J Nutr Sci Vitaminol, **54**, 61–65, 2008

Lipid Peroxidation Potential and Antioxidants in the Heart Tissue of β-Alanine- or Taurine-Treated Old Rats

Hande PARILDAR[1], Semra DOĞRU-ABBASOĞLU[1],Güldal MEHMETÇİK[2], Gül ÖZDEMİRLER[1], Necla KOÇAK-TOKER[1] and Müjdat UYSAL[1,*]

[1]*Department of Biochemistry, Istanbul Medical Faculty, Istanbul University, Çapa 34093, Istanbul, Turkey*
[2]*Near East University, Art and Science Faculty, Lefkoşa, KKTC*

(Received June 19, 2007)

Summary The aim of this study was to investigate the effect of the changes of taurine levels in the hearts of old rats on endogenous malondialdehyde (MDA) and diene conjugate (DC) levels and ascorbic acid (AA)- and NADPH-induced lipid peroxidation as well as non-enzymatic (glutathione, vitamin E and vitamin C) and enzymatic antioxidants (superoxide dismutase, glutathione peroxidase and glutathione transferase). Two groups of old (22 mo) rats were treated with β-alanine (3%, w/v; in drinking water), a taurine depleting agent, or taurine (2% w/v; in drinking water) for 6 wk. Significant decreases were observed in taurine contents of hearts in old rats as compared to young (5 mo) rats. We found that MDA and DC levels and AA- and NADPH-induced lipid peroxidation increased, but non-enzymatic and enzymatic antioxidants did not alter in heart homogenates of aged rats. β-Alanine administration resulted in significant decreases in heart taurine levels of old rats. This treatment did not cause further increases in MDA or DC levels or changes in antioxidants. However, AA- and NADPH-induced lipid peroxidation was higher than that of old rats. Taurine treatment caused significant increases in heart taurine levels of old rats. This treatment was found to decrease endogenous MDA and DC levels without affecting the antioxidant system in the heart homogenates of aged rats. AA- and NADPH-induced lipid peroxidation was also reduced in old rats when given taurine, although not statistically significantly. Our results indicate that the changes in heart taurine levels may influence the susceptibility of heart tissue to lipid peroxidation in aged rats and that taurine supplementation has protective effects on age-dependent oxidative stress in heart tissue.

Key Words taurine, aging, oxidative stress, heart, rat

Aging is associated with a decline in cardiovascular function and high cardiovascular mortality (*1*). Several reports have shown that oxidative stress plays an important role in the development of cardiac aging (*2–4*). Oxidative stress parameters such as malondialdehyde (MDA) (*3, 5, 6*), and 8-iso-prostaglandin F2α (*3*), protein carbonyl levels (*7*) and DNA damage (*2–4*) have been reported to be increased in the hearts of old rats, although some conflicting results are available (*8, 9*). Results related to non-enzymatic (*2, 5, 7, 10*) and enzymatic (*6–9, 11*) antioxidants in the heart of aged rats are conflicting.

Taurine (2-aminoethanesulfonic acid) is the major intracellular free β-amino acid, which is normally present in most mammalian tissues (*12*). It has various important biological and physiological roles such as antioxidation, osmoregulation, membrane stabilization, and regulation of intracellular calcium homeostasis (*12*). Taurine accounts for more than 50% of the total amino acid pool in the heart (*13*). Clinical and experimental studies have shown that taurine deficiency is associated with structural and functional disorders in the heart and that taurine treatment ameliorated cardiovascular disorders (*1, 13, 14*).

On the other hand, it has been reported that taurine levels decreased in plasma and tissues with aging (*15–17*). Since taurine is an important intracellular antioxidant, severe decreases in taurine levels of tissues may influence their antioxidant power and susceptibility to oxidative damage (*15, 18, 19*). Therefore, taurine deficiency in heart tissue may contribute to cardiac oxidative damage that occurs during the aging process (*16, 17*).

In this study, endogenous, ascorbic acid (AA)- and NADPH-induced lipid peroxidation and non-enzymatic and enzymatic antioxidants together with taurine levels were measured in heart homogenates of young and old rats. These measurements were also done in old rats treated with β-alanine, a taurine-depleting agent, or with taurine. Thus, we wanted to investigate how the changes in taurine levels affect endogenous and lipid peroxidation potential and antioxidant system in hearts of aged rats.

MATERIALS AND METHODS

Materials. Taurine, β-alanine and other chemicals were purchased from Sigma Chemical.

*To whom correspondence should be addressed.
E-mail: mujdatuysal@hotmail.com

Animals and treatments. Young (5 mo) and old (22 mo) male Wistar rats were obtained from the Center for Experimental Medical Research Institute of Istanbul University. The animals were allowed free access to food and water and were kept in wire-bottomed stainless steel cages. The experimental procedure used in this study met the guidelines of the Animal Care and Use Committee of the University of Istanbul.

Old rats were divided into three subgroups as untreated, β-alanine- and taurine-treated old rats. β-Alanine was applied in drinking water (3%, w/v) for 6 wk to decrease taurine levels. Taurine (2%, w/v) was given also in drinking water for 6 wk. At the end of this period, young, old, β-alanine- and taurine-treated old rats were fasted overnight and the hearts of the rats were quickly removed and washed in 0.9% NaCl and kept in ice.

The determinations of taurine levels. Heart tissues were homogenized in ten volumes of 3% sulfosalisilic acid. After centrifugation, the supernatant was filtered through a 0.45 μm filter and taurine levels were determined with a Hewlett Packard amino acid analyzer (*20*).

The determinations of lipid peroxide levels. Tissues were homogenized in ice-cold 0.15 M KCl (10%, w/v). Lipid peroxidation was assessed by two different methods in the heart homogenates. First, the levels of malondialdehyde (MDA) were determined by the thiobarbituric acid test (*21*). The breakdown product of 1,1,3,3-tetraethoxypropane was used as a standard. Second, diene conjugate (DC) levels were measured in heart lipid extracts at 233 nm spectrophotometrically and calculated using a molar extinction coefficient of $2.52\times10^4\ M^{-1}cm^{-1}$ (*22*).

To determine induced lipid peroxide levels, two different media were prepared (*23*). The incubation mixture (1 mL) for the estimation of AA-induced lipid peroxidation contained 50 μM $FeSO_4$, 1 mM KH_2PO_4, 0.15 M Tris-HCl buffer (pH 7.4), 0.4 mM ascorbic acid and 0.1 mL tissue homogenate. For the NADPH-induced system, 50 μM $FeCl_3$ instead of $FeSO_4$, and 4 mM ADP were included and 0.4 mM NADPH was added instead of ascorbic acid. After incubation at 37°C in a shaking water bath for 30 min, the amount of MDA formed was estimated according to Buege and Aust (*22*).

The determinations of non-enzymatic and enzymatic antioxidants. Heart glutathione (GSH) levels were measured with 5,5-dithiobis-(2-nitrobenzoate) at 412 nm (*24*). Vitamin E and vitamin C levels were measured in heart homogenates by the method of Desai (*25*) and Omaye et al. (*26*), respectively. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione transferase (GST) activities were determined in the postmitochondrial fraction of the heart, which was obtained by sequential centrifugation. In brief, heart homogenates were centrifuged at 600 ×*g* for 10 min at 4°C to remove crude fractions. Then, supernatants were centrifuged at 10,000 ×*g* for 20 min to obtain the postmitochondrial fraction. SOD activities were assayed by its ability to increase the effect of riboflavin-sensitized photooxidation of *o*-dianisidine (*27*). GSH-Px (*28*) and GST (*29*) activities were measured using cumene hydroperoxide and 1-chloro-2,4-dinitrobenzene as substrates, respectively. Protein levels were determined using bicinchoninic acid (*30*).

Statistical analysis. The results were expressed as mean±SD. Experimental groups were compared using the Kruskal-Wallis variance analysis test. Where significant effects were found, post-hoc analysis using the Mann-Whitney U test was performed, and $p<0.05$ was considered to be statistically significant.

RESULTS

a) Significant decreases (16.6%) were observed in taurine contents of the heart in old rats as compared to young rats (Fig. 1). Heart MDA (33.3%) and DC

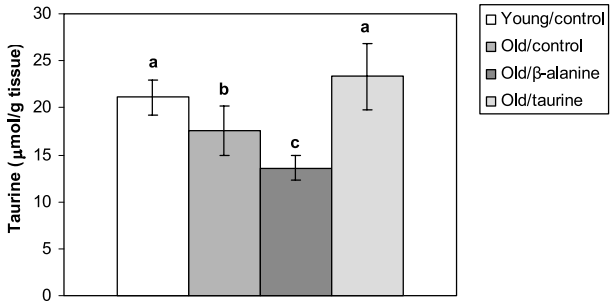

Fig. 1. Taurine levels in heart homogenates (mean±SD, $n=6$ each). Values not sharing a common superscript letter are significantly different by Kruskal-Wallis test followed by Mann-Whitney *U* test; $p<0.05$.

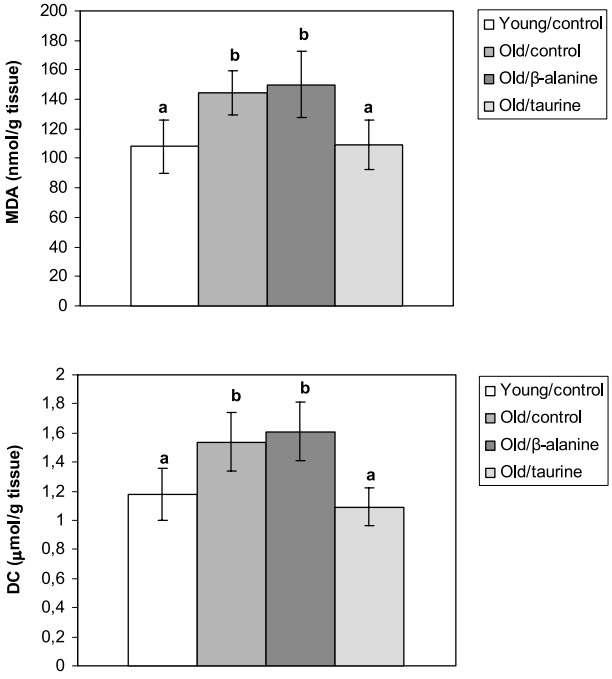

Fig. 2. Malondialdehyde (MDA) and diene conjugate (DC) levels in heart homogenates (mean±SD, $n=6$ each). Values not sharing a common superscript letter are significantly different by Kruskal-Wallis test followed by Mann-Whitney *U* test; $p<0.05$.

(30.5%) levels and AA-(113.8%) and NADPH (66.0%)-induced lipid peroxidation increased (Figs. 2 and 3), but there were no changes in GSH, vitamin E or vitamin C levels, nor in SOD, GSH-Px or GST activities in heart homogenates of aged rats (Tables 1 and 2).

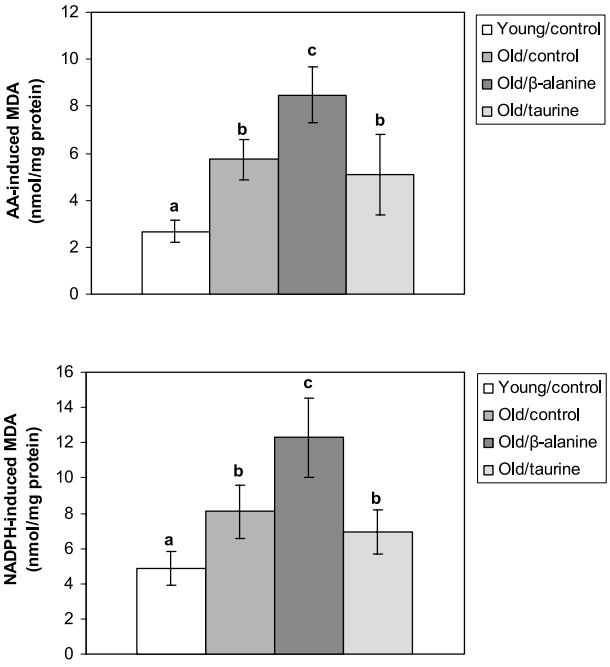

Fig. 3. Ascorbic acid (AA)- and NADPH-induced lipid peroxide (malondialdehyde=MDA) levels in heart homogenates (mean±SD, $n=6$ each). Values not sharing a common superscript letter are significantly different by Kruskal-Wallis test followed by Mann-Whitney U test; $p<0.05$.

b) Heart taurine contents were found to be decreased (22.7%) in old rats following β-alanine treatment. MDA and DC levels as well as the antioxidant system did not further change in the heart of β-alanine-treated old rats. However, AA- and NADPH-induced lipid peroxidation was found to be increased (48.0% and 51.8%, respectively) in the heart homogenates of aged rats following β-alanine treatment.

c) Taurine levels were increased (32.4%) in heart homogenates in taurine-treated old rats. This treatment decreased MDA (24.2%) and DC (29.2%) levels without affecting non-enzymatic or enzymatic antioxidants. AA- (11.3%) and NADPH (14.4%)-induced lipid peroxidation was also reduced in old rats, although not statistically significantly.

DISCUSSION

Oxygen and nitrogen species generation (*2*, *7*, *31*) as well as oxidative stress parameters (*2*, *3*, *6*, *7*, *32*) were reported to be significantly increased in the hearts of old rats. In our study, MDA and DC levels were also found increased, although no alteration was observed in non-enzymatic or enzymatic antioxidant systems, which was in accordance with other studies (*2*, *7–9*).

As it is known, AA-induced lipid peroxidation is a non-enzymatic reaction involving ferro iron and ascorbate, but enzymatic NADPH-induced lipid peroxidation is known to be mediated by the enzyme NADPH cytochrome *c* reductase and requires ferric iron chelated with ADP (*23*, *33*). Both types of lipid peroxidation show peroxidation potential of tissues and are con-

Table 1. Glutathione (GSH), vitamin E and vitamin C levels in heart homogenates of young rats and old rats treated with and without either β-alanine or taurine (mean±SD; $n=6$ each).

	Treatment	GSH (μmol/g tissue)	Vitamin E (nmol/g tissue)	Vitamin C (nmol/g tissue)
Young	—	1.73 ± 0.28^a	44.8 ± 8.04^a	1.06 ± 0.08^a
Old	—	1.42 ± 0.33^a	50.4 ± 4.43^a	1.10 ± 0.22^a
Old	β-Alanine	1.75 ± 0.14^a	47.4 ± 10.9^a	1.09 ± 0.18^a
Old	Taurine	1.72 ± 0.21^a	45.1 ± 8.62^a	1.05 ± 0.06^a

Values not sharing a common superscript letter are significantly different by Kruskal-Wallis test followed by Mann-Whitney U test; $p<0.05$.

Table 2. Superoxide dismutase (SOD), glutathione peroxidase (GSH) and glutathione transferase (GST) activities in heart homogenates of young rats and old rats treated with and without either β-alanine or taurine (mean±SD; $n=6$ each).

	Treatment	SOD (U/mg protein)	GSH-Px (nmol/min/mg protein)	GST (nmol/min/mg protein)
Young	—	20.8 ± 2.15^a	387.5 ± 38.2^a	46.0 ± 8.29^a
Old	—	21.3 ± 2.26^a	398.5 ± 48.2^a	45.8 ± 7.00^a
Old	β-Alanine	20.2 ± 2.42^a	392.5 ± 55.9^a	45.1 ± 7.33^a
Old	Taurine	22.3 ± 2.75^a	361.0 ± 49.9^a	49.0 ± 7.56^a

Values not sharing a common superscript letter are significantly different by Kruskal-Wallis test followed by Mann-Whitney U test; $p<0.05$.

trolled by several factors such as availability of substrates in the form of unsaturated fatty acids, the presence/absence of peroxidation inducers and inhibitors of free radical reactions (*32*). The heart is known to have a low total reactive antioxidant potential (*34*) and an ability to upregulate antioxidant defences and to repair accumulated peroxidative damage (7, 35). Therefore, the heart may have high susceptibility to prooxidant stimuli. However, unchanged (*32*) and decreased (*11*) AA-induced lipid peroxidation has been reported in heart homogenates in old rats. In the current study, we found that both AA- and NADPH-induced lipid peroxidation as well as endogenous lipid peroxidation increased in heart homogenates of old rats as compared to young rats.

On the other hand, some investigators have reported that taurine levels decreased in plasma and some tissues such as liver, brain and kidney with aging (*15–17*). However, no significant changes in taurine levels were detected in the heart or skletal muscles of old rats (*15*). In the current study, we observed that taurine levels were decreased in heart homogenates of aged rats. If taurine plays a role in the normal antioxidant defence system, decreases in taurine levels in heart tissue may contribute to shift the balance toward a prooxidant environment.

β-Alanine is a well known antagonist of taurine transport which produces depletion of tissue taurine levels, since both amino acids share a common transport system, selective for β-amino acids (*36*, *37*). Although some investigators have reported that taurine-deficient diets did not cause further decreases in taurine levels in tissues of old rats due to adaptive reactions (*15*, *16*), there is not sufficient knowledge in the literature about the effect of β-alanine treatment on taurine levels in tissues of old rats.

In this study, we wanted to investigate whether β-alanine treatment affected taurine levels and the susceptibility of heart to lipid peroxidation in aged rats. In these rats, heart taurine levels were found further decreased and AA- and NADPH-induced lipid peroxidation further increased following β-alanine treatment, although there was no change in endogenous MDA or DC levels or antioxidant system parameters. Therefore, taurine depletion in the heart is observed to provoke peroxidation potential, but not endogenous lipid peroxidation probably due to the unchanged endogenous antioxidant system in old rats. According to this, the balance between prooxidant and antioxidant systems seems to be highly sensitive to taurine depletion in the heart tissue of old rats. On the contrary, in our recent study, although the administration of 3% β-alanine in the drinking water of young rats decreased taurine levels in the heart (15%), this treatment was found not to alter endogenous, AA- or NADPH-induced lipid peroxidation in heart homogenates (*38*).

On the other hand, high levels of MDA and DC in the hearts of old rats were found reduced with taurine treatment, without any change in antioxidant system parameters. Therefore, the supplemented amount of taurine appears to be sufficient to decrease high endogenous lipid peroxide levels in the heart of old rats. In addition, since lipid peroxidation induction in the heart tissue is lower in taurine-treated old rats than in β-alanine treated old rats, it can be considered that taurine may play a preventive role in the induction of lipid peroxidation arising due to prooxidant stimuli in aging.

In conclusion, our results indicate that the changes in taurine levels may influence the susceptibility of heart tissue to lipid peroxidation in aged rats and that taurine supplementation may have protective effects on age-dependent oxidative stress in heart tissue.

Acknowledgments

This work was supported by the Research Fund of the University of İstanbul (Project No: BYP-969).

REFERENCES

1) Allard ML, Jeejeebhoy KN, Sole MJ. 2006. The management of conditioned nutritional requirements in heart failure. *Heart Fail Rev* **11**: 75–82.

2) Suh JH, Shigeno ET, Morrow JD, Cox B, Rocha AE, Frei B, Hagen TM. 2001. Oxidative stress in the aging rat heart is reversed by dietary supplementation with (R)-α-lipoic acid. *FASEB J* **15**: 700–706.

3) Wong YT, Ruan R, Tay FEH. 2006. Relationship between levels of oxidative DNA damage, lipid peroxidation and mitochondrial membrane potential in young and old F344 rats. *Free Radic Res* **40**: 393–402.

4) Savitha S, Pannerselvam C. 2007. Mitigation of age-dependent oxidative damage to DNA in rat heart by carnitine and lipoic acid. *Mech Ageing Dev* **128**: 206–212.

5) Van der Loo B, Bachschmid M, Spitzer V, Brey L, Ullrich V, Lüscher TF. 2003. Decreased plasma and tissue levels of vitamin C in a rat model of aging: implications for antioxidative defence. *Biochem Biophys Res Commun* **303**: 483–487.

6) Gündüz F, Şentürk ÜK, Kuru O, Aktekin B, Aktekin MR. 2004. The effect of one year's swimming exercise on antioxidant stress and antioxidant capacity in aged rats. *Physiol Res* **53**: 171–176.

7) Bejma J, Ramires P, Ji LL. 2000. Free radical generation and oxidative stress with ageing and exercise: Differential effects in the myocardium and liver. *Acta Physiol Scand* **169**: 343–351.

8) Pritsos CA, Ma J. 2000. Basal and drug-induced antioxidant enzyme activities correlate with age-dependent doxorubicin oxidative toxicity. *Chem Biol Interact* **127**: 1–11.

9) Rozenberg S, Besse S, Brisson H, Jozefowicz E, Kandoussi A, Mebazaa A, Riou B, Vallet B, Tavernier B. 2006. Endotoxin-induced myocardial dysfunction in senescent rats. *Crit Care* **10**: R124.

10) Van der Loo B, Labugger R, Aebischer CP, Skepper JN, Bashschmid M, Spitzer V, Kilo J, Altwegg L, Ullrich V, Lüscher TF. 2002. Cardiovascular aging is associated with vitamin E increase. *Circulation* **105**: 1635–1638.

11) Shinohara R, Mano T, Nagasaka A, Hayashi R, Uchimura K, Nakano I, Watanabe F, Tsugawa T, Makino M, Kakizawa H, Nagata M, Iwase K, Ishizuki Y, Itoh M. 2000. Lipid peroxidation levels in rat cardiac muscle are affected by age and thyroid status. *J Endocrinol* **164**: 97–102.

12) Schaffer S, Azuma J, Takahashi K, Mozaffari M. 2003.

Why is taurine cytoprotective? *Adv Exp Med Biol* **526**: 307–321.

13) Shiny KS, Kumar SHS, Farvin KHS, Anandan R, Devadasan K. 2005. Protective effect of taurine on myocardial antioxidant status in isoprenaline-induced myocardial infarction in rats. *J Pharm Pharmacol* **57**: 1–5.

14) Sole MJ, Jeejeebhoy KN. 2002. Conditioned nutritional requirements: Therapeutic relevance to heart failure. *Herz* **27**: 174–179.

15) Dawson R, Liu S, Eppler B, Patterson T. 1999. Effects of dietary taurine supplementation or deprivation in aged male Fischer 344 rats. *Mech Ageing Dev* **107**: 73–91.

16) Eppler B, Dawson R. 2001. Dietary taurine manipulations in aged male Fisher 344 rat tissue: taurine concentration, taurine biosynthesis, and oxidative markers. *Biochem Pharmacol* **62**: 29–39.

17) Militante J, Lombardini JB. 2004. Age-related retinal degeneration in animal models of aging: Possible involvement of taurine deficiency and oxidative stress. *Neurochem Res* **29**: 151–160.

18) Messina SA, Dawson R. 2000. Attenuation of oxidative damage to DNA by taurine and taurine analogs. *Adv Exp Med Biol* **483**: 355–367.

19) Golubnitschaja O, Moenkemann H, Kim K, Mozaffari MS. 2003. DNA damage and expression of checkpoint genes $p21^{WAF1/CIP1}$ and 14–3–3 σ in taurine-deficient cardiomyocytes. *Biochem Pharmacol* **66**: 511–517.

20) Chen W, Matuda K, Nishimura N, Yokogoshi H. 2004. The effect of taurine on cholesterol degradation in mice fed a high-cholesterol diet. *Life Sci* **74**: 1889–1898.

21) Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Anal Biochem* **95**: 351–358.

22) Buege JA, Aust JD. 1978. Microsomal lipid peroxidation. *Methods Enzymol* **52**: 302–310.

23) Devasagayam TPA, Tarachand U. 1987. Decreased lipid peroxidation in the rat kidney during gestation. *Biochem Biophys Res Commun* **145**: 134–138.

24) Beutler E, Duron O, Kelly BM. 1979. Improved method for the determination of blood glutathione. *J Lab Clin Med* **61**: 882–888.

25) Desai ID. 1984. Vitamin E analysis methods for animal tissues. *Methods Enzymol* **105**: 138–147.

26) Omaye ST, Turnbull JD, Sauberlich HE. 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. *Methods Enzymol* **62**: 3–8.

27) Mylorie AA, Collins H, Umbles C, Kyle J. 1986. Erythrocyte superoxide dismutase activity and other parameters of copper status in rats ingesting lead acetate. *Toxicol Appl Pharmacol* **82**: 512–520.

28) Lawrence RA, Burk RF. 1976. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun* **71**: 952–958.

29) Habig WH, Jacoby WB. 1981. Assays for differentation of glutathione *S*-transferases. *Methods Enzymol* **77**: 398–405.

30) Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. 1985. Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**: 76–85.

31) Kozlov AV, Szalay L, Umar F, Kropik K, Staniek K, Niedermüller H, Bahrami S, Nohl H. 2005. Skeletal muscles, heart and lung are the main sources of oxygen radicals in old rats. *Biochim Biophys Acta* **1740**: 382–389.

32) Davydov VV, Shvets VN. 2003. Age-dependent differences in the stimulation of lipid peroxidation in the heart of rats during immobilization stress. *Exp Gerontol* **38**: 693–698.

33) Pushpendran CK, Subramanian M, Devasagayam TPA, Singh BB. 1998. Study on lipid peroxidation potential in different tissues induced by ascorbate-Fe^{2+}: Possible factors involved in their differential susceptibility. *Mol Cell Biochem* **178**: 197–202.

34) Evelson P, Travacio M, Repetto M, Escobar J, Llesuy S, Lissi EA. 2001. Evaluation of total reactive antioxidant potential (TRAP) of tissue homogenates and their cytosols. *Arch Biochem Biophys* **388**: 261–266.

35) Chen Y, Saari JT, Kang YJ. 1994. Weak antioxidant defences make the heart a target for damage in copper deficient rats. *Free Radic Biol Med* **17**: 529–536.

36) Shaffer JE, Kocsis JJ. 1981. Methods of reducing tissue taurine levels. *In*: The Effects of Taurine on Excitable Tissues (Schaffer S, Baskin SI, Kocsis JJ. eds), p 219–229. Spectrum Publications, New York.

37) Dawson R, Biasetti M, Messina S, Dominy J. 2002. The cytoprotective role of taurine in exercise-induced muscle injury. *Amino Acids* **22**: 309–324.

38) Parıldar-Karpuzoğlu H, Doğru-Abbasoğlu S, Balkan J, Aykaç-Toker G, Uysal M. 2007. Decreases in taurine levels induced by β-alanine treatment did not affect the susceptibility of tissues to lipid peroxidation. *Amino Acids* **32**: 115–119.