Korean J Nutrition 30(9) : 1109~1115, 1997

Age- and Sex-Related Differences in Serum Levels of Lipid Peroxide, Retinol and α-Tocopherol in Korean Adults*

Choi, Young-Sun · Shin, Ji-Hee**

Department of Food and Nutrition, Taegu University, Kyungbuk, Korea
*Department of Food Science and Nutrition,** Catholic University of Taegu-Hyosung, Kyungbuk, Korea*

ABSTRACT

The present study was aimed to investigate whether there are age- and sex-related differences in serum levels of lipid peroxides, retinol, and α-tocopherol in Korean adults. The subjects were 441 persons, including 268 men and 173 women. Those of each sex were divided into four age groups : 20 – 29, 30 – 39, 40 – 49 and 50 – 65 years, and their lifestyles and serum levels were compared.

Men smoked and drank less as age increased, but 99.9% of women did not smoke and only 11% drank more than once a month. Lipid peroxides of males increased gradually with age, while those of females showed greater levels in the 50 – 65 years group thon younger groups. Lipid peroxides, retinol and α-tocopherol concentrations, which were adjusted for age, were significanty higher in males than in females. Lipid peroxide levels adjusted for total lipid were positively correlated with age in males but not in females. Serum levels of α-tocopherol adjusted for total lipid were positively correlated with age both in males and in females, while retinol was neither. The results indicate that serum levels of lipid peroxides, retinol, and α-tocopherol are affected by age and that the response could be different between males and females. (*Korean J Nutrition* 30(9) : 1109~1115, 1997)

KEY WORDS : lipid peroxide · retinol · *α*-tocopherol · Korean adults.

Introduction

Agressive oxygen species can damage DNA, proteins, carbohydrates, and unsaturated lipids of all body compartments. Unfortunately their direct investigation in vivo is difficult because most aggressive species have an extremely short half-life and cannot easily be trapped under physiological conditions[1]. Serious damage by agressive oxygen species could be due to insufficiency of the body's multilevel defense system against increased oxidative stress. Thus indirect information on increased oxidative stress in vivo may be obtained by comparisons of lipoperoxide and antioxidant concentrations in tissues[2].

Lipid peroxidation is a well-established general mechanism for cellular injury[3]. Increased lipoperoxide production has been demonstrated in a wide variety of clinical and toxicological conditions including acute myocardinal infarction[4], stroke[5], and diabetes mellitus[6]. Numerous studies[1,7] indicated inverse associations between essential antioxidants in plasma and incidences of cancer and cadiovascular diseases, which suggests a pathogenetic involvement of free radicals[1]. Recently Bonithon-Kopp, et al.[8] have reported that erythrocyte vitamin E level was significantly and negatively associated with intima-media thickness of

Accepted : October 22. 1997
*This research was supported by the Taegu University research grants, 1996.

the common carotid arteries in both men and women, while TBARS(thiobarbituric acid-reactive substances) which is a biological marker of lipid peroxidation, was not associated. However, TBARS was significantly higher in men with carotid plaques than in those without.

Age-related increases in lipid peroxide in rat testis and liver[9] and accumulation of lipofuscin in the testes of aging mice were observed[10]. In humans lipid peroxides were higher in older men than in younger men[11][12]. Higher levels of serum retinol in males and the association of serum vitamins A and E with age, adjusted for the effects of sex, are less consistent[13][14][15]. Serum vitamin E levels were found in several studies to increase gradually with age[15][16]. Higher levels of serum vitamin A among males than among females have been reported[13]. Vitamins A and E are lipid-soluble substances and their absorp-tion and metabolism are associated with those of lipids[17], and lipid metabolism is closely related with sex and age.

The present study was aimed to investigate whether there are age- and sex-related differences in serum levels of lipid peroxides, retinol, and α-tocopherol in Korean adults and if the differences are held even after adjustment for serum lipid.

Subjects and Methods

All blood specimens were obtained from blood donors for the biannual health examination held in Kyungpook National University Hospital from April 22 to June 23, 1994. Questionnaires on general characteristics of the subjects were provided during the examination. Fasting blood was withdrawn and serum was prepared by centrifugation of blood at 3, 000rpm for 10 minutes and kept frozen at −65℃ until analysis.

Serum total cholesterol was determined spectrophotometrically by the enzymatic method using a kit (Asan, Korea). Serum total lipid was determined by the phosphovanillin method[18]. Thiobarbituric acid-reactive substances(TBARS) for the measurement of lipid peroxides were determined fluorometrically by the Yagi's method[19]. TBARS were extracted with n-butanol and fluorescence intensity was determined at excitation 515nm and emission 533nm using 1,1,3,3-tetraethoxypropane as a standard. Retinol and α-tocopherol were determined simultaneously by high pressure liquid chromatography according to the method of Bieri, et al.[20]. A total lipid extract from 0.1ml serum containing internal standards of α-tocopheryl acetate and retinyl acetate was injected into a micro-Bondpack C_{18} column(Waters) developed with me-thanol-water(97 : 3). Vitamins were detected at UV 292nm.

Data was statistically analyzed using SPSS program. Pearson correlation coefficient was used to analyze interrelationship of variables and analysis of covariance was carried out to exclude any effects of confounding factors.

Results

The 441 persons in the study population included 268 men and 173 women, as shown in Table 1. The men and women were divided, into four age groups : 20−29(20's), 30−39(30's) 40−49(40's), and 50−65(50−60's) years. As shown in Table 2, the majority of the subjects were office workers except the male group of 20−29 years, in which 40% were labor workers and 38% did both types of work.

Fig. 1 shows the status of cigarette smoking and alcohol drinking. For men, 75% in their 20's, 60% in

Table 1. Numbers of study subjects by sex and age

Age	Sex		Male
	Total	Female	
20−29	60	59	11927.0)
30−39	58	71	129(29.3)
40−49	69	20	89(20.2)
50−65	81	23	104(23.6)
	268(60.8)	173(39.2)	441

Percentages of subjects are in parentheses

Table 2. Type of work of the subjects

	Male			Female		
	Office work	Labor	Both	Office work	Labor	Both
20−29	13	24	23	47	4	8
30−39	34	4	20	44	6	21
40−49	47	4	18	11	5	4
50−65	57	4	20	11	8	4
	151 (56.3)	36 (13.4)	81 (30.2)	113 (65.3)	23 (13.3)	37 (21.4)

Percentages of subjects are in parentheses

their 30's, 50% in their 40's, and 42% in their 50-60's were smokers. The percentage of subjects who drank more than once a month was 80% for 20's, 74% for 30's, 68% for 40's, and 65% for 50-60's. The average alcohol intake per drinking time was in the order of 20's, 30's, 40's, and 50-60's. Men smoked and drank less as age increased,. For women, only one subject smoked and only 11% drank more than once a month.

Fig. 2 shows the levels of serum total cholesterol and total lipid according to age. Serum cholesterol did not differ among the age groups of males and did not correlate with age(r=0.003), while it differed among the age groups of female subjects and correlated with age significantly(r=0.322, $p<0.001$). Mean serum cholesterol of males was significantly higher than that of females(190 vs 176 mg/dl, $p<0.001$). Each level of 20's, 30's, and 40's female groups was significantly lower than that of the corresponding male group. Serum total lipid increased by age both in males(r=0.164, $p<0.01$) and in females(r=0.332, $p<0.001$). Total lipid did not differ between males and females(5.2 vs 5.0mg/ml).

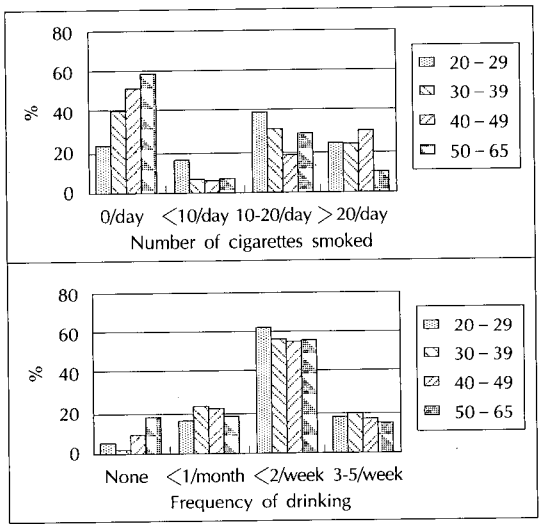

Fig. 1. Smoking and alcohol drinking habits of subjects.

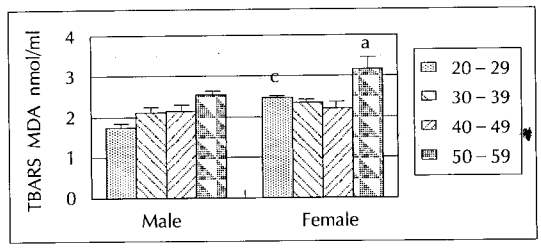

Fig. 3. Serum levels of thiobarbituric acid-reactive substances.
- a : Sex-difference of each age group is significant at $p<0.05$
- b : Sex-difference of each age group is significant at $p<0.01$
- c : Sex-difference of each age group is significant at $p<0.001$

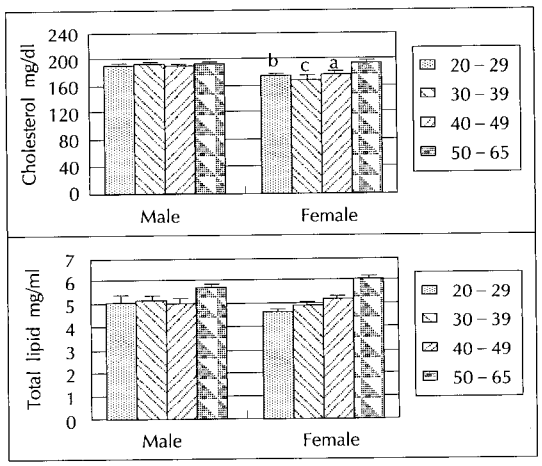

Fig. 2. Serum levels of total cholesterol and total lipid.
- a : Sex-difference of each age group is significant at $p<0.05$
- b : Sex-difference of each age group is significant at $p<0.01$
- c : Sex-difference of each age group is significant at $p<0.001$

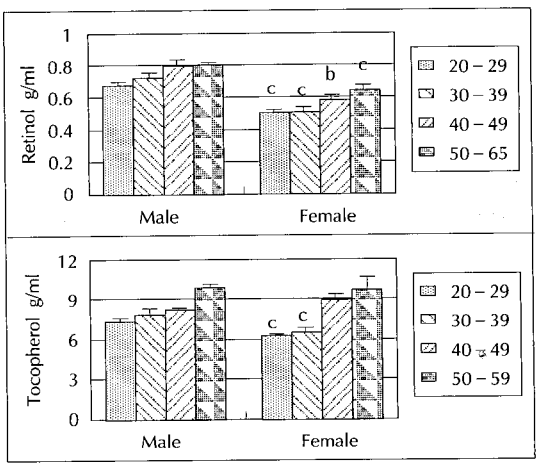

Fig. 4. Serum levels of retinol and alpha-tocopherol.
- a : Sex-difference of each age group is significant at $p<0.05$
- b : Sex-difference of each age group is significant at $p<0.01$
- c : Sex-difference of each age group is significant at $p<0.001$

Table 3. Association of serum TBARS, retinol, and α-tocopherol by age, serum total cholesterol, and total lipid

	Male			Female		
	TBARS	Retinol	α-Tocopherol	TBARS	Retinol	α-Tocopherol
Age	0.338***	0.178**	0.319***	0.172**	0.259**	0.452***
	(220)	(246)	(246)	(167)	(171)	(171)
Total	0.002	0.007	0.045	0.205*	0.037	0.215*
cholesterol	(211)	(238)	(238)	(98)	(100)	(100)
Total lipid	0.263***	0.148*	0.271***	0.056	0.380***	0.405***
	(191)	(234)	(234)	(164)	(169)	(169)

*p<0.05, **p<0.01, ***p<0.001
Numbers of subjects are in parentheses

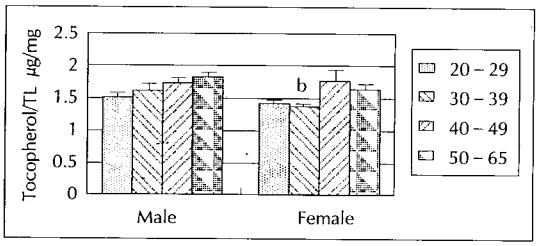

Fig. 5. Adjusted alpha-tocopherol levels for serum total lipid.
a : Sex-difference of each age group is significant at p<0.05
b : Sex-difference of each age group is significant at p<0.01
c : Sex-difference of each age group is significant at p<0.001

TBARS of males increased as age increased, while those of females were similar among 20's, 30's, and 40's(Fig. 3). TBARS levels of 20-29 and 50-65 years female groups were significantly higher than those of the corresponding male groups.

Serum levels of retinol and α-tocopherol(Fig. 4) increased as age increased. Serum retinol concentrations of all age groups were significantly lower in females than in males. Alpha-tocopherol concentration of 20-29 and 30-39 female age groups were also significantly lower than those of male age groups.

Table 3 shows overall associations of lipid peroxides, retinol, and α-tocopherol with age, total cholesterol, and total lipid. Age was positively correlated with TBARS, retinol, and α-tocopherol both in males and females. Total cholesterol was positively correlated with TBARS and α-tocopherol in females. Associations of total lipid with TBARS, retinol, and α-tocopherol were much stronger than those of total cholesterol. In this study we have found a strong correlation of serum α-tocopherol with total lipid, especially in women(r=0.405, p<0.001).

Since TBARS, retinol, and α-tocopherol were positively correlated with total lipid, and total lipid increased by age(for males, r=0.164, p<0.01 ; for females, r=0.332, p<0.001), they were divided by serum total lipid(Fig. 5) and correlated with age (Table 4). The adjusted TBARS level for total lipid was positively correlated with age in males but not in females. Serum levels of α-tocopherol after adjustment for total lipid were positiviely correlated with age both in males and in females, while serum retinol levels were not.

As shown in Table 5, Means of TBARS(2.19 vs 2.46 MDA nmol/ml, p<0.05), retinol(0.76 vs 0.53μg/ml, p<0.001), and α-tocopherol(8.58 vs 7.11μg/ml, p<0.001) were significantly higher in males than in females. Since mean ages of subjects were 41.6±11.7 years for males and 33.4±9.6 years for females and the difference was statistically significant at p<0.001, sex-related differences in serum levels of TBARS, retinol, and α-tocopherol were tested after adjustment for age. TBARS and retinol were significantly different between males and females. Alpha-tocopherol

Table 4. Association of serum TBARS, retinol, and α-tocopherol adjusted for the level of serum total lipid by age

	Male			Female		
	TBARS	Retinol	α-Tocopherol	TBARS	Retinol	α-Tocopherol
Age	0.284***	0.059	0.169**	0.018	0.042	0.222**
	(191)	(234)	(234)	(164)	(169)	(169)

*p<0.05, **p<0.01, ***p<0.001
Numbers of subjects are in parentheses

Table 5. Sex-related differences in serum TBARS, retinol, and α-tocopherol without and with adjustment for the level of serum total lipid

	Male	Female	P-value	Age-adjusted p-value
Unadjusted levels				
TBARS	2.19 ±0.97	2.46 ±1.29	<0.05	<0.001
Retinol	0.759±0.233	0.533±0.178	<0.001	<0.001
α-tocopherol	8.58 ±3.01	7.11 ±2.54	<0.001	NS
Lipid-adjusted levels				
TBARS	0.440±0.180	0.510±0.267	<0.01	<0.001
Retinol	0.152±0.056	0.111±0.036	<0.001	<0.001
α-tocopherol	1.69 ±0.576	1.47 ±0.469	<0.001	<0.05

Mean±SD NS : not significant

became insignificant after adjustment for age. However α-tocopherol/mg total lipid adjusted for age became significantly higher in males than in females.

For men, covariate analysis, in which TBARS or α-tocopherol was the dependent variable and age was covariate, showed that smoking and alcohol drinking of subjects were not significant factors. Retinol was associated with alcohol drinking significantly($p<0.01$). Subjects who drank more than 3-4 times per week had significantly higher serum retinol concentration than those who drank less frequently.

Discussion

In the present study we found age-related differences in serum lipid peroxides and α-tocopherol. Age-related increases in lipid peroxides are consistent with the results of Hagihara, et al.[11] and Knight, et al.[12] who have reported that older men had higher MDA concentrations in plasma than did younger men.

Alpha-tocopherol is mostly carried in lipoproteins[17] and serum α-tocopherol concentration is usually adjusted by serum lipids[21]. The relations of serum total lipids with lipid peroxides, retinol, and α-tocopherol levels were observed(Table 3). Populations in Western countries had a stronger correlation of serum α-tocopherol with serum total cholesterol[14][15][21], while Korean middle-aged men showed a stronger correlation of α-tocopherol with serum triglyceride[22] as compared to total cholesterol.

Age-effects on serum levels of lipid peroxides, retinol, and α-tocopherol were significant, even after adjustment for serum total lipid in our study. According to the work of Stryker, et al.[15], age was a significant predictor for serum vitamin E level, even after considering plasma lipid levels as well as vitamin E intake in women. They found that vitamin E intake, energy intake, plasma cholesterol, and plasma triglyceride in men, and vitamin E intake, energy intake, plasma total cholesterol and triglyceride, age, cigarette smoking, and alcohol drinking in women were predictors for plasma vitamin E level by multiple regression analysis. Malvy, et al.[16] reported age- and sex-specific intervals of serum retinol, β-carotene, and α-tocopherol in healthy French children and that concentrations of these micronutrients increased significantly with age, but sex had no effect.

In this study we have found sex-related differences in serum lipid proxides, retinol and α-tocopherol (Table 5). According to the work of Stryker, et al.[15] men had 19% higher plasma retinol level than women. Higher levels of lipid-adjusted serum vitamin A in males than females and higher levels of serum carotene in females than in males have been reported in a study done by Comstoek, et al.[13]. On the other hand Herbeth, et al.[14] reported that sex differences in serum levels of vitamins A and E became insignificant after adjustment for alcohol and cigarette consumption. TBARS level of females was significanty higher than that of males, whether the levels were adjusted for serum total lipid or not.

We have not found any effects of smoking on serum levels of lipid peroxides, retinol, and α-tocopherol in this study. Serum retinol was higher in the men who drank more than 3 times per week than in men drinking less frequently. In our previous studies, α-tocopherol adjusted for triglyceride was lower in heavy smokers than in moderate smokers[22], and α-tocopherol was high in subjects who drank

more than 5 times per week[23]. Stryker, et al.[15] noticed that plasma α-tocopherol was positively associated with daily alcohol intake in women but not in men. The findings on the relationship between and smoking and serum levels of antioxidant vitamins have not always been consistent[24]. Fisher and Gordon[25] have reported that there was little relation between smoking and alcohol drinking habits and nutrient intake in spite of a positive correlation between cigarette and alcohol use. Lecomte, et al.[26] observed a moderate elevation of plasma α-tocopherol with alcohol intake, but this difference disappeared after adjustment of values for plasma lipid concentrations.

Vitamin supplementation is another important factor affecting serum vitamin levels[13]. Unfortunately we don't have reliable information on vitamin supplementation. As people get older they are inclined to take more vitamin supplements so that serum levels increase with age. However that reason is unlikely in this study, because association of serum retinol with age became insignificant after adjustment for serum total lipid and most vitamin supplements contain not only vitamin E but also retinol and β-carotene.

Literature cited

1) Gey KF. Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease. *Brit Med Bull* 49 : 679-699, 1993
2) Papas AM. Determinants of antioxidant status in humans. *Lipids* 31 : s77-s82, 1996
3) Sevaian A, Hochstein P. Mechanisms and consequences of lipid peroxidation in biological systems. *Ann Rev Nutr* 5 : 365-390, 1985
4) Aznar J, Santos MT, Valles J, Sala J. Serum malondialdehyde-like material(MDA-LM) in acute myocardinal infarction. *J Clin Pathol* 36 : 712-715, 1983
5) Santos MT, Valles J, Aznar J, Vilches J. Determination of plasmamalondialdehyde-like material and its clinical application in stroke patients. *J Cin Pathol* 33 : 973-976, 1980
6) Nishigaki I, Hagihara M, Tsunekawa H, Maseki M, Yagi K. Lipid peroxide levels of serum lipoprotein fractions of diabetic patients. *Biochem Med* 25 : 373-378, 1981
7) MansonJE, Gaziano JM, Jonas MA, Hennekens CH. Antioxidants and cardiovascular disease : A Review. *J Am College Nutr* 12 : 426-432, 1993
8) Bonithon-Kopp C, Coudray C, Berr C, Touboul PJ, Feve JM, Favier A, Ducimetiere P. combined effects of lipid peroxidation and antioxidant status on carotid atherosclerosis in a population aged 59-71 y : the EVA study. *Am J Clin Nutr* 65 : 121-127, 1997
9) MIhara M, Uchiiyama M, Fukuzawa K. Thiobarbituric acid value on fresh homogenate of rat as a parameter of lipid peroxidation in aging, CCl4, intoxication, and vitamin E deficiency. *Biochem Med* 23 : 302-311, 1980
10) Miquel J, Lundgren PR, Johnson JE. Spectrophotofluorometric and elelctron microscopy study of lipofuscin accumulation in the testis of aging mice. *J Gerontol* 33 : 5-19, 1978
11) Hagihara M, Nishigaki I, Maseki M, Yagi K. Age-dependent changes in lipid peroxide levels in the lipoprotein fractions of human plasma. *J Gerontol* 39 : 269-272, 1984
12) Knight JA, Smith SE, Kinder VE, Anstall HB. Reference intervals for plasma lipoperoxides : Age-, sex-, and specimen-related variations. Clin Chem 33 : 2289-2291, 1987
13) Comstock GW, Marilyn MS, Schober SE, Vuilleumier JP, Helsing KJ. Serum levels of retinol, beta-carotene, and alpha-tocopherol in older adults. *Am J Epidemiol* 127 : 114-123, 1987
14) Herbeth B, Chavance M, Musse N, Mejean L, Vernhes G. Dietary intake and other determinants of blood vitamins in an elderly population. *Eur J Clin Nutr* 43 : 175-186, 1989
15) Stryker WC, Kaplan LA, Stein EA, Stampfer MJ, Sober A, Willett WC. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. *Am J Epidemiol* 127 : 283-296, 1989
16) Malvy DJM, Burtschy B, Dostalova L, Amedee-manesme O, Serum retinol, β-carotene, α-tocopherol and cholesterol in healthy French children. *Int J Epidemiol* 22 : 237-246, 1993
17) Kayden HJ, Traber MG. Absorption, lipoprotein transport, and regulation of plasma concentrations of vitamin E in humans. *J Lipid Res* 34 : 343-358, 1993
18) Frings CS, Dunn RT. A colormetric method of determination of total serum lipids based on the sulfo-phosphovanillin reaction. *Amer J Clin Path* 53 : 89-91, 1970
19) Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem Med* 15 : 212-216, 1976
20) Bieri G, Tolliver JJ, Catignani GL. Simultaneous determination of alpha-tocopherol and retinol in plasma or red blood cells by high pressure liquid chromatography. *Am J Clin Nutr* 32 : 2143-2149, 1979
21) Willett WC, Stampfer MJ, Underwood BA, Speizer FE, Rosner B, Hennekens CH. Validation of a dietary questionnaire with plasma carotenoid and α-tocopherol levels. *Am J Clin Nutr* 38 : 631-639, 1983
22) Cho SH, Choi YS. Relation of serum vitamin E and lipo-

peroxide levels with serum lipid status in Korean men. *Kor J Community Nutr* 2(1) : 44-51, 1997

23) Choi YS, Lee OJ, Cho SH, Park WH, Im JG, Kwon S. Serum lipid and lipoperoxide levels and their related factors in middle-aged men in Taegu. *Kor J Nutr* 28(8) : 771-781, 1995

24) Faruque O, Khan MR, Rahman M, Ahmed F. Relationship between smoking and antioxidant nutrient status. *Br J Nutr* 73 : 625-632, 1995

25) Fisher MF, Gordon T. The relation of drinking and smoking habits to diet : the Lipid Research Clinics Prevalence Study. *Am J Clin Nutr* 41 : 623-630, 1985

26) Lecomte E, Herbeth B, Pirollet P, Chancerelle Y, Arnaud J, Musse N, Paille F, Siest G, Artur Y. Effect of alcohol consumption on blood antioxidant nutrients and oxidative stress indicators. *Am J Clin Nutr* 60 : 255-261, 1994