Physiol. Res. 53: 171-176, 2004

The Effect of One Year´s Swimming Exercise on Oxidant Stress and Antioxidant Capacity in Aged Rats

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Received September 3, 2002
Accepted April 3, 2003

Summary

The effect of exercise on oxidant stress and on alterations in antioxidant defense in elderly has been investigated extensively. However, the impact of regularly performed long-term physical activity starting from adulthood and prolonged up to the old age is not yet clear. We have investigated the changes in the activities of antioxidant enzymes – superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) – and lipid peroxidation in various tissues of rats which had performed (old-trained) or had not performed (old-control) regular swimming exercise for one year. These animals were compared with young-sedentary rats. Increased lipid peroxidation was observed with ageing in all tissues (heart, liver, kidney, striated muscle) and swimming had no additional effect on this elevation of lipid peroxidation. Heart and striated muscle SOD activites, and striated muscle CAT activity increased as a consequence of ageing, whereas kidney and liver CAT activities, as well as GPx activities in kidney, liver, lung and heart were significantly decreased compared to young controls. Lung and heart SOD, liver CAT activities as well as GPx activities in liver, lung and heart were increased significantly in rats which performed exercise during ageing, compared to the old-control group. These findings suggest that lifelong exercise can improve the antioxidant defense in many tissues without constituting any additional oxidant stress.

Key words
Ageing • Antioxidant enzymes • Exercise • Lipid peroxidation

Introduction

The theory of free radicals is one of the hypotheses associated with ageing (Harman 1956). While several investigations have shown an increase in the production of reactive oxygen species which occur as consequence of electron transport chain deficiency (Feuers *et al.* 1993, Fannin *et al.* 1999), there is no uniformity in the results on how the antioxidant defense system is affected in old age (Jung and Henke 1996). Results indicating an increase or decrease, or even no change of antioxidant enzyme activities in various tissues in old age have been reported (De and Darad 1991, Matsuo *et al.* 1992, Jung and Henke 1996, Abbasoğlu-Doğru *et al.* 1997, Gomi and Matsuo 1998). In spite of this, it is believed that the antioxidant defence is generally weakened in old age (De and Darad 1991, Rikans and Hornbrook 1997, Gomi and Matsuo 1998) except for the antioxidant enzyme activities in striated muscle (Vertechy *et al.* 1989, Ji *et al.* 1990).

Physical activity is recognized as an important component of healthy life style and recommended

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throughout life by scientists and clinicians (Donaldson 2000). Exercise training is recommended for improving physiological and functional capacity in the elderly (Rhodes *et al.* 2000). However, exercise like ageing, is one of the physiological conditions characterized by increased production of free radicals (Clarkson 1995). The production of free radicals increases in parallel with the increase in oxygen consumption during exercise, and this increase is directly related to the intensity and/or the duration of exercise (Ji 1996). On the other hand, the antioxidant enzymes, which constitute a defense mechanism against free radicals produced during exercise, are also affected by the exercise (Clarkson 1995). Although contradictory views exist, it is accepted that regular physical activity leads to an increase in the activities of antioxidant enzymes especially in muscles (Ji 1996, Lawler and Powers 1998).

The effect of acute and/or chronic exercise on oxidant stress and antioxidant systems in old organisms has been investigated in a number of studies (Ji 1993, Ji *et al.* 1998, Polidori *et al.* 2000). However, the influence of long-term exercise started in adulthood and regularly performed during the rest of life on oxidant stress and antioxidant systems is not known. The aim of this study was to reveal the effects of lifelong exercise on the oxidant and antioxidant systems of various organs in aged rats.

Methods

Animals

Thirty 9-month-old male albino Wistar rats weighing 250-300 g were divided into two groups as Old-Control (n=15) and Old-Trained (n=15) groups and were housed for one year. Ten 9-month-old male rats were added to the study at the end of the experiment as the Young-Control (n=10) group. Two animals from old-control and three animals from old-trained group died before end of the experiment. All rats were given standard rat chow and tap water *ad libitum* and were housed at 23±2 ^{o}C on a 12 h dark and 12 h light cycle. All procedures were approved by the Akdeniz University Animal Care and Usage Committee and followed the guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

Exercise procedures and tissue preparation

The exercise-trained group swam one hour a day, five days a week during one year of housing (between 09:00-11:00 h on each training day). The old-control rats were housed under the same conditions as the swimming rats and they were handled as often as the exercise group. Exercise was performed by swimming in two glass tanks (length 100 cm, width 50 cm, depth 50 cm) containing tap water maintained at 32-34 ^{o}C. A maximum of only six rats was allowed to swim together. The duration of first swimming experience was limited to 10 min and increased by 10 min daily until it reached one hour. All rats were anesthetized with urethane (1 g/kg, i.p.) 48 h after the last session and tissues (heart, lung, liver, kidney, gastrocnemius and soleus muscles) were quickly removed. The excised samples were stored at –80 ^{o}C until assay.

Biochemical analyses

The tissues were homogenized with a motor-driven glass homogenizer in ice-cold phosphate (including EDTA, triton-X, butylated hydroxytoluene) buffer at 0 ^{o}C for lipid peroxidation analysis. Lipid peroxidation of all tissues was estimated by thiobarbituric acid reactive substance (TBARS) as described by Stocks and Dormandy (1971) with 1,1,3,3-tetraethoxyprophane used as standard. Results were expressed as nmol per g protein in tissues. The protein content of tissues was analyzed with the method of Lowry *et al.* (1951). The remaining tissue samples were thawed in 50 mM ice-cold phosphate buffer, (pH 7.4) and homogenized at 0 ^{o}C. The supernatants were used for antioxidant enzyme activity analysis. The activities of antioxidant enzymes namely catalase (CAT; EC 1.11.1.6), superoxide dismutase (SOD; EC 1.15.1.1) and glutathione peroxidase (GPx; EC 1. 11. 1. 9) were evaluated. CAT, SOD and GPx activities were assessed by using the methods of Paglia and Valentine (1967), Misra and Fridowich (1972) and Aebi (1983), respectively.

Statistical analyses

The results were expressed as mean ± S.E.M. and statistical analyses were done by one-way analysis of variance. The Newman-Keuls post-test for multiple comparison among means was used to compare differences among groups. $P<0.05$ was accepted as significant.

Results

Mean body weight, weight gain and heart weights at the end of the one year period did not differ between the groups, but the heart weight-to-body weight

ratio was significantly higher in the old-exercise group (0.00318±0.00058) than in the old-control group (0.00274±0.00052) ($p<0.05$).

Table 1 shows the TBARS levels as an index of lipid peroxidation. In all tissues, TBARS levels were significantly elevated with ageing in both old groups except in gastrocnemius muscle of old-trained rats. Although there was a trend towards decreasing in TBARS levels of the gastrocnemius muscles in the old-trained group, swimming did not alter the TBARS levels in tissues of old rats.

The activities of SOD in the heart and gastrocnemius muscles were significantly elevated in old-control and old-trained groups, and the lung SOD activity was also higher in the old-trained group compared with young-controls, while in the other tissues no significant difference from that of the young animals was detected (Table 2). One year´s exercise training significantly increased kidney, lung and heart SOD activities in the old-trained group compared to the old-controls.

Table 1. TBARS levels [nmol·(g protein)$^{-1}$] in the kidney, liver, lung, heart, gastrocnemius and soleus muscles of all the groups studied

	Young-Control n=9	**Old-Control n=13**	**Old-Trained n=12**
Kidney	244.7±4.69	625.6±65.78 ***	511.5±39.84 ***
Liver	121.2±12.3	205.2±13.83 *	196.9±26.57 *
Lung	60.08±2.64	113.8±12.61 **	114.8±4.09 **
Heart	72.29±3.49	137.8±8.77 ***	142.2±6.57 ***
Gastrocnemius muscle	43.78±4.16	74.25±8.69 *	56.37±7.13
Soleus muscle	39.18±4.41	81.59±17.81 *	101.2±4.89 **

Values are means ± S.E.M. * $p<0.05$, ** $p<0.01$, *** $p<0.001$; difference from Young-Control

Table 2. The kidney, liver, lung, heart, gastrocnemius and soleus muscles SOD activities [U·(mg protein)$^{-1}$] of all groups

	Young-Control n=9	**Old-Control n=13**	**Old-Trained n=12**
Kidney	38.69±1.43	35.40±2.97	43.36±1.39 †
Liver	94.08±9.16	80.79±3.93	82.42±2.68
Lung	3.10±0.11	2.94±0.19	3.61±0.11 * †
Heart	7.46±0.53	10.76±0.51 ***	12.23±0.21 *** †
Gastrocnemius muscle	5.52±0.21	6.90±0.45 *	6.83±0.25 *
Soleus muscle	2.88±0.04	3.03±0.14	3.03±0.09

Values are means ± S.E.M., * $p<0.05$, *** $p<0.001$; difference from Young-Control, † $p<0.05$; difference from Old-Control

Although the CAT activities of lung and heart were not altered with age, kidney CAT activity in both old groups and liver CAT activity in old-controls were significantly decreased compared with young animals (Table 3). On the other hand, soleus and gastrocnemius muscles had significantly higher CAT activities in both old groups compared to the young-control group. Only liver CAT activity was significantly higher in the old-trained group than in the old-controls.

The activities of GPx in both soleus and gastrocnemius muscle tissues did not differ between the groups, whereas kidney, liver, lung and heart GPx activities fell with age, and exercise training caused an elevation in liver, lung and heart GPx activities (Table 4).

Table 3. The kidney, liver, lung, heart, gastrocnemius and soleus muscles CAT activities [k·(g protein)$^{-1}$] in all groups

	Young-Control n=9	Old-Control n=13	Old-Trained n=12
Kidney	150.3±11.5	82.51±7.98 ***	101.4±8.65 **
Liver	275.9±19.75	148.5±22.26 ***	216.9±17.93 †
Lung	23.92±2.75	23.24±3.74	34.66±7.38
Heart	29.83±6.83	34.15±6.07	34.84±4.74
Gastrocnemius muscle	20.12±2.27	63.68±10.86 **	48.52±8.70 *
Soleus musle	19.02±2.51	45.7±6.77 *	45.34±7.25 **

Values are means ± S.E.M., * p<0.05, ** p<0.01, *** p<0.001; difference from Young-Control, † p<0.05; difference from Old-Control

Table 4. The kidney, liver, lung, heart, gastrocnemius and soleus muscles GPx activities [U·(g protein)$^{-1}$] in all groups

	Young-Control n=9	Old-Control n=13	Old-Trained n=12
Kidney	89.13±2.43	54.57±2.19 ***	50.02±1.29 ***
Liver	847.5±56.57	374.3±40.51 ***	610.3±32.38 *** †††
Lung	15.33±1.93	6.48±1.05 ***	11.44±1.22 †
Heart	71.67±3.22	58.74±1.37 ***	65.82±1.37 * ††
Gastrocnemius muscle	30.85±5.34	30.15±4.29	25.57±3.79
Soleus muscle	34.61±0.88	39.91±12.94	23.48±3.77

Values are means ± S.E.M., * p<0.05, *** p<0.001; difference from Young-Control, † p<0.05, †† p<0.01, ††† p<0.001 difference from Old-Control

Discussion

The effects of physical exercise on free radical production and the antioxidant system in the elderly have been widely investigated. However, it has not yet been studied how life-long physical exercise affects the oxidant-antioxidant balance. We have therefore focused our attention on how lipid peroxidation and the activities of antioxidant enzymes (SOD, CAT, GPx) in various tissues are affected in aged rats which had performed swimming exercise for one year. A prominent increase in lipid peroxidation was observed in all tissues of both old groups in our study, as it had also been shown in previous studies (Ji *et al.* 1990, Matsuo *et al.* 1992, Rikans and Hornbrook 1997, Abbasoğlu-Doğru *et al.* 1997). Free radical production and oxidant stress, which are known to increase during exercise, may contribute to the oxidant damage proposed to play a role in the ageing process. However, the TBARS levels measured in the liver, lung, kidney, heart and soleus muscle tissues in rats which were swimming for one year did not differ from those of the old-control group. It can therefore be concluded that the long-term exercise applied in our experiments did not contribute to the oxidant stress which increased with age. In addition, the gastrocnemius muscle TBARS levels of the old-trained groups were not different from those of the young-control group.

There are contradictory results on how the antioxidant enzymes are affected by ageing (Abbasoğlu-Doğru *et al.* 1997, De and Darad 1991, Gomi and Matsuo 1998, Jung and Henke 1996). The changes in the antioxidant enzymes with ageing usually show a different pattern in various tissues (Sohal *et al.* 1990). With the exception of striated muscles in our study, it can be stated that the antioxidant defense capacity is reduced in old-controls. GPx activities in old-sedentary rats decreased in all tissues except in muscles, while CAT activities were found to be lower in the liver and kidneys. Although the heart SOD activity was high in old-controls, there was a general non-significant decrease of antioxidant defense.

The reduction in the antioxidant defense was paralleled by a substantial increase of lipid peroxidation in these organs of aged rats.

The enzymatic antioxidant defense of striated muscle differs from that of other tissues in old age. Mitochondrial oxidative capacity was reduced in the muscles of old subjects, while no changes (even elevations) were observed in antioxidant enzyme activities when compared to the young ones (Ji *et al.* 1990, 1998). Although the mechanism is not yet clear, the high ability of adaptation of these enzymes in the muscle tissue has been mentioned (Ji *et al.* 1998). No change in the GPx activity of striated muscle was observed in our study in the old-controls, while there was an increase in the CAT and SOD activities. In parallel with these results, the lipid peroxidation levels in muscle tissues was not as enhanced as in other tissues. The striated muscle antioxidant enzyme activities of the rats which performed swimming exercise did not differ from those of the old-control group. Although the GPx enzyme activities were reduced to a certain extent in the exercise group, this difference was not statistically significant. The antioxidant defense of the old-trained group seems to be high compared to that of the young controls, because of the increased SOD and CAT activities in striated muscles. The absence of differences of enzyme activities in striated muscles between the two old groups in spite of the long-term exercise might be due to the fact that antioxidant enzymes in striated muscles of old animals had already reached their maximum adaptation. Increased activities of the antioxidant enzymes in various tissues with physical activity have been shown in a number of studies. GPx reveals the most prominent alteration among the antioxidant enzymes (Ji *et al.* 1990, 1998). A significant increase particularly of GPx activities with regular exercise which were affected negatively in elderly animals were also detected in our study. In addition, not only the liver CAT activity but also lung and heart SOD activities were found to be elevated following long-term exercise. It seems to be contradictory that the lipid peroxidation was not changed in the old-exercise group compared to the old-controls, although the antioxidant defense had increased. This can be explained by the reduction of activity in enzymes that eliminate lipid peroxidation products (Rikans and Hornbrook 1997).

In conclusion, the exercise performed for long periods of time did not contribute to ageing by constituting an additional oxidant stress load. In the ageing process, the augmentation of the antioxidant defense due to long-term exercise will provide a significant advantage for various pathophysiological processes in old age.

Acknowledgement

This study was supported by Akdeniz University Research Foundation.

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