

REGULAR EXERCISE ATTENUATES OXIDATIVE STRESS IN AGING RAT TISSUES: A POSSIBLE MECHANISM TOWARD ANTI-AGING MEDICINE

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Regular exercise is known to exhibit various health benefits in reducing risks of age-related diseases such as cardiovascular diseases, type 2 diabetes, cancer, and neuronal disorders. It is interesting to note that beneficial effects appear more pronounced in the elderly than in younger adults despite the fact that aging is accompanied by increased oxidative stress that is suggested to be a major cause of aging and age-related diseases. Mechanisms behind this apparent paradox are not well understood. Regular training and acute bouts of exercise upregulate activities of antioxidant enzymes and thereby can reduce oxidative stress. We have studied the effects of regular exercise on oxidative stress in the liver and brain of middle-aged and old rats. Protein carbonyl was significantly reduced and proteasome activity was upregulated in the brain with improved cognitive function by swimming training. The binding of transcription factor NF- κ B to the target DNA was reduced with concomitant increase of reduced glutathione in the liver of old rats by regular treadmill running, suggesting that inflammatory reactions are alleviated in these animals due to reduced oxidative stress. A similar exercise regimen was able to reduce 8-oxodeoxyguanosine (8-oxodG) in the nuclear and mitochondrial DNA of the liver of old rats. Based on these findings and reports by other investigators in which moderate exercise is suggested to be beneficial while excessive exercise is harmful, a hormesis-like mechanism by reactive oxygen species may be proposed that is likely to be a major mechanism of anti-aging effects and reduces risk of age-related diseases by regular exercise.

Keywords: aging, hormesis, oxidative stress, regular exercise

Introduction

In most industrialized and some developing nations, burdens of social and medical problems are caused by an ever-increasing population of elderly people who

are frail and/or sick and who may therefore need extensive help and care from their families and communities. To cope with such problems, basic as well as applied research on aging is in high demand from biological, medical, and social perspectives. A major concern of our societies, therefore, is to develop means to extend the healthy lifespan or health span rather than simply increasing longevity. Reflecting such social trends, so-called anti-aging medicine is becoming popular not only among the middle-aged and elderly people but also with clinicians as well as in industries that aim to get into the big market, often selling scientifically

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unproven remedies such as antioxidant vitamins and other chemicals.

In this brief review on aging, we discuss the current status of studies on the biological mechanisms of aging with special emphasis on proteins and DNA. Also discussed is the possible intervention of aging by regular exercise initiated late in life as viewed from age-related changes of proteins and DNA in experimental animals, based primarily on our own studies.

A brief overview of the molecular mechanisms of aging

Aging may be defined as a process of accumulation of damaged cellular constituents that are potentially harmful or useless if not removed or repaired. This could lead to physiologic deteriorations that result in the increased probability of death of an organism with gradual loss of homeostasis when exposed to internal and external stresses (Finkel & Holbrook 2000). A number of protective networks including antioxidants, antioxidant enzymes, chaperones, repair and degrading enzymes of damaged molecules are working in cells to maintain life against internal and external stresses. Nevertheless, the increase in altered potentially harmful molecules is inevitable in the long run because protective systems themselves are subjected to alterations and, therefore, never perfect. Aging may be viewed as a deteriorative process of these systems that are active in youth but progressively less active with time. One of the main molecules that can cause aging and age-related diseases is protein that is directly required for all life maintenance processes (Goto et al. 1995). In fact, altered forms of proteins do increase with advancing age (Stadtman & Levine 2000; Goto et al. 1995). They are mostly generated by post-translational modifications such as deamidation, racemization, glycation (glycoxidation), methylation, phosphorylation, and oxidation, as well as conformational changes with no apparent chemical changes, rather than by transcriptional or translational errors (Goto et al. 2003). Among these modifications, oxidation is of particular importance because oxidative modifications can occur in any cell as side reactions of oxygen metabolism, most notably in mitochondria that consume more than 90% of the

oxygen that cells require (Barja 1999; Shigenaga et al. 1994). Other oxygen metabolisms that generate reactive oxygen species (ROS) include reactions that are catalyzed by xanthine oxidase, P-450 dependent oxidase and cyclooxygenase (Boonstra & Post 2004). The oxidatively modified proteins may be removed mostly by degradation catalyzed by proteasomes or in some cases by lysosomes. Proteasomes are multicatalytic proteases that degrade altered or regulatory proteins and exist in two forms, i.e. 20 S and 26 S forms (Liu et al. 2005). The 26 S proteasome consisting of 20 S core proteasome plus two 19 S regulatory complexes is responsible for the degradation of ubiquitinated proteins, and the 20 S proteasome is involved in the degradation of non-ubiquitinated proteins. Both forms are responsible for degradation of altered proteins such as those modified by ROS and are equally reduced with age (Hayashi & Goto 1998). Reduced activities of the proteasomes would cause increased accumulation of damaged proteins that might cause aging and increased incidence of age-related diseases.

One of the other possible major molecular mechanisms of aging and age-related diseases is oxidative damage to DNA (Lombard et al. 2005). The level of oxidative modification of nuclear DNA is in the order of 10^{-6} for 8-oxoguanine, the most abundant form of oxidative modification of DNA bases, which may be too low to account for age-related general functional decline of cells even though other types of damage do occur in DNA. The low level of oxidative modifications can, however, be more than enough to induce cancer since single mutations in 30 billion bases in the genome can initiate the disease, and therefore limits lifespan. On the other hand, oxidative damage to mitochondrial DNA is reported to occur at a much higher rate, i.e. several to 10 times more than the rate for nuclear DNA (Barja & Herrero 2000). In view of the roles of mitochondria in cellular energy production, damage to the DNA that codes in part for proteins of the electron transport complexes could cause functional deterioration of cells. Furthermore, because mitochondria are the major source of ROS due to their high consumption of oxygen, the DNA and other molecules of the organelle are prone to oxidative damage that can trigger a vicious cycle of deterioration. This idea constitutes the mitochondria theory of aging (Shigenaga et al.

1994; Harman 1972), based on numerous reports on age-related alteration of mitochondrial DNA (Chan 2006; Balaban et al. 2005). The oxidatively or otherwise modified DNA may be repaired by nucleotide and/or base excision repair enzyme systems (Barnes & Lindahl 2004). Decline in repair activity can cause aging and age-related diseases (Bohr 2002).

We have studied the effects of aging and regular exercise on protein and DNA oxidation, and the degrading and repair activities for the respective damage.

Regular physical exercise

Exercise is believed to be good for health but it is often claimed to induce oxidative stress due to excessive oxygen uptake and elevated generation of ROS in the mitochondrial electron transport system, and reactions catalyzed by enzymes such as xanthine oxidase and NADPH dehydrogenase. As a result of massive generation of ROS, proteins, nucleic acids and membrane phospholipids may be more oxidatively modified in exercised tissues than in sedentary ones, possibly leading to deleterious consequences. In fact, a bout of exhaustive exercise increased protein oxidation in the skeletal muscle, lung and other tissues of sedentary animals unprepared for excessive generation of ROS (Radák et al. 1998; Reznick et al. 1992).

It has been hypothesized that moderate regular exercise can be beneficial by upregulating the protective activities against oxidative stress (Ji 2001; Powers et al. 1999). Since limited information is available for testing this hypothesis in old animals in particular, we have studied this issue using two different paradigms of regular exercise in middle-aged and old rats.

Effects of swimming training on cognitive function and oxidative modification of proteins in rat brain

Swimming exercise is an experimental paradigm to study physiologic and biochemical changes associated with forced physical activities in rodents. We have studied the effects of swimming exercise on the cognitive function and oxidative modification of brain

proteins in rats (Radák et al. 2001). Young (4 weeks old) and middle-aged (14 months old) animals were subjected to 60–90 minutes of swimming exercise per day, 5 days per week. Exercised animals showed improved cognitive function in passive and active avoidance tests in both age groups after 9 weeks of the regular exercise. These functional changes were accompanied by a decrease in protein carbonyl in the brain extracts. The proteasome activity in the brain was upregulated, suggesting that it is responsible for the decrease in the oxidatively modified proteins. Conceivably, mild oxidative stress from regular exercise might have upregulated the proteasome activity in the brain as an adaptive response. Limoli et al. (2004) reported that ROS stimulates neural cell proliferation in both primary culture and *in vivo*, which is reversed by treatment with α -lipoic acid, a potent antioxidant. These findings might be relevant to exercise-induced beneficial effects to the brain in which changes of redox state might be involved. In this context, it is interesting to note that physical activity increases cell proliferation and neurogenesis in the brain (Brown et al. 2003; van Praag et al. 1999). Furthermore, Diamond et al. (1985) reported that thickening of the brain cortex, which might reflect better neural function, can be observed in rats as old as 30 months of age when they are kept in an enriched environment for a few months, which allows the animals to stay active. We suggest that a modest increase of ROS in the brain would constitute a mechanism for beneficial consequences in brain function by regular exercise.

Regular treadmill exercise and oxidative stress in aging rat liver

It was of interest to see further whether regular exercise has beneficial influence in other tissues in aged animals. We therefore studied the effects of regular treadmill exercise (4 times a week, 60–90 minutes/day) in middle-aged (18-month-old) and old (28-month-old) male rats on oxidative status in the liver. In both age groups, the maximal oxygen uptake was increased by about 40% by 8 weeks of the exercise (Radák et al. 2002). The ROS level measured with a fluorescent probe was significantly higher in the old sedentary groups than in the middle-aged counterparts. Regular exercise

attenuated the increase (Radák et al. 2001). Redox status evaluated by glutathione level showed more than a two-fold increase of the reduced form (GSH) in exercised groups. It thus appears that the cellular milieu is shifted to a less oxidative state, suggesting a preventive effect by the exercise regimen even at old ages. We investigated the activity of nuclear factor- κ B (NF- κ B) which is an important redox sensitive transcription factor that regulates various inflammatory and immune responses (Radák et al. 2004). The binding activity of NF- κ B in nuclear extracts to deoxyoligonucleotide with the responsive element increased with age as expected from the increased oxidative stress mentioned above. The binding was reduced by the exercise regimen, suggesting that regular exercise may attenuate or reverse the age-related changes that promote inflammatory processes.

Glucocorticoids (GCs) have anti-inflammatory activities and are used to suppress inflammation in chronic diseases such as asthma and rheumatoid arthritis (Adcock 2000). GCs inhibit gene expression of pro-inflammatory 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. GC receptor (GR) is a transcription factor that directly or indirectly influences gene expression of the inflammation-related proteins. We showed that the binding activity of GR to the responsive DNA element is significantly decreased in the liver of aged animals, but 8 weeks of regular exercise reversed the change (Goto et al. 2004). 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. The serum level of GC was significantly higher in the exercised old animals than in the sedentary controls. In view of the anti-inflammatory activities of GCs, these observations also support the view that regular exercise may have an ability to reduce inflammation through the GR pathway. It is interesting to note that GR can directly interact with NF- κ B, modulating the activity of transcription (Novac et al. 2006; Smoak & Cidlowski 2004; Ray & Prefontaine 1994). It is likely, therefore, that transcription factors GR and NF- κ B synergistically downregulate the expression of inflammation-related genes by regular exercise.

Additionally, it is interesting to note that proteins in cardiac muscles of rats subjected to regular swimming training for 9 weeks were more resistant to an oxidative challenge of intraperitoneal injection of H₂O₂ (Radák et al. 2000). The exercise preconditioning increases proteasome and DT-diaphorase activities, thereby apparently reducing increased carbonyl modification of proteins due to the challenge. Regular exercise increases antioxidant enzyme activities in rat skeletal muscles (Powers et al. 1999) and the liver (Kakarla et al. 2005); taken together, these results support the view that regular exercise upregulates protection against oxidative stress.

We next studied the effect of regular exercise on oxidative modification of nuclear and mitochondrial DNA and its repair in the liver of aged rats (Nakamoto et al. unpublished). Rats were subjected to 8 weeks of regular treadmill running at 21 months of age. The amount of 8-oxodeoxyguanosine (8-oxodG) in the nuclear and mitochondrial DNA of the liver in sedentary controls was 2- and 1.5-fold higher in the two organelles, respectively, than that in young adult animals (11 months old). The mitochondrial DNA showed 10-fold higher content of the oxidative lesion than the nuclear DNA. The 8-oxodG content was reduced to levels of the young animal in both nuclear and mitochondrial DNA by the exercise. The activity of the repair enzyme OGG1 for the lesion was decreased in the nucleus but not in mitochondria with age. It was upregulated significantly by the exercise in the nucleus but downregulated in mitochondria. Thus, the repair activity was differentially regulated by exercise. The upregulation of OGG1 activity in the nucleus suggests that the reduced oxidative damage to the nuclear DNA is at least partly due to the increased repair. The reason for the downregulation of mitochondrial OGG1 activity despite the reduction of DNA oxidation is not clear. Consistent with our observation that the mitochondrial OGG1 activity is downregulated by regular exercise, Ji (1993) has reported that mitochondrial GSH peroxidase is also downregulated in chronically trained rats.

Thus, reduced oxidative damage to DNA appears to be one of the mechanisms of the anti-cancer and anti-aging effects of a physically active lifestyle as has been shown in human epidemiologic studies (Blair et al. 1989).

Conclusion

Regular physical exercise appears to retard or reverse age-related functional decline and to delay the onset of age-related diseases by attenuating potentially harmful oxidative damage and suppressing inflammatory processes, even when performed at older ages. We suggest, therefore, that regular exercise may be a form of hormesis in that while excessive generation of ROS by exhaustive exercise is obviously harmful for unprepared cells, a small increase in ROS by moderate regular exercise is beneficial, thus enabling the preparation of cells for higher stresses that may be encountered in future (Ji et al. 2006; Radák et al. 2005; Goto et al. 2004). To retard age-related physiologic decline and reduce morbidity in elderly people, we argue that regular exercise may be superior to pharmacologic anti-aging medicine in evoking reserve power by modulating the transcription of genes responsible for counteracting the outcomes of oxidative stress without much disturbance to the cellular homeostatic balance as could be the case with pharmacologic intervention.

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