USA 94:7429–7434
31. Sohal RS, Weindruch R (1996) Oxidative stress, caloric restriction, and aging. Science 273:59–63
32. Stadtman ER (1992) Protein oxidation and aging. Science 257:1220–1224
33. Takahashi R, Goto S (1987) Influence of dietary restriction on accumulation of heat-labile enzyme molecules in the liver and brain of mice. Arch Biochem Biophys 257:200–206