Exercise and Reactive Oxygen Species in Elderly
- Exercise as Prevention of Oxidative Stress -

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Oxidation produces reactive oxygen species (ROS), which cause peroxidation, enzyme inhibition and genetic damage in muscle cells. Genetic damage to cells and tissues caused by ROS facilitates aging. Therefore, the functional capacity of the antioxidant system against ROS is important to protect cells and tissues. The health benefits of regular exercise are well documented in a large number of reports. Moderate exercise can result in greater health benefits than vigorous exercise, because intense activity may be more susceptible to producing oxidative damage. Evidence would appear, as an indirect sign of muscle cell damage, such as an increase in lipid peroxidation, glutathione oxidation, and oxidative protein damage. During exercise, increased aerobic metabolism is a potential source of ROS in mitochondria. In muscle cells, mitochondria are one important source of reactive intermediates that include superoxides, hydrogen peroxide, and possibly hydroxyl radicals. Furthermore, a recent report suggested the occurrence of inter-mitochondrial complementation through exchange of genetic contents. This complementation could be a mitochondria-specific mechanism for avoiding expression of deletion-mutant mitochondria DNA from oxidative stress. Unfortunately, because research focusing on oxidative stress and antioxidants following exercise has up to now been narrow in scope, the mechanism linking oxidative stress and antioxidants in muscle tissue during exercise is not fully understood. Knowledge of the mechanism of ROS formation during exercise will be useful for health promotion for elderly as well as young exercise enthusiasts and may lead to the prevention of oxidative stress and damage associated with physical activity. This review paper provides a brief account of how exercise leads to oxidative stress and the link with antioxidants, and implies appropriate exercise regimen as prevention of oxidative stress for elderly individuals.

Keywords: elderly, exercise, free radical, health, oxidative stress, review

1. Introduction

Less exercise in our daily life with the benefits of automatization may be one of factors in the recent promotion of regular exercise to maintain and promote health. Also, the Japanese social background such as declining birthrate and increase in aging population, would make people to become more concerned about health and to try to incorporate exercise into our daily life.

This public trend is based on a premise that "Exercise is good for health." In fact, proper exercise is believed to prevent various diseases such as life-style related diseases and to improve the activity level and quality of life. Nevertheless, excessive exercise sometimes deteriorates health and may causes disorders. There is a warning that exercise is harmful to health. One of causes is reactive oxygen species (ROS).

Because ROS has much higher reaction than stable oxygen existing in the air, it causes oxidative damage (oxidative stress) to tissues if it becomes excessive in vivo. The ROS has turned out to be generated in cells and tissues as long as they consume oxygen, and
at least 1% uptake oxygen would changed to ROS (Cadenas & Boveris, 1980; McCord, 1979; Turrens & Boveris, 1980). Oxygen consumption increases 10 to 15 times the normal consumption during exercise, and oxygen inflow to active tissues reaches 100 times the normal (Sen, 1995) so that the ROS generation rate at exercise is expected to become higher than normal. Based on this issue, one may say that exercise is bad for health because it generates ROS. It is not necessarily true because a living body has a preventive system (antioxidant system) to minimize disorders brought on by ROS. Therefore, it must be interpreted that when the generation rate of ROS exceeds the permissible range of the antioxidant system, ROS generated by exercise causes disorder to tissues. Then, how much exercise causes unbalance between the generation of ROS and defense, or, how much exercise maintains a balance? These questions may be important when we think of the optimum amount of exercise. The present review outlines the effect of exercise on health by examining studies on the relationships between exercise and ROS.

2. Relationships between reactive oxygen and exercise

Although more ROS could be generated during exercise, there are few studies that directly detected the ROS generation during exercise. So far, only studies using electron spin resonance (ESR), which enables direct measurement of ROS, observed ROS to be generated more in skeletal muscles immediately after exercise than at resting (Davies, et al., 1982; Jackson, et al., 1985). However, at present, there are some difficulties to quantify the amount of ROS generation using ESR, especially by exercise. Thus, many studies have examined about exercise-generated ROS and its causative effect through indirect measurements of oxidative stress markers and/or antioxidant marker generated after or during exercise, and administration of antioxidant(s).

The oxygen molecule changes to water mediated by cytochrome oxidase in the mitochondrial electron transport chain through four-electron reduction and at least 1% could be changed to ROS (Cadenas & Boveris, 1980; McCord, 1979; Turrens & Boveris, 1980). Oxygen consumption increases as increase of exercise time and distance, and the generation rate of ROS from the mitochondrial electron transport chain may increase. In an experiment in which rats were forced to swim for 5 hrs and 8 hrs, concentration of thiobarbituric acid reactive substances ([TBARS]), which are lipid peroxidation markers in liver cells, increased dependent on the exercising time. And this time-dependent increase of [TBARS] occurred in the mitochondria (Venditti, et al. 1999). Similar phenomenon has been observed also in skeletal muscle, in which oxygen inflow into tissues may increase at exercise (Koz, et al., 1992). These results suggest that the ROS generation rate (mainly in the mitochondria) as well as the oxidative stress marker may increase by exercise depending on time and distance. Meanwhile, an experiment comparing conjugated diene (indicator of lipid peroxidation) concentration in human serum after running with maximum effort for 1 km, 10 km, and 27 km, showed conjugated diene concentration increase by -2% in 1 km, 14% in 10 km, and 11% in 27 km. The oxidative stress marker, however, did not increase proportionally with exercise distance (Vasankari, et al., 1995). Similar results were also observed in other studies (Radak, et al., 2003). Discrepancy of the results may be ascribed to the difference of the characteristics of the oxidative stress marker (metabolism, half life) and observed section (intracellular/extracellular, internal organs). Also, the use of indirect indicators such as lipid peroxides and protein peroxides as dynamics of ROS with high reaction might cause contradictory results among studies. Accordingly, it is necessary to be careful of interpreting increase and decrease of the oxidative stress marker arisen from the consequence of exercise.

Some studies have suggested that exercise intensity does not affect ROS generation (Watson, et al., 2005) while some have reported contrary observations (Alessio et al., 1988). Many of the recent studies support the latter (Gambelunghe, et al., 2001; Ji, et al., 1992; Quindry, et al. 2003). Untrained males performed maximum load exercise and submaximal exercise for thirty minutes, and had an increase in the post-exercise GSSG/total glutathione ratio (oxidative stress indicator: rate of oxidized glutathione (GSSG)) dependent on exercise intensity (Sen, et al., 1994). Concentration of pentane contained in expiration and serum in MDA (malondialdehyde) that is the indicator of oxidative stress in incremental exercise, increased dependent on exercise intensity (Leaf, et al., 1997; Lovlin, et al., 1987). In a study in which exercise intensity and duration changed...
with an equal oxygen consumption rate during exercise (40% $\text{VO}_\text{max}$-45min, 60% $\text{VO}_\text{max}$-30min, 80% $\text{VO}_\text{max}$-22.5min), post-exercise serum [TBARS] increased with a rise of exercise intensity (Toshinai, et al., 1998). It suggests a possibility that oxidative stress increase may depend more on exercise intensity than exercise duration. From these findings, exercise intensity as well as exercise duration may possibly affect the generation rate of ROS and oxidative stress.

It is also suggested that the ROS generation rate and its influence differ according to the type of exercise. In comparison to the change of serum MDA concentration after exercise between a long run and a sprint run, peak values were observed immediately after exercise in the long run, and 48 hours after exercise in the spring run (Marzatico, et al., 1997). Eccentric muscle contraction during resistance training and downhill walking (running) easily induces muscle ultrastructural disruption (Jones, et al., 1986). CK activity achieves the peak several days after eccentric contraction exercise, and degeneration of muscle fiber accompanying infiltration of macrophage is observed histologically (Jones, et al., 1986). In fact, one day after a full marathon, CK activity achieves the peak while oxidative damage is observed in leukocyte cell DNA in three days (Tsai, et al., 2001). Increased activation of these inflammatory cells is thought to promote ROS generation and oxidative stress.

Most of the increase in ROS generation and oxidative stress by a single session of exercise are temporary and they recover to the at-rest level with time (Sen, et al., 1994). This is because increased ROS and oxidative stress are removed and detoxicated by the antioxidant system possessed by cells and tissues. ROS and oxidative stress increased by exercise will not harm health if the antioxidant system functions normally and unless generation of ROS increases in excess of the antioxidant capacity.

There is a report that the energy generating capacity of cells which incorporates the mitochondria DNA (mtDNA) containing pathogenic mutations such as Alzheimer’s disease is almost the same as normal cells (Ito, et al., 1999) although it is different from exercise-derived radicals in its nature. The result of the study is contradictory to an anticipation that respiration of a cell should decrease if it contains pathogenic mutation in transplanted mtDNA, and suggests that even if mutation of mtDNA is accumulated, enzyme activation of mitochondria could be intact. It also suggests that there is a system that maintains respiratory function of mitochondria through mutual complementation of the mutation by multiple mtDNA in the event of damage to the intracellular mtDNA (Inoue, et al., 2000). Since this self-defense function of mitochondria can probably be applied to mtDNA damage by exercise-deprived radicals, influence of ROS by exercise can possibly be minimized.

3. Influence of exercise training on oxidative stress and antioxidant capacity

How does continuous exercise training affect a living body if ROS generation and oxidative stress increase with a single session of exercise?

Marzatico, et al. (1997) reported that athletes who were performing high intensity endurance exercise training or sprint training showed higher serum MDA values at rest than untrained sedentary subjects. Urinary excretion of 8-OHdG (oxidative damage indicator of DNA) after thirty-day army training (8 to 10 hrs /day) increased when compared with before training (Poulsen, et al., 1996). These results suggest that continuous exercise possibly exacerbates oxidative stress. Yet, Marzatico, et al. (1997) reported that endurance or sprint training can also increased such antioxidant enzyme activity as SOD (Superoxide dismutase) and GPx (Glutathione peroxidase). This indicates a possibility to improve antioxidant capacity by exercise training. As described above, many of the exercise trainings in which accumulation of oxidative stress increases repeat high intensity exercise loading. However, there are few reports that oxidative stress increases at rest in exercise training from low to middle intensities. To the contrary, as there are some reports that exercise training enhances the antioxidant capacity and lessens oxidative stress (Kretzschmar, et al., 1991), exercise training with appropriate exercise program can preferably affect maintenance and promotion of health in view of ROS and oxidative stress.

The degree of improvement of antioxidant capacity by exercise training affects exercise intensity and duration, which regulate the ROS generation rate at exercise. Powers, et al. (1994) examined the effect of exercise training intensity and duration on SOD activity of the rat soleus muscle by exercise with
combinations of three exercise intensities (55, 65, and 70% \( V_{\text{O}_2\text{max}} \)) and three kinds of exercise time (30, 60, and 90 min) for ten weeks. The results showed a time dependent increase in SOD activity until the exercise time of 60 minutes irrelevant to exercise intensity, and SOD activity increased in the exercise time of 90 minutes dependent on exercise intensity (Figure 1).

In our examination of relationships between the spontaneous running distance and ROS scavenging activity in skeletal muscles of rats for twenty three weeks, we found that rats which ran a longer distance showed higher ROS scavenging activity of skeletal muscle (Tanabe, et al., 2006b: Figure 2). These results suggest that antioxidant capacity possibly improves dependent on exercise training intensity and time. Further, there is a report that when rats performed forced exercise training of high intensities, the 8-OHdG levels of the internal organs (liver, lung, and heart) increased while the spontaneous exercise group showed lower 8-OHdG levels than the forced exercise group irrespective of exercise amount (Asami, et al., 1998). It implies a change of oxidative stress according to exercise training intensities, which can be interpreted, as spontaneous intensities with which exercise training is performed may not affect oxidative stress. However, as shown in Figure 1, from the increase curve of SOD activity by training, we cannot expect further improvement of antioxidant capacity by increasing exercise time (above 60 min) and intensity. In contrast to ROS with its generation to be increased, oxidative stress is possibly cumulative by exercise training if the adjustment of antioxidant system cannot follow. It could be said from a view that serum 8-OHdG content cannot be accumulated from endurance exercise training with low to middle exercise intensities but with high intensities (Goto, et al., 2003).

4. Change of oxidative stress and antioxidant capacity with aging

Age-related decline of biofunction is a complex process containing morphological and biochemical changes. Explanations for the causes of aging include many theories and hypotheses. Among which is "the free radical theory of aging." It is a tissue disorder accumulative of ROS (Harman, 1986). The theory has been examined, and the results of the following indicate the relationship between aging and ROS: longevity is longer with slower mitochondria \( \text{O}_2 \cdot \) generation speed in mammals (Ku, et al., 1993); longevity is longer with higher SOD activity (Tolmasoff, et al., 1980); and in DNA modulator...

The relationship between aging and oxidative stress has been examined in many studies. There have been many studies indicating a possibility of increase of peroxidation with aging (or, incubation period) in rat internal organs, cultured human fetal diploid fibroblasts, human serum, and human skeletal muscle (Miyazawa, et al., 1993; Suzuki, et al., 1993; Olinescu, et al., 1995; Pansarasa, et al., 1999). Most of these studies reported increase of oxidative stress with aging.

As for age-related change of antioxidant capacity, we generally think that antioxidant enzyme activity declines with age-related decline of protein metabolism and neogenesis ability of cells. However it differs according to the type of internal organs and antioxidants. In rat skeletal muscles, the protein rate became lower with age while antioxidant enzyme (Cu,Zn-SOD, Mn-SOD, intracytoplasmic GPx, mitochondrial GPx, catalase, glutathione-S-transferase, glutathione reductase) activity became higher with age (Ji, 1993). In contrast, activity of the intracytoplasmic antioxidant enzyme (Cu,Zn-SOD, intracytoplasmic –GPx, glutathione-S-transferase) in rats’ hearts all decreased with age while activity of the antioxidant enzyme (Mn-SOD, mitochondrial GPx) in mitochondria increased. Also, there are reports that concentration of the reduced form GSH (reduced glutathione) which is the main intracellular antioxidant decreased with age in the liver (Mosoni, et al., 2004), and it does not change or increase with age in rat skeletal muscle and human skeletal muscle (Leeuwenburgh, et al., 1994; Pansarasa, et al., 1999).

In age-related change, fat-soluble antioxidants, vitamin E, (α-tocopherol) increased in the liver, lung, and brain (Ji and Hollander, 2000) and did not change in skeletal muscles (Starnes, et al., 1989). Generally, the water-soluble antioxidant, vitamin C, is said to decrease with age (Ji and Hollander, 2000). Since direct measurement of ROS is difficult, change of generation rate of ROS with age has not been clarified. Yet, there is indirect proof that ROS efflux in skeletal muscle mitochondria increases with age (Bejma & Li, 1999; Ji, et al., 1990). It is presumed that the increase of age-related ROS generation or decrease of antioxidant capacity may cause age-related increase of oxidative stress.

We recorded findings about oxidative stress and antioxidant capacity with healthy middle-aged and elderly people (436 subjects including untrained young people in their twenties) who were actively participating in local exercise classes. Blood antioxidant capacity (GSH concentration) was lower in middle-aged and elderly people (45 yrs-92 yrs) and oxidative stress ([TBARS]) was significantly...
higher than young people. In the subjects who were over 45 years old, antioxidant capacity and oxidative stress showed no notable age-related change (Tanabe, et al., 2006a). Studies which explained advancement of oxidative stress in response to aging in the middle-aged and elderly (Mutlu-Turkoglu, et al., 2003; Olinescu, et al., 1995) might randomly include the subjects of middle-aged and elderly people who were potential patients and smokers so that effects of aging might not be manifested in the level of antioxidant capacity and oxidative stress in healthy middle-aged and elderly subjects. When we compared young people (in their 20s) and healthy middle-aged and elderly people (in their 60s) in antioxidant capacity (e.g.: $O_2^{-}$ scavenging activity) of muscle samples obtained from the vastus lateralis muscle, we found no age-related decrease of antioxidant capacity in skeletal muscle as we observed it in blood (unpublished data). Age-related difference of antioxidant capacity between blood and skeletal muscle might presumably be affected by ROS generation rate at each section (Bejma & Ji, 1999), and the difference of content of antioxidants (e.g.: GSH (Luo, et al., 1998)).

5. Relationship between exercise and reactive oxygen generation & antioxidant capacity in the middle-aged and the elderly

The middle-aged and the elderly accumulate more oxidative stress than the young, which may explain why they are susceptible to oxidized damage by ROS. Yet, there are few studies on the influence of exercise on oxidative stress and antioxidant capacity in the middle-aged and the elderly when compared with the young. As for availability and safety of exercise in view of ROS, examination of middle-aged and elderly subjects is important in preparing exercise programs for health maintenance and promotion.

Table 1 shows the age-related influence on the response of oxidative stress after a single session of exercise and exercise training. In two studies examining responses of oxidative stress after a single session of exercise with human subjects, no effect of aging was observed in the increase of post exercise oxidative stress (Di Massimo, et al., 1999; Sacheck, et al., 2003). Nevertheless, in an experiment with rats, there was a bigger increase of oxidative stress in older rats after acute exercise. That implied that, older rats might possibly be susceptible to oxidative stress (Bejma & Ji, 1999; Bejma, et al., 2000; Navarro-Arevalo, et al., 1999). The difference of the results between humans and rats might be ascribed to the difference of the samples. The sample was blood in the study with the human subjects while it was the cells of skeletal muscles and internal organs in rats. Therefore in skeletal and heart muscles, the older tend to increase more with age in oxidative stress after exercise. On this point, Bejima & Ji (1999) inferred that oxidative stress increased in muscle cell because ROS generation from the mitochondrial respiratory chain and NADPH oxidase increased with age. Yet, further discussion would be necessary in this respect since there is a report that ROS generation rate in the skeletal muscle mitochondria does not change with age (Tonkonog, et al., 2003). There is no study comparing the young and the elderly in oxidative stress of skeletal muscles after a single session of exercise. Future study would require examining how humans would show similar or different results to rodents.

There are studies on the influence of aging on oxidative stress response after chronic exercise training only with rodents as subjects, most of which demonstrated no influence of aging (Table 1). As previously described, despite an increase of oxidative stress in rat skeletal and heart muscles after a single session of exercise, an interesting finding is that chronic exercise does not make any influence on aging.

As we cross-sectionally examined the relationship between daily physical activity and oxidative stress and antioxidant capacity with middle-aged and elderly subjects, we did not find a strong relationship between the activities of daily life and oxidative stress and antioxidant capacity (Tanabe, et al., 2002). In the meantime, middle-aged and elderly people who are performing daily endurance exercise training tended to show a higher antioxidant capacity marker (GSH concentration) in the blood than age-matched normal people (Tanabe, et al., 2002). In a longitudinal examination of a twelve week of endurance exercise training with relatively low intensity (80%VT load, 30min, 5 times per week) by middle-aged and elderly people, we found an increase in the antioxidant capacity marker (GSH concentration) in the blood at rest after training, without change in serum lipid peroxidation concentration. It can be interpreted that the exercise
Table 1 The effects of aging on acute and chronic exercise-induced oxidative stress.

<table>
<thead>
<tr>
<th>Author</th>
<th>Subject</th>
<th>Sample</th>
<th>Exercise</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute exercise</td>
<td></td>
<td></td>
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<tr>
<td>Di Massimo et al.</td>
<td>human, old: 52-70 yr,</td>
<td>plasma</td>
<td>cycling</td>
<td>TBARS ↑: old = young</td>
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<tr>
<td>1999</td>
<td>young: 20-30 yr</td>
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<tr>
<td>Sachen et al. 2003</td>
<td>human, old: 65-80 yr,</td>
<td>serum</td>
<td>downhill running 75%VO₂ max</td>
<td>MDA ↑: old = young</td>
</tr>
<tr>
<td></td>
<td>young: 18-35 yr</td>
<td></td>
<td></td>
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<tr>
<td>Navarro-Arevalo et</td>
<td>rat, old: 24-27 mo,</td>
<td>heart,</td>
<td>running, to exhaustion</td>
<td>TBARS ↑: old &gt; adult</td>
</tr>
<tr>
<td>1999</td>
<td>adult: 3-5 mo</td>
<td>skeletal muscle</td>
<td></td>
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<tr>
<td>Bejma &amp; Ji 1999</td>
<td>rat, old: 25 mo,</td>
<td>skeletal muscle</td>
<td>running 75%VO₂ max</td>
<td>ROS production rate ↑: old &gt; adult</td>
</tr>
<tr>
<td></td>
<td>adult: 8 mo</td>
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<tr>
<td>Bejma et al. 2000</td>
<td>rat, old: 25 mo,</td>
<td>heart</td>
<td>running 75%VO₂ max</td>
<td>ROS production rate: old ↑, adult→</td>
</tr>
<tr>
<td></td>
<td>adult: 8 mo</td>
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<tr>
<td>Chronic exercise (Training)</td>
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<tr>
<td>Radak et al. 1999</td>
<td>rat, adult: 14 mo,</td>
<td>skeletal muscle</td>
<td>swimming 9 wk</td>
<td>8-OHdG ↑: adult = young</td>
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<tr>
<td></td>
<td>young: 4 wk</td>
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<tr>
<td>Radak et al. 2001</td>
<td>rat, adult: 14 mo,</td>
<td>brain</td>
<td>swimming 9 wk</td>
<td>RCD ↓: adult = young</td>
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<td></td>
<td>young: 4 wk</td>
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<tr>
<td>Radak et al. 2002</td>
<td>rat, old: 30 mo,</td>
<td>skeletal muscle</td>
<td>running 8 wk</td>
<td>old: RCD ↓, 8-OHdG ↑, adult: RCD→, 8-OHdG→</td>
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<tr>
<td></td>
<td>adult: 20 mo</td>
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<tr>
<td>Radak et al. 2004</td>
<td>rat, old: 28 mo,</td>
<td>liver</td>
<td>running 8 wk</td>
<td>MDA=HNE(↑), GSH ↑: old = adult</td>
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<tr>
<td></td>
<td>adult: 18 mo</td>
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<tr>
<td>Kakarla et al. 2005</td>
<td>rat, older adult: 12 mo</td>
<td>liver</td>
<td>running 12 wk</td>
<td>MDA ↑, SOD ↑, GR ↑, CAT ↑, GSH ↑: older = younger</td>
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<tr>
<td></td>
<td>younger adult: 3 mo</td>
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<tr>
<td>Rosa et al. 2005</td>
<td>mouse, older adult: 18 mo</td>
<td>skeletal muscle</td>
<td>running 15 mo</td>
<td>MDA: older &gt;&gt; younger &gt; training older adult</td>
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<tr>
<td></td>
<td>younger adult: 3 mo</td>
<td></td>
<td>(during 3-18 mo old)</td>
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</table>

Oxidative stress marker; TBARS: thiobarbituric acid reactive substance, MDA: malondialdehyde, ROS: reactive oxygen species, RCD: reactive carbonyl derivatives, 8-Ohdg: 8-hydroxy-2′ deoxyguanosine, 4-HNE: 4-hydroxy-nonenal Antioxidant marker; GSH: reduced glutathione, SOD: superoxide dismutase, GR: glutathione reductase, CAT: catalase ↑ or ↓: significantly increase or decrease after acute exercise or training, →: no change after acute exercise or training, (↑): trend to decrease after training

Some of the other studies with elderly subjects have indicated that middle-aged and elderly people may not affect oxidative stress.

program was not a type of exercise training that might increase oxidative stress (Tanabe, et al., 2003) (Figure 3). These findings suggest that middle-aged and elderly people can enhance antioxidant capacity by chronic exercise training, and that spontaneous exercise and physical activities of daily life, or endurance exercise training with relatively low intensity continuously performable by middle-aged and elderly people may not affect oxidative stress. Some of the other studies with elderly subjects have
reported that endurance training improves antioxidant capacity and suppresses oxidative stress (Fatouros, et al., 2004; Jessup, et al., 2003).

The fact that endurance exercise for the middle-aged and the elderly improves antioxidant capacity without increasing oxidative stress is a useful finding in promoting exercise for the middle-aged and the elderly.

A recent study demonstrated direct proof of increase in generation of ROS in venous blood during resistance exercise (Bailey, et al., 2004). There are few studies on the effect of repeated resistance training to oxidative stress or antioxidant capacity of the elderly (Rall, et al., 2000). We reported in our study of middle-aged and elderly people performing resistance training that the antioxidant capacity of blood level improved and oxidative stress was restrained (Tanabe, et al., 2003: Figure 3). In another study, we have verified that comparatively low intensity resistance training, if performed frequently and continuously, can also improve antioxidant capacity in the blood and suppress oxidative stress (unpublished data). Repeated increase of ROS generation by continuous exercise, in spite of its difference in form from endurance exercise training, can presumably promote accommodation of antioxidant capacity in the blood and suppress oxidative stress. A similar result was obtained in a study performing resistance training of 50% to 80% 1RM by the elderly (Vincent, et al., 2002).

6. Limitations of our previous studies and future study

As described in the result of an epidemiological survey that "Exercise habit positively relates to the human life span (Wannamethee, et al., 1998), exercise is an indispensable factor in considering health. Still, there is no clear answer to the question whether an increase of ROS generation at exercise may harm the health of the elderly with much cumulative oxidative stress. The result of our study did not bring any proof of an adverse effect of exercise-derived ROS on living cells and tissues.

As previously mentioned, the balance of generation and deletion of ROS decides accumulation of oxidative stress. Hindering examination of the relationship between aging and oxidative stress, or between exercise of the elderly and oxidative stress, are the following two questions: Does ROS generation increase with age?; and, How does antioxidant capacity change with age? Despite many studies on these questions, they are still controversial. The reason for no conclusive findings may include difficulty in standardizing individual background because of such greater environmental influences as life habits as they grow older, and presumption of ROS measured by indirect indicators because its direct measurement is difficult. In future studies, it is necessary to standardize individual or populational background as much as possible and to find direct proof of the relationship between in vivo ROS generation and the antioxidant system. To do this,
many issues remain such as development of methods and identification of the marker.

Electron spin resonance (ESR) can solely make direct detection of ROS radicals with unpaired electrons and has generally been used to understand the structure and the nature of chemical substances mainly on engineering science. Recently, with advancement of the accuracy of ESR equipment, development of a spin trap agent, a spin label agent and a spin probe agent that can temporarily stabilize radicals, selective quantification of radicals such as cells and serum existing in liquid substances became possible \textit{in vitro} or \textit{in situ} (Noda, et al., 1997). These also have applied to medicine and biology. Also, with the improvement of accuracy of L-band ESR (\textit{in vivo} ESR) permitting quantifiable ROS generated \textit{in vivo}, noninvasive capturing of \textit{in vivo} ROS is possible even if there is little evidence (Hirayama, et al., 2005). Now, ROS generation movement in disease models in the internal organs is shown in 3D images and ESR imaging studies have been developed (Hirayama, et al., 2005). In sports science and sports medicine, however, there are only a few studies using ESR that examine the relationship between aging or exercise and ROS. There are few studies focusing on skeletal muscles, which are the source of ROS generation during exercise. So far, by using both ESR and the spin trap agent (Green, et al., 1979) we measured $\text{O}_2^-$ scavenging activity of rat skeletal muscles, which are different in metabolic characteristics, and verified the validity (Masuda, et al., 2003: \textbf{Figure 4}). Using the same technique, we also examined the relationship between the distance of voluntary exercise and ROS scavenging activity of skeletal muscles (\textbf{Figure 2}) and the change of ROS scavenging activity in skeletal muscles before and after exercise training with the young and elderly subjects. Although this technique neither detected \textit{in vivo} radicals directly nor measured their reducing activities, it should be regarded as a physiological parameter of \textit{in vivo} ROS scavenging.

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