ELSEVIER

Mechanisms of Ageing and Development
104 (1998) 91–102

mechanisms of ageing and development

Age and exercise-related changes in lipid peroxidation and superoxide dismutase activity in liver and soleus muscle tissues of rats

Ana Navarro-Arévalo *, María Jesús Sánchez-del-Pino

Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad de Cádiz, Plaza Fragela s/n, E-11003 Cádiz, Spain

Received 12 December 1997; received in revised form 27 April 1998; accepted 30 April 1998

Abstract

Thiobarbituric acid-reactive substances (TBARS) level, as marker of lipid peroxidation, and superoxide dismutase (SOD) activity, as endogenous antioxidant enzyme, were examined in liver and soleus muscle tissue of young and old male Wistar rats. We established different types of exercise running on a treadmill both for young and old rats, investigated the effect of aging, exhaustion and training on these groups. The hepatic TBARS levels were raised in the short-training young group and in the long-training old group. On the other hand, the TBARS content decreased in soleus muscle in the short-training young group, and long-training exercise enhanced lipid peroxidation in old rats. SOD activity increased in liver in short-training group, while this activity showed the lowest values in long-training old rats. With respect to soleus muscle tissue, SOD activity was elevated after exhaustive exercise in young rats and old rats had the highest activity in the long-training old group. These findings suggest that free radicals play a role in aging and that the different type, intensity and duration of exercise modify the lipid peroxidation level and antioxidant enzyme activity.

Keywords: Aging; Exercise; Lipid peroxidation; Superoxide dismutase

* Corresponding author. Tel.: + 34 956 225207; fax: + 34 956 223904; e-mail: ana.navarro@uca.es

0047-6374/98/$19.00
PII S0047-6374(98)00061-X

1. Introduction

Oxygen free radicals are involved in aging and exercise. Aging has been proposed to be a result of continuous reactions of the cell components with oxygen free radicals throughout the life span (Reznick et al., 1992).

Aging is a biological phenomenon described as a progressive loss of the physiological functions, with resulting accumulation of tissue changes and increasing susceptibility to disease and death (Harman, 1981). According to Miquel (1989) cell aging is related to mitochondria damage resulting from free radical attack, and to diminished effective mechanisms of cellular repair, and Nohl (1993) refers to aging as a loss of organism homeostatic capacity. These concepts are related to the free radical theory, proposed by Rebeca Gershman (1954), on the deleterious action of the reactive oxygen species linked to aerobic metabolism. Accordingly, aging could depend on the capacity of limiting the oxidative damage of DNA, and other biomolecules.

On the other hand, antioxidant enzyme play an important role in defending the cells against free radicals mediated oxidative damage. However, the relationship between aging and antioxidant enzyme systems is still a controversial and unresolved subject (Ji et al., 1990). Two factors may interact and confound the search for a consensus. First, aging is associated to a decline in protein synthesis and cell proliferation, thereby affecting the turnover of antioxidant enzymes (Ji et al., 1988). Second, age-related oxidative stress may cause adaptational changes in the antioxidant enzyme status within the cell (Lawler et al., 1993). Thus, a systematic approach to study all antioxidant enzymes in various cell compartments may be required to provide better insight into the problem.

In the aging process, prooxidant status is involved due to increased oxygen free radical generation and decreased antioxidant defenses. In the same way, it is widely accepted that free radical production is increased during strenuous physical exercise and this produces oxidative damage affecting various tissues as skeletal muscle, liver, blood and other tissues (Duthie et al., 1990; Laughlin et al., 1990). However, exercise training may induce antioxidant enzymatic defenses (Jenkins, 1988). Little and controversial information exists to explain the relationship between oxidative damage and antioxidant capacity, produced by exercise, both in young and old animals (Hammeren et al., 1992). Ji (1993) explains how the antioxidant enzymatic mechanisms showed a general decline in liver and an uniform increase in skeletal muscle at older age. Ji and Fu (1992) report a significant increase in all enzymes after exhaustion and it seems that, during exercise bouts, superoxide dismutase (SOD) undergoes the highest rise, according to Lawler et al. (1994).

It is also widely acepted now that free radical generation is enhanced during strenous exercise. This undoubtedly may cause alterations in cellular antioxidant status, both acutely and in the form of chronic adaptation. In the last decade evidence has been accumulated showing that antioxidant enzyme adaptation is one of the fundamental changes of skeletal muscle in response to exercise training, much the same as mitochondrial oxidative enzyme adaptations (Jenkins, 1988; Sjodin et al., 1990). However, the significance of these findings has not been fully appreciated by exercise physiologists.

These experiments tested the hypothesis that the type, duration and intensity of exercise affect biomarkers of free radicals and antioxidant activity, and the modification with age.

2. Materials and methods

2.1. Animals

We employed 104 male Wistar rats; 43 animals were young rats (3–5 months) and 61 old rats (24–27 months). They were divided into seven groups: rest young (CY; $n = 16$), exhausted young (EY; $n = 16$), short-training young (STY; $n = 11$), rest old (CO; $n = 24$), exhausted old (EO; $n = 15$), short-training old (STO; $n = 12$) and long-training old (LTO; $n = 10$). The animals were maintained under conventional conditions and standard diet ad libitum in room temperature 22–28°C on a 12:12 h light–dark cycle.

The animals were obtained from the Central Service of Experimental Animal Department of the University of Cadiz, and all the experiments were approved by the University Committee for Use of Animals in Research and followed guidlines established by the European Regulation on animal treatment submitted to experiment.

2.2. Exercise protocols

The different types of exercise were performed in a treadmill, with different speed and slope. The group of the rest rats were considered as the control groups. These animals were sedentary and were sacrificed without pre-exercise by decapitation.

The group of the exhausted rats (EY and EO) were in rest until they were extenuated before their sacrifice. The protocol of extenuation was the following: the exercise started at 10 m min^{-1}, 0% grade, followed by gradual increases of treadmill speed and grade every 5 min up to 30 m min^{-1}, 15% grade. Average run time to exhaustion for old rats (EO) was about 27 min. The young rats (EY) time was about 55 min.

We also studied the training exercise. We established the protocols for short and long-training. All animals were extenuated before decapitation, with a similar protocol for extenuation without training. The group of short-training rats was accustomed to running on a treadmill in a 4-week period during which intensity of exercise was increased gradually to 20 m min^{-1}, 15% grade for 1 h each day, 5 days per week. This intensity was maintained for another 8 weeks. The total time of training was 3 months. The animals were extenuated before their sacrificie with the same protocol of the other group. Average run time to exhaustion was about 40 min for the old rats (STO), and 80 min for the young rats (STY).

The protocol for long-training rats was as follows: the animals started this protocol as 1 year olds. The group of long-training rats (LTO) was exercised with the same protocol as the group of short-training, but the total time of training was

1 year. The rats were extenuated and sacrificed when they were 27 months old. The protocol of extenuation was the following: the exercise started at 10 m min^{-1}, 0% grade, followed by gradual increases of treadmill speed and grade every 5 min up to 30 m min^{-1}, 15% grade. Average run time to exhaustion was about 55 min (LTO).

Animals were considered exhausted when serum lactate was more than 6.1 mM. This compound was determined according to the Wahlefeld method (Wahlefeld, 1988).

2.3. Tissue preparation

All rats were killed by decapitation inmediatly after treadmill running. Control groups were treated similarly. The time of the day when rats were killed was consistent so that possible diurnal effects of the enzymes were eliminated.

After the rats were decapited and exanguinated, the abdominal cavity was quickly opened and the liver was excised. The skeletal muscle soleus was then dissected. All tissues were frozen in liquid N_2 as soon as possible, and kept under -80°C until analysis.

2.4. Assay methods

For analysis, a section of frozen liver or muscle tissue was tawed in a medium containing sodium phosphate buffer 200 mM (pH 7.8) and homogenized at 4°C inmediatly prior to assays.

The thiobarbituric acid-reactive substances (TBARS), as lipid peroxidation marker, were assayed in homogenized tissues according to the Buege and Aust method (Buege and Aust, 1978). The results were expressed as μmol/g tissue.

The superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed according to Elstern's method (Elstern et al., 1988) in supernatant. One unit enzyme is determined by the SOD units per gram of proteins in one mililiter of supernatant which are able to reduce to half the value of maximal absorbance. The values were expressed as U SOD/ g protein.

The protein concentration was determined using the Lowry et al. method, with bovine serum albumin as the standard (Lowry et al., 1951).

2.5. Statistical analysis

Means and standard desviation (S.D.) were determined for all data, and analysis of variance was used (ANOVA). Significance levels were set at $P < 0.05$.

3. Results

The TBARS levels in the liver and soleus muscle are shown in Table 1. Old rest rats (CO) presented double the TBARS levels in liver with respect to young rest

group (CY), and this difference was statistically significative. After extenuation, the TBARS levels in liver were higher in old rats (EO) than young rats (EY) with significant difference.

If we compare young and old rats, TBARS levels were not modified in the liver when the rats did short-training exercise (STY vs STO).

In soleus muscle, the young control rats (CY) had similar TBARS levels to the old control group (CO). The young exhausted rats (EY) presented lower TBARS levels than the old exhausted rats (EO), and this difference was significant. Also, short-training young rats (STY) showed lower TBARS levels than short-training old rats (STO), and the difference was significant.

For the different groups of young rats, STY had the highest TBARS values in liver tissue (STY vs CY and STY vs EY). No significant difference was seen between CY and EY. As shown in Table 1, lipid peroxidation level in soleus muscle decreased in the short-training group, and the differences were significant between this group and exhausted young rats (STY vs EY).

With respect to the old groups, LTO presented the TBARS levels higher than other old rats, in both liver and soleus muscle (LTO vs CO, vs EO and vs STO). TBARS levels in liver were similar on comparing the control group of old rats (CO) with exhausted old rats (EO) and short-training old rats (STO). In soleus muscle, the significant difference was shown between control and exhausted old rats (CO vs EO) and between (EO vs STO).

The results of SOD activity are shown in Table 2. SOD activity was higher in rest old rats (CO) than rest young (CY), in both liver and soleus muscle, and these

Table 1
TBARS levels in the liver and soleus muscle in control young (CY), control old (CO), exhausted young (EY), exhausted old (EO), short-training young (STY), short-training old (STO) and long-training old (LTO) groups

Groups	TBARS in liver (μmol/g tissue)	TBARS in soleus muscle (μmol/g tissue)
Young rats (3–5 months)		
CY ($n=16$)	33.68 ± 9.46	23.94 ± 5.27
EY ($n=16$)	35.03 ± 16.40	33.14 ± 10.94[a]
STY ($n=11$)	46.74 ± 15.36[a]	18.6 ± 8.99
Old rats (24–27 months)		
CO ($n=24$)	66.37 ± 18.79**	23.78 ± 5.44
EO ($n=15$)	54.47 ± 21.32**	45.91 ± 14.61**[b]
STO ($n=12$)	57.03 ± 6.45	27.33 ± 5.59*
LTO ($n=10$)	101.61 ± 10[c]	74.08 ± 4.3[c]

Values are mean $\pm$ S.D.
[a] $P<0.01$ STY versus CY and STY versus EY; [b] $P<0.05$ EO versus CO and EO versus STO; [c] $P<0.01$ LTO versus CO, LTO versus EO and LTO versus STO.
** $P<0.01$ CY versus CO and EY versus EO; * $P<0.05$ STY versus STO.

Table 2
SOD activity in the liver and soleus muscle in control young (CY), control old (CO), exhausted young (EY), exhausted old (EO), short-training young (STY), short-training old (STO) and long-training old (LTO) groups

Groups	SOD activity in liver (U SOD/g prot)	SOD activity in soleus muscle (U SOD/g prot)
Young rats (3–5 months)		
CY ($n=16$)	572.7 ± 370.4	138.2 ± 36.4^{c}
EY ($n=16$)	3511.9 ± 1199.2^{a}	248.9 ± 62.1
STY ($n=11$)	4262.9 ± 1207.6^{a}	147.5 ± 76.4^{b}
Old rats (24–27 months)		
CO ($n=24$)	2560.7 ± 841.7**	335.4 ± 110.2**
EO ($n=15$)	2151.0 ± 752.6**	153.5 ± 73.3**^{e}
STO ($n=12$)	1468.7 ± 622.6**^{d}	62.2 ± 24.8^{d}
LTO ($n=10$)	135.1 ± 20.2^{f}	547.7 ± 38.2^{f}

Values are mean $\pm$ S.D.
[a] $P<0.01$ CY versus EY and CY versus STY; [b] $P<0.01$ EY versus STY; [c] $P<0.05$ CY versus EY; [d] $P<0.01$ CO versus STO; [e] $P<0.05$ EO versus STO; [f] $P<0.01$ LTO versus CO, versus EO and versus STO.
** $P<0.01$ CY versus CO, EY versus EO and STY versus STO.

differences were statistically significant. In exhausted old rats, SOD activity decreased with respect to exhausted young rats in both liver and soleus muscle (EY vs EO). The short-training young group (STY) presented higher values than the short-training old group (STO), but the differences were only significant in liver tissue.

On comparing the control group of young rats (CY) with exhausted young rats (EY), the SOD values were higher in the exhausted than in the control group of young rats. The short-training for young rats had bigger values in liver than the other young groups, but the significant differences were with the control young group (CY vs STY). In soleus muscle, the biggest SOD activity was in the exhausted young rats (EY), showing a significant difference between short-training and exhausted young groups (EY vs STY). The exhausted young group (EY) had a significant difference with respect to the control young group (CY).

The rest old animals (CO) presented the highest SOD activity in liver. Exhausted old rats (EO) presented similar values. SOD activity decreased in short-training old rats (STO) with respect to rest old group (CO vs STO). SOD activity was the lowest in long-training old rats (LTO) in liver, when we compared it with respect to the other old groups. In soleus muscle, the short-training group (STO) had the lowest SOD activity The differences were significant in both exhausted old rats (STO vs EO) and rest old rats (STO vs CO). However, SOD activity in soleus muscle in long-training old rats (LTO) was higher than the other groups.

4. Discussion

4.1. Age-related changes

Lipid peroxidation secondary to free radicals is considered to be the best indication of the amount of molecular lesions produced by ROS (Cutler, 1986; Ji et al., 1990; Kretzschmar et al., 1990; Viani et al., 1991). The TBARS levels in liver tissue of old control rats showed a 2-fold increase. This data is in disagreement with Barja de Quiroga et al. (1992) where they refer to TBARS levels greater in young rats, however, we are in agreement with Liu and Mori (1993).

Literature concerning hepatic antioxidant enzyme response to aging is confusing. Liver has one of the highest antioxidant enzyme activities in the body and is involved in major detoxification functions. The difficulty in studying aged liver is subject to numerous influences such as metabolic state and nutritional status. In our experiences, liver SOD activity showed well grouped data and reveals a significant increase in old control rats versus young control rats. These data are in agreement with the results reported by Yen et al. (1994) and Ji et al. (1990).

These data can be interpreted as a purely aging-related effect. The possible contribution of peroxidation to the aging process has been confirmed by the fact that free radical-induced peroxidation promotes strong modifications of membrane fluidity, lipid composition, and Na^+/K^+-ATPase activity just as aging does (Viani et al., 1991). The SOD activity increase may be the expression of a self-protective mechanism from superoxide-generated radicals operating in aging (Sawada and Carlson, 1987).

In muscle tissue age-related changes in TBARS levels were not significant. Age-related changes in muscular SOD activity, a well defined parameter in young rats, showed in old control rats not only an increase in statistical mean but also a very large standard deviation. So aging has a more variable effect on muscular SOD activity levels without effects on normal limits of lipid peroxidation indexes.

The generation of oxygen radicals is directly related to the metabolic rate of the cell. Liver and skeletal muscle tissues have different metabolic rate and protein turnover, thus the influence of aging on the antioxidant enzymes in these two tissues is to be expected different (Harman, 1986). Our results are in agreement with this proposal.

4.2. Effects of maximal performance

Exhaustive exercise has metabolic correlates well defined in several studies but secondary effects of sustained metabolic stress has seldom been reported (Rogers and Evans, 1993; Cartee, 1994). Strenuous physical exercise has been shown to impose an oxidative stress on the body due to oxygen free radical generation including an increase of lipid peroxidation in various tissues (Ji and Fu, 1992; Leaf et al., 1997). Young exhausted rats showed a 7-fold increase in liver SOD activity levels, when compared to the control young group, and they also showed a lesser significative increase in muscle SOD activity. This ability of liver tissue to generate

a protective SOD response to exercise metabolic stress is seldom shown by the muscular tissue. Our results are in agreement with the research of Ji and Fu (1992). TBARS levels as liver as muscle are not modified in young exhausted rats with respect to control group. Many investigators have reported increased lipoperoxidation after strenuous exercise (Davies et al., 1982; Duthie et al., 1990; Ji et al., 1990) but there is some controversy in the literature. Ji and Fu (1992) showed significant lipid peroxidation in liver mitochondria but not in whole liver homogenate, and this agrees with our results.

Thus, young exhausted rats are capable of inducing antioxidant enzymes under oxidative stress (Fielding and Meydani, 1997). Storz et al. (1990) showed that oxidative stress could upregulate a series of antioxidant enzymes. If these results found in the prokaryotes could be extrapolated to the eukaryotes and mammalian cells, the exercise-related increases in skeletal muscle and hepatic antioxidant enzyme activities may be explained by a similar mechanism that reactive oxygen species serve as inducers.

Recently, Oh-ishi et al. (1997) in a research about the effects of endurance training on SOD activity, and content and mRNA expression in rat muscle, suggest that adequate endurance training increases both the activity and content of Mn-SOD and that untrained rats are rather susceptible to oxidative stress during physical exercise. It thus appears that Mn-SOD provides a reliable index of physical training.

In the old exhausted rats only the TBARS levels in muscle change, with respect to the control group. Our results suggest the lack of capacity to adapt in old animals, since as opposed to what occured in the young rats, an increase in antioxidant activity is not induced and a greater muscular peroxidation appears. This increase in lipoperoxidation could be thoght to contribute to the damage shown by other factors in the skeletal muscle of old individuals after exhaustive exercise, with modification to the microtubules, sarcoplasmic reticulum, and mitochondria, and also a reduction in various enzyme activities like the SOD (Cartee, 1994). And these would agree with the theory of the age threshold in exercise suggested by Reznick et al. (1992).

4.3. Effects of short-training on exhaustion

Young short-training rats showed a significant increase in TBARS levels in liver with respect to control and exhausted young rats. The SOD activity in liver is increased with respect to the young control group. We can not compare our results because we have not been able to find equivalent models in the consulted bibliography. This increase in lipoperoxidation in this group as opposed to the exhausted rats could be explain by the greater endurance time due to the fact that the animals have been previously trained. This would lead to the production of lipoperoxidation in spite of the increase of antioxidant SOD capacity.

In skeletal muscle, TBARS levels were not modified in young short-training rats, with respect to the control group, but the levels were lower than in the young exhausted rats. The SOD activity was similar to the control group, but lower than

in young exhausted rats. There is a lack of data on the effects of training on lipoperoxidation. Because training involves a chronic intermittent increased exposure to oxygen it is of interest to know whether the SOD activity of the trained subject is able to adapt. Alessio and Goldfarb (1988) and Alessio (1993) carried out a study in rats to determine whether endurance training would influence the production of lipoperoxides products at rest and after an acute exercise run. The training programme increased oxidative capacity in skeletal muscle. After exercise, the endurance trained group did not show an increase in lipoperoxidation. The activity of SOD was unaffected by chronic exercise. Our results agree with these authors, and disagree with Higuchi et al. (1985), Jenkins (1988), Criswell et al. (1993). Our data suggest that training can result in a reduction of TBARS levels. It might be suggested that these disparate results are related to differences in training intensity, duration, or mode.

Old short-training rats showed a significant decrease of SOD activity levels, in liver and muscle tissue, but TBARS levels were not modified in these tissues, when we compared them with the old control group. Aging per se already caused a significant elevation of basal antioxidant enzyme status, which might raise the threshold of training adaptation. Moreover, we employed a rather conservative training regime for the aged rats compared with the other authors.

Muscular tissue in short-training rats has a very similar response to exhaustion protocol in old and young animals. In both groups a significative decrease in lipoperoxidation effects of extenuated exercise was showed and a very similar proportion of reduced SOD activity was evident. In old rats, the short-training protocol prevents the TBARS elevation typical of this age group, and, in some sense, they respond as if they were younger. In young rats, the training protocol showed also the same reduction in lipoperoxidation produced by exercise, so the effect of training on extenuation is the same for both ages, although its effects seem to be different in older rats because, in this case, it prevents the otherwise inevitable effects of strong metabolic stress in old muscle. The concomitant reduction of SOD activities after previous training is not well explained and perhaps other free radicals scavenger systems play some role. In either case training seems to protect both age groups against muscular injury by oxidative stress, a most marked effect in old rats.

4.4. Effects of long-training on exhaustion

Old rats subjected to a long-training protocol had been nearly half of their lives under a chronic physical exercise, and they showed a very different response to maximal performance. It is not possible to compare our results with other, as there exists no study of training of old animals with such a long duration in the consulted bibliography.

In the liver, lipoperoxidation effects of extenuation are very marked and they showed a 2-fold increase in TBARS levels as compared with previously short-training rats of the same age. Chronic exercise protocol is much more lesive for hepatic tissue metabolic response because of a very prolonged maximal performance. The

same lipoperoxidation effects were noticed in muscular tissue where a 2-fold increase of TBARS levels was evident also. Sustained training could result in a more prolonged endurance and a substantial worsening of secondary molecular lessions of exercise. An alternative, or perhaps complementary explanation, could be the presumed aging effects of prolonged exercise.

More difficult is to explain the very low levels of hepatic SOD activity after extenuation protocol in long-training rats, because they did not show a large deviation and they obtain even lower values than normal resting young rats. Muscular response of old rats after a very long-training is more surprising because it showed a very large elevation of SOD activity that does not seem to protect muscle cells from free radical metabolic damage. This unexplained elevation of SOD is exactly opposed to the expected effect of training on exhaustion in muscular cells of old rats. The muscle of long-training rats responds to exhaustion protocol in an opposite way to old short-training animals, i.e. with a marked elevation of TBARS and a very intense activity of SOD. Perhaps long term changes in muscular fibre composition or metabolic adaptation to chronic exercise in these rats deserves some explanation.

In summary, these findings suggest that free radicals play a role in aging and that the different type, intensity and duration of exercise modify the lipid peroxidation level and antioxidant enzyme activity.

Acknowledgements

This research project was supported in part by the grant CTS 194 from Plan Andaluz de Investigación PAI (Junta de Andalucía).

References

Alessio, H.M., 1993. Exercise-induced oxidative stress. Med. Sci. Sports Exerc. 25, 218–224.

Alessio, H.M., Goldfarb, A.H., 1988. Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. J. Appl. Physiol. 64, 1333–1336.

Barja de Quiroga, G., López Torres, M., Pérez Campos, R., 1992. Relationship between antioxidants, lipid peroxidation and aging. In: Emerit, I., Chance, B. (Eds.), Free Radicals and Aging. Birkhauser Verlag, Basel, Switzerland, pp. 109–123.

Buege, S.D., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 52, 302–310.

Cartee, G.D., 1994. Aging skeletal muscle: response to exercise. Exerc. Sports Sci. Rev. 22, 91–120.

Criswell, D., Powers, S., Dodd, S., Lawler, J., Edwards, W., Renshler, K., Grinton, S., 1993. High intensity training-induced changes in skeletal muscle antioxidant enzyme activity. Med. Sci. Sports Exerc. 25, 1135–1140.

Cutler, R.G., 1986. Aging and oxygen radicals. In: Taylor, A.E., Matalon, S., Ward, P.A. (Eds.), Physiology of Oxygen Radicals. American Physiology Society, Bethseda, MD, pp. 251–284.

Davies, K.J.A., Qitanilha, A.T., Brooks, G.A., Packer, L., 1982. Free radicals and tissue damage produced by exercise. Biochem. Biophys. Res. Commun. 107, 1198–1205.

Duthie, G.G., Robertson, J.D., Maughan, R.J., Morrice, P.C., 1990. Blood antioxidant status and erythrocyte lipid peroxidation following distance running. Arch. Biochem. Biophys. 282, 78–83.

Elstern, E.F., Youngman, R.J., Oßwald, W., 1988. Superoxide dismutase. In: Bergmeyer, H.V., Graßl, M. (Eds.), Methods of Enzymatic Analysis. Wenheim, Germany, pp. 293–302.

Fielding, R.A., Meydani, M., 1997. Exercise, free radical generation, and aging. Aging (Milan) 9, 12–18.

Gershman, R., 1954. Oxygen poisoning and X-irradiation: a mechanism in common. Science 119, 623–629.

Hammeren, J., Powers, S., Lawler, J., Criswell, D., Martin, D., Lowenthal, D., Pollocok, M., 1992. Exercise training-induced alterations in skeletal muscle oxidative and antioxidant enzyme activity in senescent rats. Int. J. Sports Med. 13, 412–416.

Harman, D., 1981. The aging process. Proc. Natl. Acad. Sci. USA 78, 7124–7128.

Harman, D., 1986. Free radical theory of aging: role of free radicals in the origination and evolution of life, aging, and disease processes. In: Johnson, J., Walford, R., Harman, D., Miquel, J. (Eds.), Biology of Aging. Liss, New York, pp. 3–50.

Higuchi, M., Cartier, L., Chen, M., Holloszy, J.O., 1985. Superoxide dismutase and catalase in skeletal muscle: adaptive response to exercise. J. Gerontol. 40, 281–286.

Jenkins, R.R., 1988. Free radicals chemistry: relationship to exercise. Sport Med. 5, 156–170.

Ji, L.L., 1993. Antioxidant enzyme response to exercise and aging. Med. Sci. Sports Exerc. 25, 225–231.

Ji, L.L., Stratman, F.W., Lardy, H.A., 1988. Enzymatic down regulation with exercise in rat skeletal muscle. Arch. Biochem. Byophys. 263, 137–149.

Ji, L.L., Dillon, D., Wu, E., 1990. Alteration of antioxidant enzymes with aging in rat skeletal muscle and liver. Am. J. Physiol. 258, R918–R923.

Ji, L.L., Fu, R., 1992. Responses of glutathione system and antioxidant enzymes to exhaustive exercise and hydroperoxide. J. Appl. Physiol. 72, 549–554.

Kretzschmar, D., Müller, D., Hubscher, J., Martin, E., Klinger, W., 1990. Influences of aging, training and acute physical exercise on plasma glutathione and lipid peroxidation in man. Int. J. Sports Med. 12, 218–222.

Laughlin, M.H., Simpson, T., Sexton, W.L., Brown, O.R., Smith, J.K., Korthuis, R.J., 1990. Skeletal muscle oxidative capacity, antioxidant enzymes, and exercise training. J. Appl. Physiol. 68, 2337–2343.

Lawler, J.M., Powers, S.K., Visser, T., van Dijk, H., Kordus, M.J., Ji, L.L., 1993. Acute exercise and skeletal muscle antioxidant and metabolic enzymes: effects of fiber type and age. Am. J. Physiol. 265, R1344–R1350.

Lawler, J.M., Powers, S.K., van Dijk, H., Visser, T., Kordus, M.J., Ji, L.L., 1994. Metabolic and antioxidant enzyme activities in the diaphragm: effects of acute exercise. Respiration Physiol. 96, 139–149.

Leaf, D.A., Kleinman, M.T., Hamilton, M., Barstow, T.J., 1997. The effect of exercise intensity on lipid peroxidation. Med. Sci. Sport Exerc. 29, 1036–1039.

Liu, J., Mori, A., 1993. Age-associated changes in superoxide dismutase activity, thiobarbituric acid reactivity and reduced glutathione level in the brain and liver in senescence accelerated mice (SMA): a comparasion with ddY mice. Mech. Ageing Dev. 71, 23–30.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Miquel, J., 1989. Historical introduction to free radical and antioxidant biomedical research. In: Miquel, J., Quintanilha, A.T., Weber, H. (Eds.), Handbook of Free Radicals and Antioxidants in Biomedicine. CRC Press, Boca Raton, FL, pp. 3–13.

Nohl, H., 1993. Involvement of free radicals in ageing: a consequence or cause of senescence. Free Rad. Med. 49, 653–667.

Oh-ishi, S., Kizaki, T., Nagasawa, J., Izawa, T., Komabayashi, T., Nagata, N., Suzuki, K., Taniguchi, N., Ohno, H., 1997. Effects of endurance training on superoxide dismutase activity, content and mRNA expression in rat muscle. Clin. Exp. Pharmacol. Physiol. 24, 326–332.

Reznick, A.Z., Witt, E.H., Silbermann M., Packer, L., 1992. The threshold of age in exercise and antioxidants action. In: Emerit, I., Chance, B. (Eds.), Free Radicals and Aging. Birkhauser Verlag, Basel, Switzerland, pp. 423–427.

Rogers, M.A., Evans, W.J., 1993. Changes in skeletal muscle with aging: effects of training. Exerc. Sport Sci. Rev. 21, 65–102.

Sawada, M., Carlson, J.C., 1987. Changes in superoxide radical and lipid peroxide formation in the brain, heart and liver during the life time of the rat. Mech. Ageing Dev. 41, 125–137.

Sjodin, B., Westing, Y.H., Apple, F.S., 1990. Biochemical mechanisms for oxygen free radical formation during exercise. Sports Med. 10, 236–254.

Storz, G., Tartaglia, L.A., Ames, B.N., 1990. Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation. Science 248, 189–194.

Viani, P., Cervato, G., Fiorilli, A., Cestaro, B., 1991. Age-related differences in synaptosomal peroxidative damage and membrane properties. J. Neurochem. 56, 253–258.

Wahlefeld, A.W., 1988. UV-method with L-lactate and NAD. In: Bergmeyer, H.V., Graßl, M. (Eds.), Methods of Enzymatic Analysis. Wenheim, Germany, pp. 126–133.

Yen, T.Ch., King, K.L., Lee, H.Ch., Yeh, S.H., Wei, Y.H., 1994. Age-dependent increase of mitochondrial DNA deletions together with lipid peroxides and superoxide dismutase in human liver mitochondria. Free Rad. Biol. Med. 16, 207–214.