AGE-RELATED ALTERATIONS IN LIPID PEROXIDATION AND ACTIVITIES OF ERYTHROCYTE CYTOPROTECTIVE ENZYMES IN WOMEN

Farshad Amirkhizi[(1)], Fereydoun Siassi[(2)], Sara Minaie[(3)], Mahmoud Djalali[(4)] Abbas Rahimi[(5)], Maryam Chamari[(6)]

Abstract

INTRODUCTION: The incidence of atherosclerosis increases with age, as do various indices of free-radical-mediated damage, e.g. lipid peroxidation. Because Antioxidant enzymes are the major defense system of cells in normal aerobic reactions, we aimed to assess the age-related alterations in the activity of erythrocyte cytoprotective enzymes among women.

METHODS: One hundred sixty 20-45-year-old women were randomly selected among women receiving the services of rural health centers of Kerman Province, Iran. Data were gathered by using questionnaires and face-to-face interviews. We assessed lipid peroxidation by measuring the concentrations of plasma malondialdehyde (MDA), total antioxidant capacity (TAC), and the activities of erythrocyte copper-zinc superoxide dismutase (CuZn-SOD), glutathione peroxidase (GPX) and catalase (CAT).

RESULTS: Those individuals in the highest quartiles of age and number of pregnancies presented the highest levels of plasma MDA ($P<0.001$). We also observed an inverse relationship between age and erythrocyte CuZn-SOD and GPX activities. Although we found no significant difference between age groups in respect of erythrocyte CAT activity and/or plasma TAC levels, erythrocyte GPX activity was negatively correlated with the number of pregnancies ($P<0.001$) . No significant difference was found between age groups and/or between quartiles of number of pregnancies for either energy or nutrient intake. Plasma MDA levels were positively related to age ($r=0.307$; $P<0.0001$), number of pregnancies ($r=0.250$; $P<0.001$), fat intake ($r=0.281$; $P<0.05$) and Vitamin E intake ($r=0.356$; $P<0.01$). Furthermore, there were negative correlations both between age and GPX activity ($r= -0.280$; $P<0.0001$) as well as with CuZn-SOD($r= -0.228$; $P<0.005$).

CONCLUSIONS: Lipid peroxidation and antioxidants were affected by age. Erythrocyte cytoprotective enzymes have an important role in detoxification of free radicals in the body; the age-related decrease in the activities of these enzymes might contribute to atherogenesis, along with classic risk factors.

Keywords: Age, lipid peroxidation, cytoprotective enzymes, oxidative stress, women.

ARYA Atherosclerosis Journal 2007, 3(2): 81- 86

Date of submission: 18 Apr 2007, *Date of acceptance:* 30 Jun 2007

Introduction

Aging can be defined as a process of deterioration, [1] or a decrease in adaptive abilities due to progressive failure of maintenance[2]. The free radical theory of aging provides an attractive mechanistic viewpoint that may explain molecular changes associated with the aging process.[3]

Cardiovascular diseases (CVDs), often associated with aging, have been a leading cause of morbidity and mortality in developed and developing countries[4]. CVD, including coronary heart disease, stroke, and peripheral vascular disease are the clinical expression of advanced atherosclerosis.[4] Increased level of low-density lipoprotein-cholesterol (LDL-C) is a major risk factor in the pathogenesis of atherosclerosis and its subsequent sequelae.[5]

(1) Farshad Amirkhizi, M.Sc., Nutritionist, Department of Nutrition and Biochemistry, School of Public Health, Tehran University of Medical Sciences (TUMS), Tehran, Iran.
(2) Fereydoun Siassi, Ph.D., Professor. Department of Nutrition and Biochemistry, School of Public Health, TUMS, Tehran, Iran. Tel: +9821 88954924, E-mail: Siassif@hotmail.com
(3) Sara Minaie, M.Sc., Nutritionist, Department of Nutrition and Biochemistry, School of Public Health, TUMS, Iran.
(4) Mahmoud Djalali, Ph.D., Associate Professor, Department of Nutrition and Biochemistry, School of Public Health, TUMS, Iran.
(5) Abbas Rahimi, Ph.D., Assistant professor, Department of Epidemiology and Public Health, School of Public Health, TUMS, Iran.
(6) Maryam Chamari, M.Sc., Nutritionist, Department of Nutrition and Biochemistry, School of Public Health, TUMS, Iran.
Corresponding author: Fereydoun Siassi

A number of recent publications have focused attention on the risk of CVD events in women[6,7] and epidemiological studies have demonstrated a relationship between the number of pregnancies and an increased risk of CVD later in life.[8]

Oxidative stress is being increasingly recognized as an important CVD risk factor in high-risk individuals.[9] Oxidative stress can be defined as the imbalance between free radical damage,e.g. the oxidation of lipids and antioxidant protection. Likewise, it is assumed that lipid peroxidation, subsequent to free-radical damage, is involved in the process of aging.[10] The superoxide anion ($O_2^{\bullet-}$) is known to inactivate enzymes and initiate the damaging chain reactions of lipid peroxidation.[11] Cellular defensive mechanisms against superoxides include series of linked enzyme reactions which remove the toxic radicals and repair radical-induced damage. The first of these enzymes is superoxide dismutase (SOD) which converts the superoxide anion into hydrogen peroxide. Hydrogen peroxide, also toxic to cells, is removed by catalase.[12] Although animal studies have shown that oxidative stress levels increase and endogenous antioxidant enzymes activities decrease with age,[13,14] to the best of our knowledge, there is no report in the literature of the effect of increased age *per se* on lipid peroxidation and erythrocyte cytoprotection in humans.

A number of prospective cohort and case-control studies have reported that increased intake of dietary antioxidants including vitamins E and Vitamin C is associated with reduced risk of CVD.[15,16]

The objective of this study was to examine the association of age and number of pregnancies with lipid peroxidation level and activities of erythrocyte cytoprotective enzymes in selected groups of women.

Materials and methods

The subjects used in this study were recruited from among women receiving the services of rural health centers of Kerman Province, Iran. A number of 160 women aged 20-45 years (mean age: 31.5 years) were randomly selected. We excluded pregnant and lactating women and those with history of cancer, CVD, diabetes and hypertension, renal and/or liver diseases, as well as those taking vitamin or mineral supplements. Informed written consent was obtained from subjects before entering the study. General data were gathered using questionnaires and face-to-face interviews. Food intake was assessed using a 24-hour recall questionnaire, extensively described elsewhere. The Food Processor® Program (Version 2) was used to analyze the data from the 24-hour recall. Average daily intakes were calculated for each participant for energy (Kcal/day), as well as the following nutrients: protein (g/day), fat (g/day), vitamin E (mg/day) and iron (mg/day).

Venous blood samples were collected from the median cubital vein into standard tubes containing ethylene diamine tetra acetic acid (EDTA). Blood samples were centrifuged at 3000 rpm. for 10 minutes at 4°C and plasma was separated for MDA assay. The buffy coat was removed and the remaining erythrocytes were washed three times in cold saline (9.0 g/l NaCl) and hemolyzed by the addition of cold deionized water. The subjects' plasma and hemolysate samples were stored at -70°C until analysis.

Plasma malondialdehyde (MDA) concentrations were assayed by measurement of thiobarbituric acid reactive substances (TBARS) according to the Satoh method.[17] The pink chromogen produced by the reaction of thiobarbituric acid with MDA was measured at 530 nm.

In order to express enzyme activities per gram hemoglobin (Hb), Hb concentration was measured in the hemolysate samples with a standard kit using the cyanmethemoglobin method (Drabkin's method).

Catalase (CAT, E.C.1.11.1.6) activity was determined according to Hygo Aebi.[18] Activity of CAT was determined by following the decomposition of H_2O_2 in phosphate buffer pH 7.2 spectrophotometrically at 230 nm.

Glutathione peroxidase (GPX, E.C.1.11.1.9) activity was measured according Paglia and Valentine method[19] and copper zinc-superoxide dismutase (CuZn-SOD) (SOD, E.C.1.15.1.1) activity was assayed by RAN-SOD kit (cat. NO. SD 125). Plasma total antioxidant capacity (TAC) levels were determined by colorimetric assay using 2, 2'-Azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS).[20] The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS to $ABTS^{\bullet+}$ by a peroxidase.

The amount of $ABTS^{\bullet+}$ produced can be monitored by reading the absorbance at 600 nm.

Data were expressed as mean ± standard deviation (SD). Statistical differences between the groups were evaluated by analysis of variance (ANOVA).

The association between variables was evaluated by Pearson correlation coefficients. Significance level was set at $P<0.05$. All statistical analyses were done with the SPSS for Windows version 12.5 statistical package (SPSS.Inc, Chicago IL).

Results

Mean values of MDA and TAC plasma levels and activities of erythrocyte CuZn-SOD, GPX and CAT according to quartiles of age and number of pregnancies are shown in Table 1. Subjects in the highest quartiles of age presented the highest levels of plasma MDA (P<0.001).

Those in the highest quartiles of number of pregnancies also had the highest mean values of plasma MDA (P<0.05). We also observed an inverse relationship between age and the activities of erythrocyte CuZn-SOD and GPX (Table 1).

The results showed that subjects with the highest quartiles of age had lowest erythrocyte CuZn-SOD and GPX activities (P<0.01).

Furthermore, erythrocyte GPX activity was negatively correlated with the number of pregnancies (P<0.001). No significant difference was found between age groups in erythrocyte CAT activity and plasma TAC levels. There were no significant differences between age groups and/or between quartiles of number of pregnancies for either energy or nutrient intake (Table 2). Table 3 shows a statistically positive correlation between plasma MDA levels and age (r=0.307; P<0.0001), number of pregnancies (r=0.250; P<0.001), fat intake (r=0.281; P<0.05) and vitamin E intake (r=0.356; P<0.01). Negative correlations were found between age and GPX activity (r = -0.280; P<0.0001) and between age and CuZn-SOD (r= -0.228; P<0.005).

TABLE 1. Mean (SD) of MDA and TAC plasma levels and activities of erythrocyte CuZn-SOD, GPX and CAT according to quartiles of age and number of pregnancies

	MDA (µmol/L)	CAT (K/gHb)	GPX (U/gHb)	CuZn-SOD (U/gHb)	TAC (µmol/L)
Age groups (years)					
<27	1.83 ± 0.61	197.2 ± 68	56.6 ± 16.5	684 ± 98	2.01 ± 0.41
27-31	2.05 ± 0.94	208.3 ± 71	49.3 ± 12.3	643 ± 71	1.97 ± 0.30
32-39	2.36 ± 0.82†	202.6 ± 65	47.2 ±11.4	624 ± 84‡	1.89 ± 0.43
>39	2.68 ± 0.63†	192 ± 85	44.5 ± 12.1‡	612 ± 77‡	1.88 ± 0.36
Number of pregnancies					
0	1.95 ± 0.70	185.4 ± 62	55.9 ± 15.2	661 ± 106	1.98 ± 0.40
1-2	2.28 ± 0.72	209 ± 70	51.4 ± 13.7	648 ± 104	1.92 ± 0.37
3-4	2.42 ± 0.81§	195 ± 81	48 ± 14.7	642 ± 98	1.91 ± 0.28
>5	2.56 ± 0.78§	197 ± 77	44 ± 15.1†	621 ± 89	1.88 ± 0.32

Significantly difference from first quartile; †P< 0.001; ‡P< 0.01; §P< 0.05

TABLE 2. Mean (SD) daily energy and nutrient intakes according to quartiles of age and number of pregnancies in participants

	Energy (Kcal)	Protein (g)	Fat (g)	Vitamin E (mg)	Iron (mg)
Age groups (years)					
<27	1948 ± 428	54.3 ± 14.2	86.5 ± 19.3	6.3 ± 3.1	14.5 ± 5.7
27-31	2257 ± 396	49.6 ± 13.1	95.7 ± 17.5	5.9 ± 2.7	16.6 ± 6.2
32-39	2097 ± 529	51.7 ± 16.4	104.4 ± 26.4	6.1 ± 3.4	15.2 ± 7.1
>39	2138 ± 759	50.6 ± 15.6	98.1 ± 20.4	5.3 ± 2.1	14.7 ± 5.9
Number of pregnancies					
0	2059 ± 587	48.6 ± 14.6	88.2 ± 21.3	6.7 ± 2.9	14.1 ± 5.4
1-2	2143 ± 625	50.3 ± 15.1	92.6 ± 19.6	7.1 ± 3.1	15.1 ± 8.2
3-4	2254 ± 625	49.8 ± 17.6	102.6 ± 27.3	6.1 ± 2.8	16.2 ± 7.2
>5	2368 ± 495	51.3 ± 16.2	98.4 ± 23.4	5.8 ± 2.1	15.9 ± 7.6

TABLE 3. Pearson correlation coefficients between dependent and independent variables (N=160).

Variables	MDA (μmol/L)		CAT (K/gHb)		GPX (U/gHb)		CuZn-SOD (U/gHb)		TAC (μmol/L)	
	r	p	r	p	r	p	r	p	r	p
Age (years)	0.307	<0.0001	0.046	N.S.	-0.280	<0.0001	-0.228	<0.005	0.040	N.S.
Number of pregnancies	0.250	<0.001	0.058	N.S.	-0.064	N.S.	-0.048	N.S.	0.029	N.S.
Energy (Kcal/day)	0.032	N.S.*	0.095	N.S.	0.088	N.S.	-0.056	N.S.	0.036	N.S.
Protein intake (g/day)	0.074	N.S.	0.065	N.S.	0.042	N.S.	0.038	N.S.	0.112	N.S.
Fat intake (g/day)	0.281	<0.05	0.077	N.S.	0.071	N.S.	-0.124	N.S.	0.025	N.S.
Vitamin E (mg/day)	0.356	<0.01	0.056	N.S.	0.092	N.S.	0.082	N.S.	0.073	N.S.
Iron intake (g/day)	0.179	N.S.	0.032	N.S.	0.021	N.S.	-0.130	N.S.	-0.10	N.S.

*N.S: Not significant

Discussion

Lipid peroxidation is a free radical-generating process which occurs on every membranous structure of the cell. Free radicals are known to be involved in a number of human pathologies including atherosclerosis,[21] cancer,[22] and hypertension.[23] [First, the most important finding and its interpretation should be provided, then the following details:] In our comparison of age groups, we found significant elevations in plasma MDA with increasing age. Two possible factors may have led to the increased lipid peroxidation: the increasing production of free radicals and the declining activity of the antioxidant system. Antioxidant enzymes are the major defense system of cells in normal aerobic reactions.[13] In this study, the activities of erythrocyte CuZn-SOD and GPX decreased with age. This finding is consistent with the studies of Benzi et al.[24] and Carrilo et al.[25] that have demonstrated that the activities of antioxidant enzymes, total SOD, and Mn SOD decreased with aging in brain cortices and the cerebellum. Sawada and Carlson [26] report that superoxide radical formation increases with age; hence decreased protection against toxic radicals may have serious consequences for cell membranes. In contrast, the animal model of Matsuo et al.[27] implies that activities of catalase and SOD remain relatively stable throughout the life span. In the present study, mean CAT activity did not differ significantly with age. Vertechy et al.[28] point out that in general, the activity of catalase declines during the maturation of the animal to adulthood. These controversies may be due to differences in methodologies for tissue preparation and enzyme activity determination. These discrepancies may also be due to differences in organs, sex, species and ages of the animals studied.

Tsay et al.[13] found that the rate of mitochondrial $O_2^{\bullet-}$ generation increased with age. On the other hand, the loss of properly balanced antioxidant defense may cause further oxidative stress in tissues. In our study, the non-enzymatic antioxidant levels, e.g. of glutathione, were not investigated. However, these findings support the notion that both the rate of lipid peroxidation and oxidative stress increased with age.

The Framingham Heart Study, consisting of 28 years of patient follow-up, and the National Health and Nutrition Examination Survey (NHANES), with a 12-year follow-up, both found a consistent relationship between the number of pregnancies and the subsequent development of CVD.[8] In our study, the number of pregnancies had positive relationship with plasma MDA levels, and negative correlation with erythrocyte GPX activity. Hence, it is suggested that the relationship between the number of pregnancies and development of CVD might be partially linked to increased lipid peroxidation and decreased activity of antioxidant enzymes.

Although we did not document any significant difference between age groups and/or between the number of pregnancy quartiles for either energy or nutrient intake, but fat intake was significantly correlated with plasma MDA levels. A possible mechanism to explain this observation is that increased polyunsaturated fatty acids (PUFA) in tissues promotes lipid oxidation by increasing the amount of substrate available for peroxidation. Double bonds in the fatty acid molecules are vulnerable to oxidation reactions and consequently may cause lipid peroxidation.[29]

Several epidemiological studies have reported that increased intake of dietary antioxidants including vitamin E is associated with reduced risk of CVD.[15,16] In contrast, our data showed a positive correlation between vitamin E intake and plasma MDA levels. Vitamin E becomes a pro-oxidant under certain conditions.[30] An increase in oxidative stress by free radicals or imbalance in antioxidant status may be one of the causes of aging.[31] With age and increasing oxidative stress, endogenous antioxidant systems may be challenged beyond their ability to maintain the pro-oxidant-to-antioxidant equilibrium. Because of these intrinsic age-related changes, it has been

suggested that endogenous antioxidants may be useful for maintaining the redox status in the body.[7] However, antioxidant vitamins may be ineffective once LDL oxidation has progressed, i.e. atherosclerosis has progressed to a certain point where vitamin E might be harmful[31,32] without limiting vitamin C. However, since serum vitamin E and C levels were not measured in our subjects, we can make no judgment as to whether plasma vitamin E and C concentrations correlated with plasma MDA levels. However, our results were in agreement with those of Nakamura[33] who found a positive relation between daily vitamin E intake and serum MDA levels in young and elderly humans.

Our findings show that in women , activities of erythrocyte cytoprotective enzymes decrease with age. These changes may contribute to increased free radical-mediated damage in the body. The results also bear out the concept that increased number of pregnancies and age could increase the risk of coronary artery disease by promoting lipid peroxidation. Longitudinal studies are warranted to determine whether or not these MDA levels are related to vascular disease and/or mortality.

Acknowledgements

This study was funded by the Research Council of the School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences. We extend our sincerest thanks to all women who participated in this study.

References

1. Vanfletern JR, Braeckman BP. Mechanisms of life span determination in Caeno-rhabditis elegans. Neurobiol Aging. 1999;20:487-502.
2. Rattan SI. Applying hormesis in aging research and therapy. BELLE Newslett 2001;9:2-5.
3. Granot E, Kohen R. Oxidative stress in childhood in health and disease states. Clin Nutr. 2004;23:3-11.
4. Schwenke D.C. Antioxidants and atherosclerosis. J Nutr Biochem.1998;9: 424-45.
5. Jira W, Spiteller G, Schramm A. Increased in hydroxyl fatty acids in human low density lipoproteins with age. Chem Phys Lipids 1996; 84: 165-73.
6. Edmunds E, Lip GYH. Cardiovascular risk in women: the cardiologist' perspective. Q J Med 2000;93:1435-45.
7. Hu FB, Stampfer MJ, Manson JE, Grodstein F, Colditz GA. Trends in the incidence of coronary heart disease and changes in diet and life style in women. New Engl J Med. 2000;343:530-7.
8. Ness R.B, Harris T, Cobb J, Kelsey J.L, Balanger A, Stunkard A.J, D'Agostino R.B. Number of pregnancies and the subsequent risk of cardiovascular disease. New Engl J Med 1993; 328: 1528-33.
9. Steinberg D, Witztum JL. Is the oxidative modification hypothesis relevant to human atherosclerosis? Circulation. 2002;105:2107-11.
10. Schwenke D.C, Antioxidants and atherosclerosis. J Nutr Biochem.1998;9: 424-45.
11. Lenaz G. Role of mitochondria in oxidative stress and aging. Biochem Biophys Acta. 1998;1366:53-67.
12. Vertechy M, Cooper MB, Ghirardi O, Ramacci MT. Antioxidant enzyme activities in heart and skeletal muscle of rats of different ages. Exp Gerontol .1989;24:211-8.
13. Tsay HJ, Wang SL, Hai Ku H. Age-associated changes of superoxide dismutase and catalase activities in the rat brain. J Biomed Sci. 2000;7:466-74.
14. Apler G, Sozmen E, Kanit L. Age-related alterations in superoxide dismutase and catalase activities in rat brain. Tr J Med Sci 1998; 28: 491-4.
15. Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Ronser B, Willett WC. Aprospective study of Vitamin E consumption and risk of coronary disease in women. N Engl J Med. 1993;328:1444-9.
16. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med. 1993;328:1450-6.
17. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta. 1978;90(1):37-43.
18. Abei H. Catalase invitro. Methods Enzymol 1984; 105:121-6.
19. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967; 70:158-69.
20. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci. 1993;84:407-12.
21. Steinberg D. A critical look at the evidence for the oxidation of LDL in atherogenesis. Atherosclerosis.1997;13:5-7.
22. Cerutti PA. Oxy-radicals and cancer. Lancet .1994;344:796-8.
23. Russo C, Oliviri O, Girelli D, Faccini G, Zenari MI, Lombardi S, Corrocher R. Anti-oxidant status and lipid peroxidation in patients with essential hypertension. J Hypertens. 1998;16:1267-1271.
24. Benzi G, Marzatico F, Pastoris O, Villa RF. Relationship between ageing, drug treatment and the cerebral enzymatic antioxidant system. Exp Gerontol. 1989;24:137-48.
25. Carrilo MC, Kanai S, Satoh Y, Kitani K. Age-related changes in antioxidant enzyme activities is region and organ, as well as sex, selective in the rat. Mech Ageing Dev. 1992;65:187-98.
26. Sawada M, Carlson JC. Changes in superoxide radical and lipid peroxide formation in the brain, heart and liver during the life time of the rat. Mech Ageing Dev. 1987;41:125-37.
27. Matsuo M, Gomi F, Dooley MM. Age related alterations in antioxidant capacity and lipid peroxidation in brain, liver and lung homogenates of normal and vitamin E-deficient rats. Mech Ageing Dev. 1992;64:273-92.

28. Vertechy M, Cooper MB, Ghirardi O, Ramacci MT. The effect of age on the activity of enzymes of peroxide metabolism in rat brain. Experimental Gerontology. 1993;28:77-85.
29. Vincent HK, Powers SK, Stewart DJ, Shanely RA, Demirel H, Nalto H. Obesity is associated with increased myocardial oxidative stress. Int J Obes Relat Metab Disord. 1999;23:67-74.
30. Carr A.C, Zhu B, Frei B. Potential antiatherogenic mechanisms of ascorbate (vitamin C) and alpha-tocopherol (vitamin E). Circu Res 2000; 87: 349-54.
31. Mezzeti A, Auliani G, Romano F, Costantini F, Pierdomenico S.D, Cuccurullo F, Fellini R. Vitamin E and lipid peroxide plasma levels predict thr risk of cardiovascular events in a group of healthy very old people. J Am Geriatr Soc. 2001;49:533-7.
32. Terentis AC, Thomas SR, Burr JA, Liebler DC, Stocker R. Vitamin E oxidation in human atherosclerotic lesions. Circul Res. 2002;90:333-9.
33. Nakamura Y, Read MH, Jeffry WE, Omaye ST. Oxidation of serum low-density lipoprotein (LDL) and antioxidant status in young and elderly humans. Arch Geron Geriatr. 2006;42:265-76.