J Invest Biochem. 2013;2(1):37-43 *ISSN: 2146-8338*

GESDAV

Journal of Investigational Biochemistry

available at www.scopemed.org

Original Research

Alteration of antioxidant enzymes and oxidative stress in elderly patient

Seema L. Jawalekar[1], Anil K. Bhutey[2], Aarti Karnik[3], Vasant T. Surve[4]

[1]Sree Narayana Institute of Medical Sciences, Ernakulam
[2]P.D. Medical College, Department of Biochemistry, Amravati
[3]A C P M Medical college, Dhule
[4]Dr. Ulhas Patil Medical College, Department of Biochemistry, Jalgaon

Received: December 21, 2012

Accepted: January 29, 2013

Published Online: February 11, 2013

DOI: 10.5455/jib.20130129093838

Corresponding Author:
Seema Laxman Jawalekar,
Sree Narayana Institute of Medical Sciences
Ernakulam
seems2april@rediffmail.com

Key words: Lipid peroxidation, Glutathione peroxidase, Malondialdehyde, Total antioxidant capacity, Nitric oxide.

Abstract

Reactive oxygen spaces are common by-products of many oxidative biochemical and physiological processes. Therefore the present study was carried out to evaluate the total antioxidant capacity, lipid peroxidation and status of superoxide dismutase in elderly patients with hypertension, Diabetes with hypertension and cardiovascular diseases. Total 85 patients of both sexes were included in the study and further classified into 3 groups as hypertensive Diabetes with hypertension and cerebrovascular disease/stroke. The 26 healthy subjects who were not on any kind of prescribed medication or dietary restrictions were included in the control group. Malondialdehyde is estimated as a marker of lipid peroxidation, levels were significantly increased in all groups than controls ($p<0.001$). Superoxide dismutase and glutathione reductase activities was significantly lower in all groups than control ($p<0.001$). Glutathione peroxidase levels were decreased in all groups except hypertension ($p<0.001$), nitric oxide level were decreased in all the groups except cerebrovascular disease / stroke when compared to the control. Significantly lower level of total antioxidant capacity and prominent scavenger of superoxide anion radicals suggests that failure of antioxidant defense mechanism against oxidative stress may be an important factor in the pathogenesis of cardiovascular diseases.

INTRODUCTION

Oxidative stress is intensified with the process of aging, and in the elderly, this is accompanied by a more common occurrence of primary hypertension, cardiovascular diseases (CVD) and diabetes [1-3]. Oxidative stress has a potential role in diabetogenesis, development of diabetic complications like atherosclerosis and cardiovascular disease. Among the sequel of hyperglycemia, dyslipidemia, and high blood pressure; oxidative stress has been suggested as a potential mechanism for accelerated atherosclerosis. The mechanisms of these effects are, however, still unknown.

Recent studies show that oxidative stress and increased superoxide anion production from vascular NAD(P)H oxidases play critical role in the pathogenesis of hypertension and endothelial dysfunction [4]. Both NAD(P)H oxidase activity and expression are increased in animal models of hypertension and in hypertensive individuals [4]. Hypertension is associated with impaired nitric oxide (NO) production/degradation. Under pathological conditions and during aging, an accelerated inactivation of nitric oxide caused by the superoxide anion ($O_2\bullet^-$) may be related to hypertension [1,5,6]. The most important source of the superoxide anion in the vessel wall is membrane-bound NAD(P)H oxidase [1,6]. NO can be scavenged by $O_2\bullet^-$ to form (peroxynitrite) ONOO–, which can be transformed to a highly reactive oxygen species –peroxynitrous acid and

ONOO– may also induce oxidation reactions of endogenous compounds. Both ONOO– and peroxynitrous acid may be involved in numerous pathophysiological processes. The path mechanism of hypertension has close relevance with an impaired bioavailability of NO and a large amount of ONOO– [1,5,6].

Many experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. In fact, diabetes is typically associated with increased generation of free radicals and/or impaired antioxidant defense qualifications, representing a central contribution for reactive oxygen species in the onset, progression, and pathological consequences of diabetes. There are multiple sources of oxidative stress in diabetes including nonenzymatic, enzymatic and mitochondrial pathways. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defence mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus. Changes in oxidative stress biomarkers, including superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, vitamins, lipid peroxidation, nitrite concentration, nonenzymatic glycosylated proteins [7,8]. The antioxidant defence system comprises a number of interconnected, overlapping components that include both enzymatic and non-enzymatic factors. Vitamin E, the major lipid-soluble antioxidant, protects against lipid peroxidation. Vitamin C can quench free radicals as well as singlet oxygen and can also regenerate the reduced antioxidant form of vitamin E. Together with uric acid, carotenoids, flavonoids and ubiquinol, these antioxidants make up the total antioxidant capacity (TAC) in plasma [9].

AIMS AND OBJECTIVES

Aim of our study was to assess the antioxidant status by measuring the antioxidant enzymes and lipid peroxidation in the elderly individuals compared to that of young people.

MATERIALS AND METHODS

Patients who were attending Diabetic OPD as well as Patients admitted in Medicine wards at Dr. Ulhas Patil Medical College, Jalgaon Maharashtra, India during a period of Dec. 2008-2009 were taken for evaluation of oxidants, antioxidants and nitric oxide content in their blood. Total 85 subjects were included for study at the age group of 65 ± 10 yrs (63 males and 22 females) out of 26 age sex matched healthy human volunteers, age group of 62 ± 8 yrs (18 males and 8 females) were not on any kind of prescribed medication or dietary restrictions were included in the control group I and 30 young people ageing 28 ± 5 yrs (19 males and 11 females), working in the hospital, were taken as control group II. The procedures were approved by the local ethics committee.

The subjects were classified into 3 groups:

Group I: Subjects with hypertension. Total patients 30 out of which 22 were males and 8 were female. This group consists of patients who have been diagnosed as essential hypertension for the first time > 140/90 mm/Hg.

Group II: Subjects with Diabetes and hypertension. Total patients 30 out of which 20 were males and 10 were female.

Group III: Subjects with cardiovascular diseases. Total patients 25 out of which 20 were males and 5 were female.

Group IV: Healthy age sex matched controls groups I. Total healthy Volunteers 26 out of which 18 were males and 08 were female.

Group V: Healthy Young people Control group II. Total healthy Volunteers 30 out of which 19 were males and 11 were female.

All subjects gave written consent before the beginning of the study. Information regarding chronic illness, smoking, alcohol consumption and drug intake was obtained by questionnaires. All the subjects were subjected to a thorough clinical examination and biochemical investigations to detect signs and symptoms of chronic diseases such as hypertension, diabetes mellitus etc. Individuals suffering from any chronic disease and/or under drug treatment and patients taking antioxidant therapy that interferes with study parameters like oxidative stress were excluded from the study.

The subjects in our study were non smokers and non alcoholics. The purpose of our study was explained to the subjects and their consent was taken. The study was approved by the institutional ethical committee.

Five mL of venous blood was collected in EDTA bottles using disposable syringes, plasma was separated. Concentration of serum nitric oxide [10], Glutathione peroxidase (GPX) in RBCs [11], Glutathione Reductase (GR) in RBCs [12], Superoxide dismutase (SOD) in RBCs [13] and serum Malondialdehyde (MDA) in serum [14] were analyzed.

The RA- 50 Chemistry analyzer was used to carry out all analysis. Total antioxidant capacity (TAC) was accordingly determined [15], the assay measures the capacity of the serum to inhibit the production of thiobarbituric acid reactive substances (TBARS) from sodium benzoate, under the influence of the oxygen free radicals derived from Fenton's reaction. The reaction was measured spectrophotometrically at 532 nm. Antioxidants from the added sample cause suppression of the production of TBARS and the inhibition of colour development is defined as to TAC.

RESULTS

Table 1. Status of nitric oxide and antioxidant enzymes in various groups

Parameters	Group I (Hypertension)	Group II (Diabetes with hypertension)	Group III (Cardiovascular diseases/stroke)	Control vs Group I	Control vs Group II	P Value
NO (µmol/L)	38.59 ± 3.68	36.24 ± 8.17	30.23 ± 6.87	51.0 ± 5.81	59.87± 6.34	<0.001
GPX (U/gHb)	26.71 ± 7.88	22.01 ± 4.38	24.09 ± 2.97	33.31 ± 5.25	38.63 ± 6.78	<0.001
GR (U/gHb)	3.72 ± 1.62	3.12 ± 1.52	3.99 ± 1.53	9.68 ± 4.68	11.53 ± 3.67	<0.001
SOD (U/gHb)	471.59 ± 206.46	421.46 ± 109.46	462.23 ± 148.53	728.91 ± 208.86	984 ± 229	<0.001
MDA (nmol/mg protein)	4.99 ± 0.91	4.86 ± 0.89	5.88 ± 0.74	3.89 ± 0.94	2.65 ± 0.50	<0.001

Data were given as mean±SD.

Table 2. Shows total antioxidant capacity (TAC)

	TAC (mmol/L)	p
Group I (Hypertension)	1.09 ± 0.10	<0.001
Group II (Diabetes with hypertension)	1.00 ± 0.26	<0.001
Group II (Cardiovascular diseases/stroke)	0.86 ± 0.08	<0.001
Control vs Group I	1.63 ± 0.25	<0.001
Control vs Group II	1.83 ± 0.25	<0.001

DISCUSSION

According to the free radical theory of aging, free radicals play an important role in the aging process and contribute many common diseases [1]. Ageing is a progressive accumulation of physiological and morphological changes, responsible for an increasing susceptibility to disease [16]. Oxygen free radicals are implicated in the ageing process. These highly reactive species can cause cell damage including lipid peroxidation, inactivation of enzymes, alteration of intra-cellular oxidation-reduction state and damage to DNA [3,17]. Ageing is a process of irreversible changes associated with accumulation of these oxidative damages in the cell.

ROS can stimulate oxidation of low-density lipoprotein (LDL), and ox-LDL, which is not recognized by the LDL receptor, can be taken up by scavenger receptors in macrophages leading to foam cell formation and atherosclerotic plaques [18]. $O_2^{\bullet-}$ can activate several damaging pathways in diabetes including accelerated formation of advanced glycation end products (AGE), polyol pathway, hexosamine pathway and PKC(protein kinase C), all of which have been proven to be involved in micro- and macrovascular complications. In endothelial cells, H_2O_2 mediates apoptosis and pathological angiogenesis [19]. Furthermore, $O_2^{\bullet-}$ immediately reacts with $^{\bullet}NO$ generating cytotoxic $ONOO^-$ and this reaction itself has several consequences. First, $ONOO^-$ alters function of biomolecules by protein nitration as well as causing

lipid peroxidation [20]. For example, potassium channels, which regulate the vaso relaxation response, are inhibited by nitration [21,22]. It also decreases $^{\bullet}NO$ bioavailability causing impaired relaxation and inhibition of the antiproliferative effects of $^{\bullet}NO$ [7]. Furthermore, $ONOO^-$ oxidizes tetrahydrobiopterin (BH_4), an important cofactor for NOS, and causes uncoupling of NOS, which produces $^{\bullet}O_2^-$ instead of $^{\bullet}NO$ [7]. ROS-induced peroxidation of membrane lipids alters the structure and the fluidity of biological membranes, which ultimately affects function [7,19, 23-24]. All these pathological modifications contribute to the pathogenesis of vascular dysfunction.

The antioxidant defence system comprises a number of interconnected, overlapping components that include both enzymatic and non-enzymatic factors. But free radicals do not go unchecked. Mounted against them is a multilayer defence system manned by anti-oxidants that react with and disarm these damaging molecules. The human body has a complex antioxidant defence system that includes enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, Glutathione reductase (GR).

The form of geriatric hypertension and diabetes is associated with increased cardiovascular morbidity and mortality in the form of coronary artery disease, stroke, congestive heart failure, end-stage renal disease, and total cardiovascular deaths [25,26]**.** Our results indicate that there is increase in free radical generation and decrease in antioxidant defense mechanism in elderly people when compared to normal subjects. Highly significant increase in MDA and decrease in antioxidants was observed in elderly people when complicated with diabetes and hypertension. Increased MDA and decreased antioxidants with ageing indicate that peroxidative damage increases with ageing process. In this study, malonyldialdehyde levels (TBARS) were increased in all patients, serving as a marker of massive oxidative stress at the onset of illness.

Lipid peroxidation (MDA) levels of Cardiovascular / stroke patients are higher than in normal people as seen in our study. The oxidative destruction of lipids (lipid peroxidation) is a destructive, self perpetuating chain reaction, releasing Malonyldialdehyde (MDA) as the end product. The reason for increased MDA levels in all patients may be due to increased reactive oxygen products and decreased antioxidants. Insufficient neutralization of free radicals causes the oxidation of cellular lipids, proteins, nucleic acids, glycolipids and glycoprotiens [27]. This oxidative effect also causes damage to the vascular endothelial cells, as evident from increased MDA levels observed in our study in elderly patients.

Increase in MDA and decrease in SOD GPX, and GR activities ageing patients might be due to its inactivation by increased oxidative stress . This decrease in SOD activity may be explained with the effect of increased oxygen-derived free radicals on SOD. It's known that, lower $O_2\bullet^-$ concentrations induce the SOD activity while higher $O_2\bullet^-$ concentrations inhibit. Furthermore catalase is activated in higher H_2O_2 concentrations while SOD is inhibited.

SOD activity is undoubtedly important to the regulation of oxidative status in diabetes. However, there is variation as to the status of this enzyme in the diabetic state. Some studies have reported decreased SOD activity [28,29]. While others have shown increases [30] or no change in the enzyme [31,32].

GPx catalyses the reduction of variety of hydrogen peroxide (ROOH and H_2O_2) using glutathione as a substrate, thereby protecting mammalian cells against oxidative stress .It is well reported that low activity of this enzyme may render the tissue more susceptible to lipid peroxidation damage accompanied by a significant increase in MDA level. It seems that in elderly people the GPx status is largely related to their health status [33-35], potentially through a relation with selenium status [36] and reduced levels have also been related to presence of illness. The level of erythrocyte SOD and the decrease of GR activity can be interpreted as an insufficient antioxidant defence in erythrocytes to cope with enhanced ROS production in aging, DM and CVD. The increase in lipid peroxidation is in accordance with previous studies which showed that lipid oxidative damage increases in animal models and human erythrocytes with age DM and CVD. Lipid oxidative damage resulted in the degeneration of membrane structure and the loss of membrane integrity and functionality. Maritim and colleagues recently reviewed in detail that diabetes has multiple effects on the protein levels and activity of these enzymes, which further augment oxidative stress by causing a suppressed defence response [7] For example, in the heart, which is an important target in diabetes and prone to diabetic cardiomyopathy leading to chronic heart failure, SOD and glutathione peroxidase expression as well as activity are decreased whereas catalase is increased in experimental models of diabetes [7,37,38]. The reason for increased MDA levels in all patients may be due to increased reactive oxygen products and decreased antioxidants and had a lower NO (nitrite/nitrate) levels in plasma in all patients when compared with controls. Superoxide reacts rapidly with NO resulting in the formation of peroxynitrite and loss of NO bioavailability. Furthermore, exposure of endothelial cells to high glucose leads to augmented production of superoxide anion, which may quench nitric oxide. Decreased nitric oxide levels result with

impaired endothelial functions, vasodilation and delayed cell replication. The availability of NO in diabetes is reduced, and this leads to vasoconstriction, an altered vascular redox state, abnormal growth of vascular smooth muscle cells, and prothroembotic changes in the vessel wall [39].

eNOS is a major source of superoxide in diabetes, where it preferentially transfers electrons to molecular oxygen, thus 'uncoupling' itself and producing superoxide instead of NO. Linking oxidant stress with NO-related endothelial dysfunction is a potentially fruitful concept when applied to geriatric vessels. Superoxide generation impairs NO bioavailability, most likely through inhibition of the NO-synthesizing enzyme, endothelial NO synthase [40,41]. Furthermore, large vessel superoxide generation increases and NO bioavailability decrease with age. If indeed the aging large blood vessel increases the production of ROS leading to oxidative stress, therapies might be targeted to the reduction of the offending agents, thereby restoring NO vasodilation capability and either reducing existing hypertension or preventing its development with aging [42]. Lowered concentration of NO indicates damage to the endothelium in hypertension. The study not only showed significantly lower nitrate/nitrite serum levels in patients with essential arterial hypertension than in younger and older controls but also significantly lower nitrate/nitrite serum levels in older normotensive subjects than in younger ones. We measured TAC as an indicator of total antioxidant capacity in the plasma. The majority of the TAC is composed of uric acid. Uric acid tends to increase with age, which may cover part of the antioxidant deficiency in old age [43]. Decrease in TAC implies a diminution of anti-oxidative capacity which may be caused due to the reduction in individual antioxidants. Decrease in SOD and glutathione peroxidase in all three groups main enzymatic defence systems, against free radicals. Several studies have reported an age-related decline in erythrocytic SOD. Therefore, the decreased total TAC found in our study can be attributed to the diminution of the individual antioxidants. The total antioxidant capacity is not a simple sum of the activities of the various antioxidant substances but the cooperation of the antioxidants in human serum that provides a greater protection against attacks by free radicals. It is the dynamic equilibrium between various antioxidants. A non equilibrium or poor cooperation between the antioxidants may possibly result in low TAC.

Summing up, this study confirms the intensification of oxidative stress in elderly patients, with associated essential arterial hypertension, DM and CVD. Moreover, this study indicates the possible participation of reactive oxygen species, not only in the physiological aging process, but also, most importantly, in the pathogenesis of old age diseases, including arterial hypertension, DM and CVD which may accelerate the aging process. The antioxidant defence mechanisms are not sufficient to prevent age related increase in oxidative damage and dietary intake of a variety of antioxidants might be beneficial for preserving the normal function in elderly people.

REFERENCES

1. Harman D. The ageing process. Proc Natl Sci. 1981;78:7124-8.
2. Laila G,Yues A, Bernad H, Claude J, Gerard C, Gerard S. Biological variability of superoxide dismutase, glutathione peroxidase and catalase in blood. Clin Chem. 1991;37:1932-7.
3. Harman D. Free radicals in aging. Mol Cell Biochem. 1988;84:155-61.
4. Zalba G., Jose GS, Moreno MU, Fortuno MA, Fortuno A, Beaumont FJ, Diez J. Oxidative stress in arterial hypertension. Role of NAD(P)H oxidase. Hypertension. 2001;38:1395-9.
5. Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc Natl Acad Sci USA. 2000;97:8841-8.
6. Touyz RM. Oxidative stress and vascular damage in hypertension. Curr Hypertens Rep. 2000;2:98-105.
7. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol. 2003;17:24-38.
8. Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. Reactive oxygen species, cell signaling, and cell injury. Free Radic Biol Med. 2000;28:1456-62.
9. Vega-Lopez S, Devaraj S, Jialal I: Oxidative stress and antioxidant supplementation in the management of diabetic cardiovascular disease. J Investig Med. 2004;52**:**24-32.
10. Cortas NK , Wakid NW. Estimation of total serum nitrite in biological samples. Clin Chem. 1990;36:1440-3.
11. Pagila DS, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte Glutathione peroxidase. J Lab Clin Med. 1967;70:158-69.
12. Mannervik B. Glutathione reductase. Methods in Enzymology. 1985;113:484-90.
13. Marklund S, Marklund G. Involvement of the superoxide anion radical in acute oxidation of pyrogallol as a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47:469-74.
14. Byrne JA, Grieve DJ, Cave AC, Shah AM. Oxidative stress and heart failure. Arch Mal Coeur. 2003;96:214-21.
15. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxi- dant activity in human fluids. J Clin Pathol. 2001;54:356-61.
16. Matsubara LS, Machado PE. Age-related changes of glutathione content, glutathione reductase and glutathione peroxidase activity of human erythrocytes. Braz J Med Biol Res. 1991;24:449-54.
17. Ceballos-Picot I, Trivier JM, Nicole A, Sinet PM, Thevenin M. Age-correlated modifications of copper-zinc superoxide dismutase and glutathione-related enzyme activities in human erythrocytes. Clin Chem. 1992;38:66-70.
18. Boullier A, Bird DA, Chang MK, Dennis EA, Friedman P, Gillotre-Taylor K, Hörkkö S, Palinski W, Quehenberger O, Shaw P, Steinberg D, Terpstra V, Witztum JL. Scavenger receptors, oxidized LDL, and atherosclerosis. Ann N Y Acad Sci. 2001;947:214-22; discussion 222-3.
19. Taniyama Y, Griendling KK: Reactive oxygen species in the vasculature: Molecular and cellular mechanisms. Hypertension. 2003,42**:**1075-81.
20. Turko IV, Marcondes S, Murad F. Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA:3-oxoacid CoA-transferase. Am J Physiol Heart Circ Physiol. 2001;281**:**H2289-94.
21. Liu Y, Gutterman DD. The coronary circulation in diabetes: influence of reactive oxygen species on K+ channel-mediated vasodilation. Vascul Pharmacol. 2002;38**:**43-9.
22. Liu Y, Terata K, Chai Q, Li H, Kleinman LH, Gutterman DD. Peroxynitrite inhibits Ca2+-activated K+ channel activity in smooth muscle of human coronary arterioles. Circ Res. 2002;91**:**1070-6.
23. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part I: Basic mechanisms and in vivo monitoring of ROS**.** Circulation. 2003;108**:**1912-6.
24. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part II: animal and human studies. Circulation. 2003;108:2034-40.
25. Meeks WM. Pathophysiology of hypertension in the elderly. Semin Nephrol. 2002;22:65-70.
26. Sander G**.** High blood pressure in the geriatric population: treatment considerations. Am J Geriatr Cardiol. 2002;11:223-32.
27. Afanas'ev IB. Mechanism of superoxide-mediated damage relevance to mitochondrial aging. Ann N Y Acad Sci. 2004;1019:343-5.
28. Kedziora-kornatowska K, Szram S, Kornatowski T, Szadujkis-Szadurski L, Kedziora J, Bartosz G: Effect of vitamin E and vitamin C supplementation of antioxidative state and renal glomerular basement membrane thickness in diabetic kidney. Exp Nephrology 2003;95**:**134-43.
29. Obrosova I, Fathallah L, Greene D. Early changes in lipid peroxidation and antioxidative defense in rat retina. Eur J Pharm. 2000;398**:**139-46.
30. Rauscher F, Sanders R, Watkins JI. Effects of coenzyme Q10 treatment on antioxidant pathways in normal and streptozotocin-induced diabetic rats. J Biochem Mol Toxicol. 2001;15**:**41-6.
31. Mekinova D, Chorvathova V, Volkovova K, Staruchova M, Grancicova E, Klvanoca J, Ondreicka R. Effect of intake of exogenous vitamins C, E and beta-carotene on the antioxidative status in kidneys of rats with streptozotocin-induced diabetes. Nahrung. 1995;39**:**257-61.

32. Maritim A, Sanders R, Watkins JI. Effects of alpha-lipoic acid on biomarkers of oxidative stress in streptozotic-induced diabetic rats. J Nutr Biochem. 2003;14:288-94.

33. Wright AJ, Southon S, Bailey AL, Finglas PM, Maisey S, Fulcher RA. Nutrient intake and biochemical status of non-instutionalized elderly subjects in Norwich: comparison with younger adults and adolescents from the same general community. Br J Nutr. 1995;74:453-75.

34. Cristol JP, Abderrazick M, Favier F, Michel F, Castel J, Leger C, Descomps B. Impairment of antioxidant defense mechanisms in elderly women without increase in oxidative stress markers: "a weak equilibrium." Lipids. 1999;34 suppl:S289 .

35. Kasapoglu M, Ozben T. Alterations of antioxidant enzymes and oxidative stress markers in aging. Exp Gerontol. 2001;36:209-20.

36. Bates CJ, Thane CW, Prentice A, Delves HT. Selenium status and its correlates in a British national diet and nutrition survey: people aged 65 years and over. J Trace Elem Med Biol. 2002;16:1-8.

37. Kaul N, Siveski-Iliskovic N, Hill M, Khaper N, Seneviratne C, Singal PK. Probucol treatment reverses antioxidant and functional deficit in diabetic cardiomyopathy. Mol Cell Biochem. 1996;160-161**:**283-8.

38. Hayden MR, Tyagi SC. Myocardial redox stress and remodeling in metabolic syndrome, type 2 diabetes mellitus, and congestive heart failure. Med Sci Monit. 2003;9:SR35-52.

39. Harrison DG. Cellular and molecular mechanisms of endothelial cell dysfunction. J Clin Invest. 1997;100:2157.

40. Rengasamy A, Johns RA. Inhibition of nitric oxide synthase by a superoxide generating system. J Pharmacol Exp Ther. 1993;267:1024-7.

41. Sheehy AM, Burson MA, Black SM. Nitric oxide exposure inhibits endothelial NOS activity but not gene expression: a role for superoxide. Am J Physiol Lung Cell Mol Physiol. 1998;274:L833-41.

42. Elmarakby AA, Williams JM, Pollock DM. Targeting sources of superoxide and increasing nitric oxide bioavailability in hypertension. Curr Opin Investig Drugs. 2003;4:282-92.

43. Bunker VW. Free radicals, antioxidants and ageing. Med Lab Sci. 1992;49:299-312.