RESEARCH PAPER Asian Journal of Bio Science, Vol. 4 Issue 1 : 73-78 (April to September, 2009)

Modulation of brain antioxidant enzymes in response to treadmill exercise training with reference to aging

N. SOMASEKHARA REDDY[1], K.R. SHANMUGAM[1], K. MUNIKRISHANA REDDY[1], K. MALLIKARJUNA[2] AND K. SATHYAVELU REDDY

[1]Division of Molecular Biology and Exercise Physiology, Department of Zoology, Sri Venkateswara University, TIRUPATI (A.P.) INDIA

[2]Exercise Biochemistry Lab, Taipei Physical Education College, Taipei City, TAIWAN

(Accepted : February, 2009)

Aging is an universal, intrinsic, progressive, irreversible and deleterious phenomenon. As age advances, several enzymes show an increase, while some decrease and some do not show any change in their activities. These specific alterations in the enzymes must have inflicted a great impact on the process of aging. This process is thought to be related to increase in free radicals generation and oxidative stress. In the present study an attempt was made to investigate the impact of exercise training on aging by selecting two age groups (3months as "young" and 18 months as "old"). Standard protocols were followed for the assay of selective antioxidant enzymes with rat as an animal model. Superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), and glutathione (GSH) levels were decreased with the advancement of age. The decline in antioxidant enzymes with age could be due to the disturbances of intracellular prooxidant-antioxiadnt homeostasis, which leads to cell death. However, the activities of SOD, CAT, GR and GPx and levels of GSH were augmented with exercise training in both age groups of rats. The elevation of SOD observed might be aimed at the removal of superoxide anion radicals generated due to aging process. Where as, the increase in GPx activity indicate its active participation in the decomposition of hydrogen peroxide and organic hydroperoxides generated via dismutation of super oxide anion by SOD. It was concluded that 2 months treadmill exercise is beneficial in order to avoid detrimental effects of free radical generation during the process of aging.

Key words : Aging, Exercise, Antioxidant enzymes, Rats.

INTRODUCTION

It is well known fact that every organism, which has come into existence, has to face death sooner or later. However, scientists with the hallmark of enquiring nature could not stop, wondering about this inevitable phenomenon of aging and death (Subba Rao, 1990). Aging is an inevitable biological process and characterized by a general decline in physiological functions. In 1956, Harman suggested that free radicals produced during aerobic respiration cause cumulative oxidative damage, resulting in aging and death. In recent years, convincing (believable) data have been accumulated to suggest that mitochondria act like a timer that ticks all the way through the aging process (Wei and Lee, 2002). Age related damage from oxidative stress could be elicited through increased ROS, decreased antioxidant enzyme activities or a combination of both. Free radical oxidation of proteins also increases with age (Stadtman, 1992; Haramaki and Packer, 1994). Several investigations have shown an increase in production of ROS in various tissues of older animals (Davidge *et al.*, 1996).

Exercise is an elixir of life. Many studies show that aerobic activity continuing at least 20 minutes is necessary for obtaining psychological well being. Either high or low intensity exercise lead to positive changes in mood (Kennedy and Newton, 1997). Exercise can be accomplished only through the series of complex interactions within the body involving all the body systems. Exercise is a general term that refers to many types of physical exertions that may vary in its duration, intensity and type. The benefits of regular physical exercise include reduce risk of cardiovascular disease, cancer, osteoporosis and diabetes (McCarteer, 2000). It is well known that regular performed moderate exercise has many beneficial effects, whereas, acute exercise can produce significant damage in the tissue (McCutcheon *et al.*, 1992). Nevertheless, an age related decrease in the expression of several genes involved in mitochondrial bioenergetics and mitochondrial biogenesis occurs during aging.

To cope with oxidative stress, like other organs of the body, brain also well equipped with highly sophisticated and complex defense mechanism known as "antioxidant defense system". This defense system includes antioxidant enzymes such as, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-

Px), glutathione reductase (GR) and non-enzymatic antioxidants like glutathione (GSH) (Mallikarjuna, 2005). Both aging and exercise have been found to produce changes in antioxidant defense system. It is logical, therefore, that differences in the brain tissue response to exercise, when comparing young with older individuals may exist. So far, less much work has been focused on the interaction of exercise training and aging, but with reference to brain antioxidant system and also aging no work has been carried out. Hence, in the present study an attempt was made to know the effect of exercise on aging process, with reference to antioxidant enzymes.

MATERIALS AND METHODS

Animal care and treatment:

Pathogen free, Wistar strain male albino rats (n = 24) of two different age groups *i.e.*, young (3 months old) weighing 170±10gm and moderately aged/old (18 months old) weighing 240±10gm were used in the current investigation. [Approved by the Institutional Animal Ethics Committee [(Regd.No. 438/01/a/CPCSEA/dt 17.7.2001) in its resolution number 9/IAEC/ SVU/ Zool, dated 4.3.2002]. Such division of the age groups was assumed according to the studies of Cao and Cutler (1995). The rats were housed in clean polypropylene cages, 6 rats per cage and maintained under temperature controlled room (27 ± 2^0C) with a photoperiod of 12 hrs light and 12hrs dark cycle. The rats were fed with a standard rat pellet diet and water *ad libitum*.

Chemicals:

All the chemicals used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fischer (Pitrsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

The animals (total n = 24) of both the age groups *i.e.*, young (n = 12) and moderately aged/old (n = 12) were divided in to two groups of six each.

Group I – Normal control (SC) :

Six rats were put on a six-channel, motor driven treadmill for 5 days / week for a period of 2 months and given 23 m / min exercise for 5 mm for equivalent handling, and also the rats received normal (0.9%) saline orally via orogastric tube.

Group II - Exercise training (Ex) :

Six rats were given exercise training on a six-channel, motor driven treadmill for 5 days / week for a period of 2 months at the running speed of 23m / 30 min at a constant gradient of 7.5%. Treadmill was custom-built at University Scientific Instrumentation Center (USIC) - Sri Venkateswara University Campus.

After completion of 2 months month treatment the animals were sacrificed by cervical dislocation and the brain tissue was excised at 4^0C. The tissue was washed with ice-cold saline, immersed in liquid nitrogen and immediately stored in deep freezer at -80°C for further biochemical analysis. The selected antioxidant enzymes such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione reductase (GR), activities and Glutathione (GSH), levels were monitored by the methods of Misra and Fridovich (1972), Aebi (1984), Flohe and Gunzler, (1984), Carlberg and Mannervik (1985), Theodorus *et al.* (1981), respectively. Where as total proteins were estimated by the method of Lowry *et al.* (1951). The experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee (Regd. No. 438/01/a/CPCSEA/ dt.17.07.2001) in its resolution number 9/IAEC/SVU/2001/dt. 4.03.2002).

Statistical analysis:

The data has been analyzed by using SPSS (Version 13.5; SPSS Inc., Chicago, IL, USA) and M.S. Office, Excel Software for the significance of the main effects (factors), and treatments along with their interactions. The data has been compared using one way ANOVA with Dunnett's multiple comparison test and differences were considered significant at $p < 0.001$.

RESULTS AND DISCUSSION

In the present study, the SOD activity in the brain tissue was decreased in old age rats compared to young rats. Endurance exercise training significantly ($p<0.001$) elevates the SOD activity in the brain of both young (42%) and old (56%) rats.

Table 1 depicts that as age advances CAT activity was decreased in brain tissue. But with exercise training a significant elevation of CAT activity was noticed in both age (Young 75%, Old 82%) groups of brain.

GPx activity was greater in young animals compared to old animals in brain tissue. It is reported that significant elevation ($p<0.001$) in GPx activity was due to exercise. The per cent elevation of GPx was more (33%) in young rats. The same trend was also observed in moderately aged/old exercised rats (21%).

As result of age the activity of GR was decreased in brain compared to their young rats. In the present study

a significant ($p<0.001$) augmentation of GR activity in brain (39%) in young rats, and (72%) in moderately old rats was observed with exercise training when compared to their respective normal controls.

In this investigation the concentrations of GSH observed as a foot marker of oxidative stress, was dropped with advancement of age and elevated with exercise training. Exercise induced elevation of GSH content even in old (26%) and Young (39%) animals promotes the antioxidant status brain.

The purpose of this study was to examine the beneficial role of exercise in two different age groups of male albino rats. To examine the effect endurance exercise training in different age groups (Young and Old) with reference to antioxidant defense system. The rat brain was studied in this investigation. The brain was chosen for the study because they are actively recruited during treadmill exercise and represent the major coordinating organ of the body. The major findings of this study were as follows.

Cells are equipped with a host of enzymes that are directly or indirectly involved in the antioxidant defense against ROS. Enzymes that provide primary defenses include SOD, CAT, GPx, GR and GSH.(Yu, 1994) The catalytic mechanism and regulation of various antioxidant enzymes have been reviewed extensively by previous authors (Halliwell and Gutteridge, 1989; Jenkins, 1988) Thus the present study focuses on the effects of exercise on the antioxidant enzymes in the brain tissue of two different age groups.

The purpose of this study was to examine the beneficial role of exercise in two different age groups of male albino rats. To examine the effect endurance exercise training in different age groups (Young and Old) with reference to antioxidant defense system. We studied the rat brain in this investigation. The brain was chosen for the study because they are actively recruited during treadmill exercise and represent the major coordinating organ of the body. The major findings of this study were as follows.

Among various antioxidative mechanisms in the body SOD is thought to be one of the major enzymes which protects against tissue damage caused by the potentially cytotoxic reactivities of radicals (Carrillo *et al.*, 1992). In this study, age related decrease in SOD activity was observed in brain tissue, indicates either reduced synthesis of enzyme or elevated degradation or inactivation of enzyme as age advances. It is, therefore, possible that the decreases in SOD activities with age may be closely related to the aging of the organism. Sawada and Carlson (1987) reported that superoxide radical formation increases with age, therefore, a decreased protection against toxic radical may have serious consequences for aging tissues. However, with exercise training SOD activity was increased in both age groups. This elevation was more pronounced in old age rats than young age. Previously several authors reported that an acute bout of exercise has been shown to increase in SOD activity in various tissues including brain. (Ji, 1993; Powers *et al.*, 2004). This elevation in SOD activity was proposed to

Table 1 : Changes in SOD, CAT, GPx, and GR, activities and GSH level in the brain tissue of Normal Control (NC), Exercised (ExT) (23 m / min, 30 min/day, 5 days/week for 2 months), rats of two age groups. *i,e.* Young (Y), and Old (O)

Parameters	Normal control (NC)		Exercise training (ExT)	
	Young	Old	Young	Old
SOD[a]	6.293±0.090	3.879±0.143	8.926±0.191* (+41.840)	6.066±0.329* (+56.380)
CAT[b]	0.083±0.004	0.045±0.004	0.145±0.007* (+74.698)	0.082±0.006* (+82.222)
GPx[c]	0.393±0.056	0.182±0.027	0.524±0.044* (+33.333)	0.221±0.011* (+21.428)
GR[d]	0.114±0.006	0.050±0.004	0.158±0.005* (+38.596)	0.086±0.005* (+72.000)
GSH[e]	25.291±0.898	12.478±0.322	35.261±0.764* (+39.421)	15.761±0.660* (+26.310)

All the values are mean, ± SD of six individual observations,
[a] values are expressed in units of superoxide anion reduced/mg protein/minute,
[b] values are expressed in μ moles of H_2O_2 degraded/mg protein/minute,
[c,d] values are expressed in μ moles of NADPH oxidized/mg protein/minute,
[e] values are expressed in μmoles of glutathione/gram wet weight of the tissue
values in the parenthesis denote per cent change over normal control,
* indicate significance of value at $P< 0.001$ with normal control.

result from increased superoxide anion radicals production during exercise (Leeuenbergh *et al.*, 1994). The increased generation of free radicals would have triggered the SOD enzyme and hence this enzyme activity was elevated during exercise.

In the present investigation, decrease in the activity of CAT was observed in the brain of old aged rats when compared with young rats. The lower activity of CAT may be due to lower levels of SOD or may be due to inactivation of catalase owing to excess production of free radicals, especially hydrogen peroxide. The decreased CAT activity in the present study may be because of high reactive oxygen metabolites production especially $O_2^{\bullet-}$ and H_2O_2 during aging process and cause oxidative stress to the tissue. Evidences suggest that $O_2^{\bullet-}$ it self affect directly the CAT activity (Kono and Fridovich, 1982). Where as with exercise training CAT activity was increased in both age groups. The increased catalase activity indicates its active involvement in the decomposition of hydrogen peroxides with exercise training. The rise in CAT activity indicates major role of this enzyme in organic peroxide detoxification to prevent the secondary effects of peroxides formed during exercise (Indira Sriram and Jhansi Lakshmi, 2001). The combination of SOD and CAT provide an efficient mechanism for removal of free radicals from the cell (Somani *et al.*, l996).

In the current study, we observed, a decrease in the specific activity of GPx in the brain tissue of old rats was observed when compared to young rats. The production of free radicals and other ROS are believed to increase with age in most cells (Lee and Wei, 2007). The age induced increased free radicals especially hydrogen peroxide may be responsible for the low activity of GPx in older rats. Vohra *et al.* (2001) reported the decreased SOD and GPx activities in aged guinea pigs. The results obtained from the present study reveals that endurance exercise training enhanced the brain glutathione peroxidase activity to a significant levels in both age groups of rats. GPx activity increased in brain tissue at a high level indicating an efficient elimination of organic peroxides (Ohkuwa *et al.*, 1997). The increased activity of GPx might account in part for the decline in protein oxidation in exercise trained animals and efficient elimination of organoperoxides (Leeuwenburgh and Heinecke, 2001).

In this study, GR activity was dropped in the brain tissue of older rats. Recently Mallikarjuna *et al.* (2007) and Pushpalatha *et al.* (2007) reported the decreased hepatic and cardiac GR activity with advancement of age. The decrease in enzyme activity also suggests the possible free radical mediated oxidative stress and consequent damage to the brain tissue. Age induced oxygen derived free radicals, which cause the disturbance of pro-oxidants and antioxidant homeostasis in the tissues and leads to decrease all the antioxidant enzymes including GR. Glutathione reductase involved in the detoxification of peroxides. The activity of brain glutathione reductase was significantly augmented with exercise training in both age groups of rats. Young rats showed (39%) elevation and old age rats showed (72%) elevation of GR activity with exercise training. GR activity has been shown to increase in rat brain after acute bout of exercise training along with increased GPx activity (Ji and Fu, 1992; Ji and Leichtweis, 1997).

The results show decrease in the levels of glutathione in the ageing brain. Glutathione is a substrate for the enzyme glutathione peroxidase which metabolizes hydrogen peroxide in living cells. Glutathione is the most abundant intracellular thiol based antioxidant present in milli molar concentration, and plays an important role in maintaining the integrity of cells (Powers *et al.*, 2004). Exercise training represents a significant augmentation of brain GSH levels in both age groups of rats than that of their respective control rats. Previously some studies reported that elevated hepatic GSH (Sen *et al.*, 1993) and myocardial GSH content after treadmill exercise training (Somani *et al.*, 1995). Although a substantial amount of GSH is oxidized to GSSG in the tissues during exercise due to increased ROS production. GSH redox status *i.e.*, GSH : GSSG is not altered significantly because GSSG can be reduced back to GSH by the enzyme glutathione reductase using NADPH as the co-factor (Ji, 1999; Lawler and Demaree, 2001). Furthermore, exercise training upregulates the GR activity, suggests that whatever oxidized GSSG is reduced back to GSH in the same concentration in the brain tissue.

From the above results it is concluded that exercise is beneficial in countering the free radicals generated during aging process. With exercise training the antioxidant enzymes like SOD, CAT, GPx, and GR activities and GSH levels were elevated which shows that these antioxidant enzymes eliminates the free radicals which are produced during aging process. Further studies are needed to know the impact of exercise during aging.

REFERENCES

Aebi, H. (1984). Catalase. *Methods in Enzymology*, **105** : 125-126.

Carlberg, I. and Mannervik, B. (1985). Glutathione reductase. *Methods in Enzymol.*, **106** : 484-490.

Cao, G. and Catler, R.G. (1995). Protein oxidation and aging: II. Difficulties in measuring alkaline protease activity in tissues using the fluorescamine procedure. *Arch. Biochem. Biophys.*, **320** (1), 195-201

Carrillo, M.C., Kanai. S., Sato, Y. and Kitani, K. (1992). Age-related changes in antioxidant enzyme activities are region and organ as well as sex. Selective in the rat. *Mech. Ageing Dev.*, **65**:187-198.

Davidge, S., Hubel, C. and McLaughlin, M. (1996). Impairment of vascular function is associated with an age related increase of lipid peroxidation in rats. *American J. Physiol.*, **266** : H1280-H1285.

Flohe, L. and Gunzler, W.A. (1984). Glutathione Peroxidase. *Methods in Enzymol.*, **105** : 115-121.

Halliwell, B., and Gutteridge, J.M.C. (1989). *Free Radicals in Biology and Medicine*, 2nd Ed. Oxford, Clarendon Press.

Haramaki, N. and Packer, L. (1994). *Oxidative Stress Indices in Exercise*. In: Sec, C., Packer, L. and Hanninen, O. ed., Exercise and oxygen toxicity. *Elsevier*, **20** : 145-147.

Harman, D. (1956). Aging: Theory based on free radical and radiation chemistry. *J. Gerontol.*, **11**: 298-300.

Indira Sriram, K. and Jhansi Lakshmi, CH. (2001). Endurance exercise-induced alterations in antioxidant enzymes of old albino male rats. *Curr. Sci.*, **80**(8): 921-923.

Jenkins, RR. (1988). Free radical chemistry: relationship to exercise. *Sport Med.*, **5** : 156-70.

Ji, L.L. (1993). Antioxidant enzyme response to exercise and aging. *Med. Sci. Sports Exer.*, **25** : 225-231.

Ji, L.L. (1999). Antioxidants and oxidative stress in exercise. *Exp. Biol. Med.*, **222** : 283-292.

Ji, L.L. and Fu, R. (1992). Response of glutathione system and antioxidants enzymes to exhaustive exercise and hydroperoxides. *J. Appl. Physiol.*, **72** : 549-554.

Ji, L. L. and Leichtweis, S. (1997). Exercise and oxidative stresss: sources of free radicals and their impact on antioxidant system. *Age*, **20** : 90-106.

Kennedy, M.M. and Newton, M. (1997). Effect of exercise intensity on mood in step aerobics. *J. Sport Med. Phys. Fitness*, **37**: 200-204.

Kono Y. and Fridovich I. (1982). Superoxide radical inhibits catalase. *J. Biol. Chem.*, **257** : 5751-5754.

Lawler, J.M., and Demaree, S.R. (2001). Relationship between NADP-specific isocitrate dehydrogenase and glutathione periodase in aging rat skeletal muscle. *Mech. Aging Develop*., **122** : 291-304.

Lee H.C. and Wei, Y.H. (2007). Oxidative stress, mitochondrial DNA mutation and apoptosis in aging; *Exp. Biol. Med*., **232** : 592-606

Leeuwenburgh, C. and Heinecke, J.W. (2001). Oxidative stress and antioxidants in exercise; *Curr. Med. Chem.*, **8** : 829-838

Leeuwenburgh, C., Fiebig, R., Chandwaney, R. and Ji, L.L. (1994). Aging and exercise training in skeletal muscle: response of glutathione and antioxidant systems. *American J. Physiol.*, **267**: R439-R445.

Lowry, O.H., Rosebrough, N.C., Farr., A. L. and Randall, R.J. (1951). Protein measurements with folin-phenol reagent. *J. Biol. Chem.*, **193** : 265-275.

Mallikarjuna, K. (2005). Alcohol effects and exercise benefits: hepatic antioxidant enzyme homeostasis in two different groups of male albino rats. Ph.D. Thesis, Sri Venkateswara University, Tirupati. India.

Mallikarjuna, K., Nishanth, K. and Sathyavelu Reddy, K. (2007). Hepatic glutathione mediated antioxidant system in ethanol treated rats: Decline with age; *Pathophysiol*., **14** : 17-21.

McCarteer, R.J.M. (2000). In: *Studies in Handbook of Oxidants and Antioxidants in Exercise*. Eds. Sen C., Packer L., Hannine O., Amsterdam. 797-830.

McCutcheon, L.J., Byrd, S.K. and Hodgson, D.R. (1992). Ultra structural changes in skeletal muscle after fatigue exercise. *J. Appl. Physiol.*, **72**: 1111-1117.

Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.*, **247** : 3170-3175.

Ohkuwa, T., Sato, Y. and Naoi, M. (1997). Glutathione status and reactive oxygen generation in tissues of young and old exercised rats. *Acta Physiol. Scand.*, **159** : 237-244.

Powers, S.K., DeRuisseau, K.C., Quindary, J. and Hamilton, K.L. (2004). Dietary antioxidants and exercise; *J. Sports Sci*., **22** : 81-94

Pushpalatha, K, Nishanth, K. and Sathyavelu Reddy, K. (2007). Myocardial antioxidant status and oxidative stress after combined action of exercise training and ethanol in two different age groups of male albino rats. *Acta Biol. Hung*., **58** (2) : 173-85

Sawada, M. and 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.

Sen, C.K., Rahkila, P. and Hanninnen, O. (1993). Glutahione metabolism skeletal muscle derived cell of the L6 line. *Acta. Physiol. Scand.*, **148** : 21-26.

Somani, S.M., Husain, K., Dalz Philips, L., Lanzotti, D.J., Karets, K.R. and Trammell, G.L. (1996). Interaction of exercise and ethanol on antioxidant enzymes in brain regions of the rat. *Alcohol.*, **13**(6): 603-610.

Somani, S.M., Ravi, R. and Ryback, L.P. (1995). Effect of exercise training on antioxidant system in brain regions of rat. Pharmo. *Biochem. Behav.,* **50** (4) : 635-639.

Stadtman, E.R. (1992). Protein oxidation and aging. *Sci.*, **257**: 1220-1224.

Subba Rao, K. (1990). Health aging- a myth or possibility. Bio Spectra. 61-65.

Theodorus, P.M., Akerboom and Helmut Sies (1981). Assay of Gluthathione, Glutathione disulfide and Gluthathione mixed disulfide in biological samples. *Methods in Enzymol.,* **77** : 373-382.

Vohra, B.P.S., Sharma, S.P. and Kansal, V. (2001). Age-dependent variations in mitochondrial and cytosolic antioxidant enzymes and lipid peroxidation in different regions of central nervous system of guinea pigs; *Indian J. Biochem. Biophys.*, **38** : 321-326

Wei, Y.H. and Lee, S.C. (2002). Oxidative stress, mitochondrial DNA mutation and impairment of antioxidant enzymes in aging. *Proce. Soc. Expe. Biol. Med.,* **217**: 53-63.

Yu, B.P. (1994). Cellular defenses against damage from reactive oxygen species. *Physiol.Rev.,* **74** : 139-162.