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Serum Concentrations of α-Tocopherol, Carotenoids and Retinol of Normal Koreans*

Yang Cha Lee-Kim and Mi Kyung Kim

Research Institution of Food & Nutritional Sciences, Yonsei University, Seoul 120-749, Korea

ABSTRACT

Five hundred and seventy-eight healthy subjects (351 men and 227 women) with a mean age of 44.8 years (45.2 for men, 44.3 for women) participated in this study. The serum concentrations of α-tocopherol, β-carotene, α-carotene, zeaxanthin+lutein, cryptoxanthin, lycopene and retinol of normal Koreans were measured, and their relation to gender, age, BMI, alcohol consumption, cigarette smoking, and menopausal status were evaluated. The concentrations of α-tocopherol, carotenoids and retinol in serum were measured simultaneously by reverse phase HPLC (multi-wavelength, gradient and computerized automatic system). Average serum levels of α-tocopherol, β-carotene and retinol were 10.1±0.41 μg/ml, 33.1±1.24 μg/dl, and 82.0±1.63 μg/dl for men and 11.1±0.74 μg/ml, 48.1±1.60 μg/dl and 64.5±1.96 μg/dl for women, respectively. Serum concentrations of α-tocopherol, β-carotene, α-carotene, cryptoxanthin and lycopene appeared to be higher in women than in men. The serum concentrations of zeaxanthin+lutein increased with the increase in age of men and those of α-carotene and β-carotene increased with the increase in age of women. For men, current smokers showed significantly lower serum concentrations of β-carotene and α-carotene than ex-smokers ($p<0.05$). Current drinkers also showed significantly lower serum β-carotene and zeaxanthin+lutein concentrations than ex-drinkers. For women, current smokers showed significantly lower serum concentrations of zeaxanthin+lutein and cryptoxanthin than ex-smokers ($p<0.05$). Men with BMI≥24 showed significantly lower serum concentrations of β-carotene, α-carotene, lycopene and cryptoxanthin than men with BMI<20. The mean concentrations of α-tocopherol, β-carotene, α-carotene and lycopene for postmenopausal women were higher than those for premenopausal women ($p<0.05$). In conclusion, there were obvious differences in serum α-tocopherol and carotenoids levels depending on gender, age, cigarette-smoking habits, alcohol consumption, BMI and menopausal status. Further studies are required to establish the normal levels of these vitamins for children and the elderly and to elucidate their roles in different disease states.

KEY WORDS : α-tocopherol · carotenoids · retinol · Koreans

INTRODUCTION

Functions of α-tocopherol and carotenoids in prevention of the major chronic diseases have been the focus of scientific investigation during the last decade.[1-3] Interest in the absorption and metabolism of carotenoids, especially α-carotene, has been stimulated by several reports which indicated that these compounds protect against certain cancers and degenerative diseases, such as heart disease and muscular degeneration.[4]

In the early 1980s, β-carotene was proposed to reduce the risk of cancer and coronary heart disease.[5] Epidemiologic support for this hypothesis has come from three types of studies : retrospective studies, intervention studies of dietary intake and disease,[6-12] and studies of blood antioxidant vitamin levels and disease.[13-15]

Various observational studies have shown that cancer incidence rates were higher among subjects with low[16-20] serum levels of β-carotene and/or α-tocopherol. Although a few studies carried out in Korea[21-24] have also shown a significant correlation between the serum levels of β-carotene and α-tocopherol and cancer incidences, more detailed investigation should be pursued in this area.

It is necessary to establish the normal concentration of antioxidant vitamins in different races. In this study, the normal average serum concentrations of α-tocopherol, β-carotene, α-carotene, zeaxanthin+lutein, cryptoxanthin, lycopene and retinol among healthy Koreans were measured and compared between genders and different ages. Other factors affecting the serum concentrations of antioxidant vitamins were also studied.

METHODS

1. Research subjects

The subjects for this study were normal healthy adults between the age of 18 and 87 who lived in rarious urban areas of Korea. Subjects who were diagnosed with endocrinologic diseases and those who were taking vitamin

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supplements were excluded. The method of stratification with 10 strata (5 age strata of 20's, 30's, 40's, 50's, and 60+'s within male and female) was applied for sampling.

Healthy male (n=351) and female (n=227) volunteers (aged 18-87 years) who visited the Health Promotion Center of the A Medical Center from July 1, 1994 to July 22, 1994 were included in this analysis. Subjects were ambulatory, independent, healthy people.

2. Measurements

1) Demographic information

Items for socioeconomic status of subjects were included in a questionnaire for this study, because certain socioeconomic variables could affect the major outcome of an observational study. Height and weight were measured and body mass index (BMI=kg/m^2) was calculated. A brief medical history including history of metabolic diseases was also obtained.

2) Experimental procedure

The concentrations of α-tocopherol, carotenoids, and retinol in serum were measured simultaneously by reverse-phase high performance liquid chromatography (HPLC).[25] Serum total cholesterol (Chol), triglyceride (TG), and High density lipoprotein-cholesterol (HDL-Chol) were also measured. Low density lipoprotein-cholesterol (LDL-Chol) was calculated by the equation of Friedewald.[26]

(1) Chemical products

dl-α-Tocopherol, retinol, β-carotene, α-carotene, zeaxanthin+lutein, cryptoxanthin, lycopene, retinol, and retinyl acetate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solutions were prepared immediately before use. HPLC solvents (obtained from Burdick & Jackson Co. and Riedel Co. USA) were filtered through a 0.5 μm membrane filter (Waters, Millipore, MA, USA) before use.

(2) HPLC procedure

dl-α-Tocopherol, β-carotene, α-carotene, zeaxanthin+lutein, cryptoxanthin, lycopene, and retinol were analyzed using reverse-phase HPLC. Two hundred μl aliquots of serum were extracted using $CHCl_3$: CH_3OH (2 : 1, v/v). Retinyl acetate was added as an internal standard. A 200 μl portion of 0.9% NaCl was also added. After vortexing for 1 min, the mixtures were centrifuged for 10 min at 2500 rpm and 4℃. The chloroform layer was separated and 1 ml hexane was added to the residue and stirred. The mixture was centrifuged again for 10 min at 2500 rpm and 4℃. The hexane layer was added to the first extraction and evaporated to dryness under N_2 and was then dissolved in 150 μl EtOH. A 50 μl aliquot of the final extract was injected into the HPLC system (Fig. 1).[27-29] All extractions were performed under red light.

The HPLC system consisted of two Waters 510 pumps (Waters Chromatography Division of Millipore Corp., Milford, MA, USA), an WISP 710 auto injector, a Waters 991 photodiode array detector, a Waters C18 Novapak 3.9 cm×15 mm column, and a Waters 991 data station. The HPLC mobile phases were mixture of CH_3CN : tetrahydrofuran (THF) : H_2O (50 : 20 : 30, v/v/v, solvent A) and CH_3CN : THF : H_2O (50 : 44 : 6, v/v/v, solvent B). To simultaneously separate seven vitamins (five carotenoids, α-tocopherol, and retinol), a gradient mobile phase was used. The gradient procedure at a flow rate of 1.2 ml/min was as follows : a 10-min linear gradient of 100% solvent A to 100% solvent B followed by a 6-min run with 100% solvent B, then a 4-min gradient back to 100% solvent A. For 2 min, 100% solvent A was

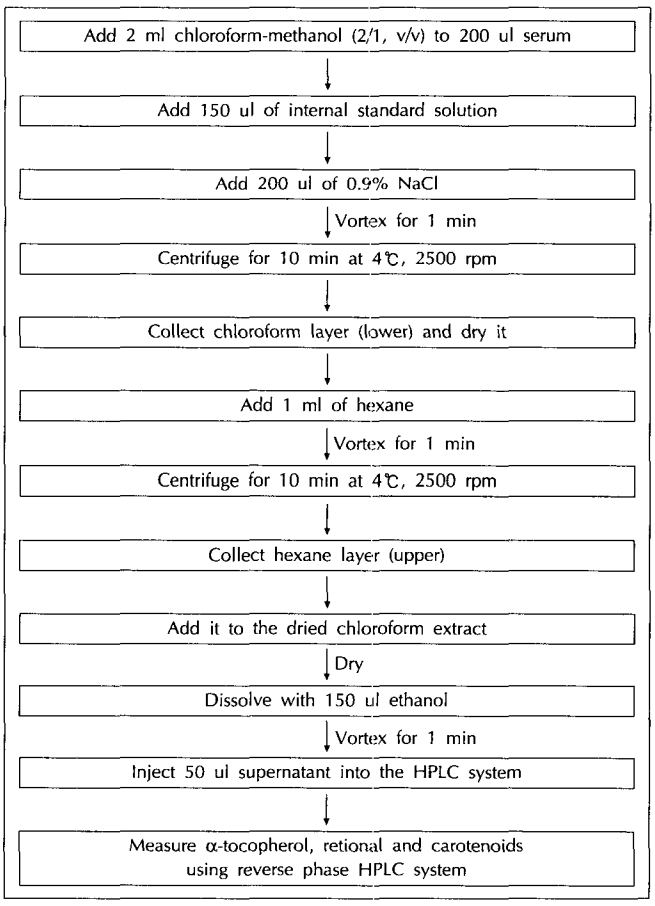

Fig. 1. Extraction and automatic measurements of α-tocopherol, carotenoids, and retinol by HPLC.

maintained, and the column was re-equilibrated until the next run. The detector was set at 292 nm for α-tocopherol and 340 nm for retenoids and 450 nm for carotenoids. dl-α-Tocopherol, retinol, retinyl acetate, β-carotene, α-carotene zeaxanthin+lutein, cryptoxanthin, and lycopene were quantitated by determining peak areas calibrated against known amounts of standards. Because zeaxanthin and lutein are isomers and are not separated by the method employed, they were analyzed together and hereafter are referred to as zeaxanthin+lutein.

3. Statistical analysis

Statistical analyis was performed using Strategic Application Software (SAS) version 6.12, and the results were expressed as mean±SEM. To compare differences of mean values between men and women, student's t test was used. Comparisons between the cigarette smoking and alcohol drinking subgroups were made using one-way analysis of variance and significance by the Duncan test. The maximal tolerable chance of committing an error for tests was 5%.

RESULTS

1. Age and gender distribution of the subjects

Table 1 shows the age and gender distribution of the subjects. The proportion of subjects ranging from 31 years to 50 years was 68.7% for men and 70.0% for women. Mean age and serum concentrations of cholesterol were similar between men and women. However, the mean serum triglyceride concentration of men was significantly higher than that of women (Table 2). Body mass indexes were not significantly different between men and women.

2. Serum concentrations of antioxidant vitamins

The serum concentrations of antioxidant vitamins by gender are shown in Table 3. The mean concentrations of β-carotene ($p<0.001$), α-carotene ($p<0.001$), lycopene ($p<0.001$), and cryptoxanthin ($p<0.01$) for women were significantly higher than for men. On the other hand, the mean concentration of retinol for men was significantly higher than for women ($p<0.001$). The serum concentrations of α-tocopherol, retinol, and β-carotene, when based on the serum cholesterol and triglyceride levels for women, were also statistically different from those of men.

Figs. 2 and 3 show serum antioxidant vitamin levels by gender and age groups. The serum concentrations of zeaxanthin+lutein increased with the increase in age of men, and those of α-carotene and β-carotene increased with the increase in age of women.

Table 1. Age distribution of the subjects

	Men	Women	Total
≤30	14 (4.0)*	9 (4.0)	23 (4.0)
31 - 40	87 (24.8)	69 (30.4)	156 (27.0)
41 - 50	154 (43.9)	90 (39.6)	244 (42.2)
51 - 60	62 (17.7)	40 (17.6)	102 (17.6)
≥61	34 (9.6)	19 (8.4)	53 (9.2)
Total	351 (100.0)	227 (100.0)	578 (100.0)

*Number of subjects. Percentage in parenthesis

Table 2. Anthropometric variables and serum lipid concentrations of the subjects

	Men (n=351)	Women (n=227)	Total (n=578)
Age (years)	45.2±0.56	44.3±0.64	44.8±0.42
Height (cm)	163.6±0.66	156.2±1.05*	160.7±0.59
Weight (kg)	61.7±0.55	55.4±0.59*	59.2±0.43
BMI (kg/m²)	22.9±0.14	22.8±0.19	22.8±0.11
Cholesterol (mg/dl)	186.2±3.07	186.9±3.45	186.0±2.33
Triglyceride (mg/dl)	163.5±6.05	119.3±6.09*	147.3±4.53

Values are Mean SEM.
BMI : Body Mass Index[=body wt (kg)/ht (m²)]
Significantly different from men (*$p<0.05$).

Table 3. Serum vitamin concentrations of the subjects

	Men (n=351)	Women (n=227)	Total (n=578)
α-Tocopherol (μg/ml)	10.1 ±0.41	11.1 ±0.74	10.4 ±0.38
Retinol (μg/dl)	82.0 ±1.63	64.5 ±1.96***	75.1 ±1.30
β-Carotene (μg/dl)	33.1 ±1.24	48.1 ±1.60***	38.9 ±1.02
Lycopene (μg/dl)	8.01±0.43	10.9 ±0.48***	9.15±0.33
Zeaxanthin+ Lutein (μg/dl)	66.1 ±1.83	67.6 ±1.82	66.7 ±1.32
Cryptoxanthin (μg/dl)	62.6 ±2.62	71.9 ±2.02**	66.2 ±1.78
α-Carotene (μg/dl)	1.01±0.10	1.65±0.13***	1.26±0.08
α-Tocopherol/ (Chol+TG) (mg/mg)	29.4 ±1.28	34.2 ±1.90**	31.1 ±1.07
Retinol/ (Chol+TG) (mg/mg)	251.9 ±6.29 105.9 ±4.95	222.7 ±10.0** 166.8 ±7.71***	241.2 ±5.43 128.1 ±4.43

Values are Mean±SEM.
Significantly different from men (**$p<0.01$, ***$p<0.001$).

3. Serum concentrations of antioxidant vitamins according to alcohol-drinking and smoking habits

The subjects were classified into three categories according to their alcohol-drinking and smoking habits ; non-drinker, ex-drinker, and current drinker ; non-smoker, ex-smoker, and current smoker.

The proportions of current drinkers were 53.0% for men and 18.1% for women, respectively. The proportions of current smokers were 31.0% for men and 4.8% for women. Non-mokers numbered 53.0% of men and 93.4%

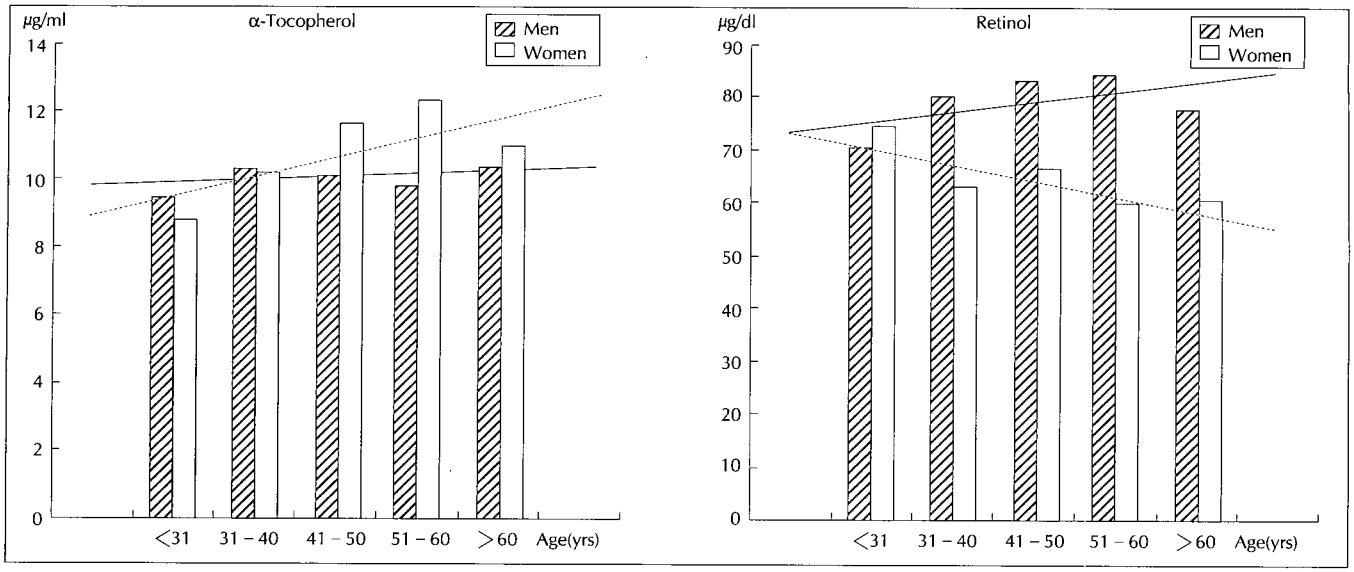

Fig. 2. Changes of serum α-tocopherol and retinol concentrations by age.

of women.

The mean concentrations of serum vitamins according to alcohol-drinking habits are shown in Table 4. For men, current drinkers showed significantly lower serum concentrations of β-carotene ($p<0.001$) and zeaxanthin+lutein ($p<0.05$) than ex-drinkers, mean serum concentrations of cryptoxanthin of current drinkers were lower than those of non-drinkers, but these differences were not statistically significant. When based on the serum cholesterol and triglyceride level, and the serum concentration of β-carotene for current-drinker were significantly lower than those of ex-drinker. For women, the mean concentrations of antioxidant vitamins did not differ between subgroups of alcohol drinking. But when based on the serum cholesterol and triglyceride levels, the concentrations of α-tocopherol, and β-carotene were significantly lower in current drinkers than in non-drinkers.

The mean concentrations of serum vitamins according to cigarette-smoking habits are shown in Table 5. For men, current smokers showed significantly lower serum concentrations of β-carotene and α-carotene than ex-smokers. And the mean concentrations of α-carotene, β-carotene, lycopene and cryptoxanthin for current smokers were lower than for non-smokers, but these results were not statistically significant. The serum concentrations of β-carotene based on the serum cholesterol and triglyceride level for current smokers were significantly lower than those of ex-smoker in men. For women, the mean concentrations of α-carotene ($p<0.05$), β-carotene ($p<0.01$), zeaxanthin+lutein ($p<0.05$) and cryptoxanthin ($p<0.05$) in current smokers were significantly lower than in non-smokers. Also, the serum concentrations of β-carotene based on the serum cholesterol and triglyceride level for current smoker were significantly lower ($p<0.05$) than those of non-smokers in women.

4. Serum concentrations of antioxidant vitamins according to BMI

The range of Quetelet index values was divided into tertiles for analysis, that is lean (BMI<20), normal (20≤ BMI<24) and overweight (BMI≥24).

Table 6 shows the mean serum concentrations of antioxidant vitamins in terms of body mass index. Men in the highest tertile of BMI (BMI≥24) showed significantly lower serum concentrations of β-carotene, α-carotene, lycopene, and cryptoxanthin than for lean men (BMI<20) and these differences increased when expressed concentrations on the basis of serum lipid level. The serum concentrations of α-tocopherol based on the serum cholesterol and triglyceride levels for overweight men was also statistically lower than for lean men ($p<0.05$). And the serum concentrations of β-carotene based on the serum lipid level for lean men was about twice as high as for overweight men. For women, however, there were no differences in serum concentrations between BMI tertiles whether or not expressed on the basis of serum lipid level.

5. Serum concentrations of antioxidant vitamins according to menopausal status

The serum concentrations of antioxidant vitamins in menopausal subjects are shown in Table 7. The mean concentrations of α-tocopherol, α-carotene, and lycopene for postmenopause women were higher than those of the premenopause women ($p<0.05$). These differences disap-

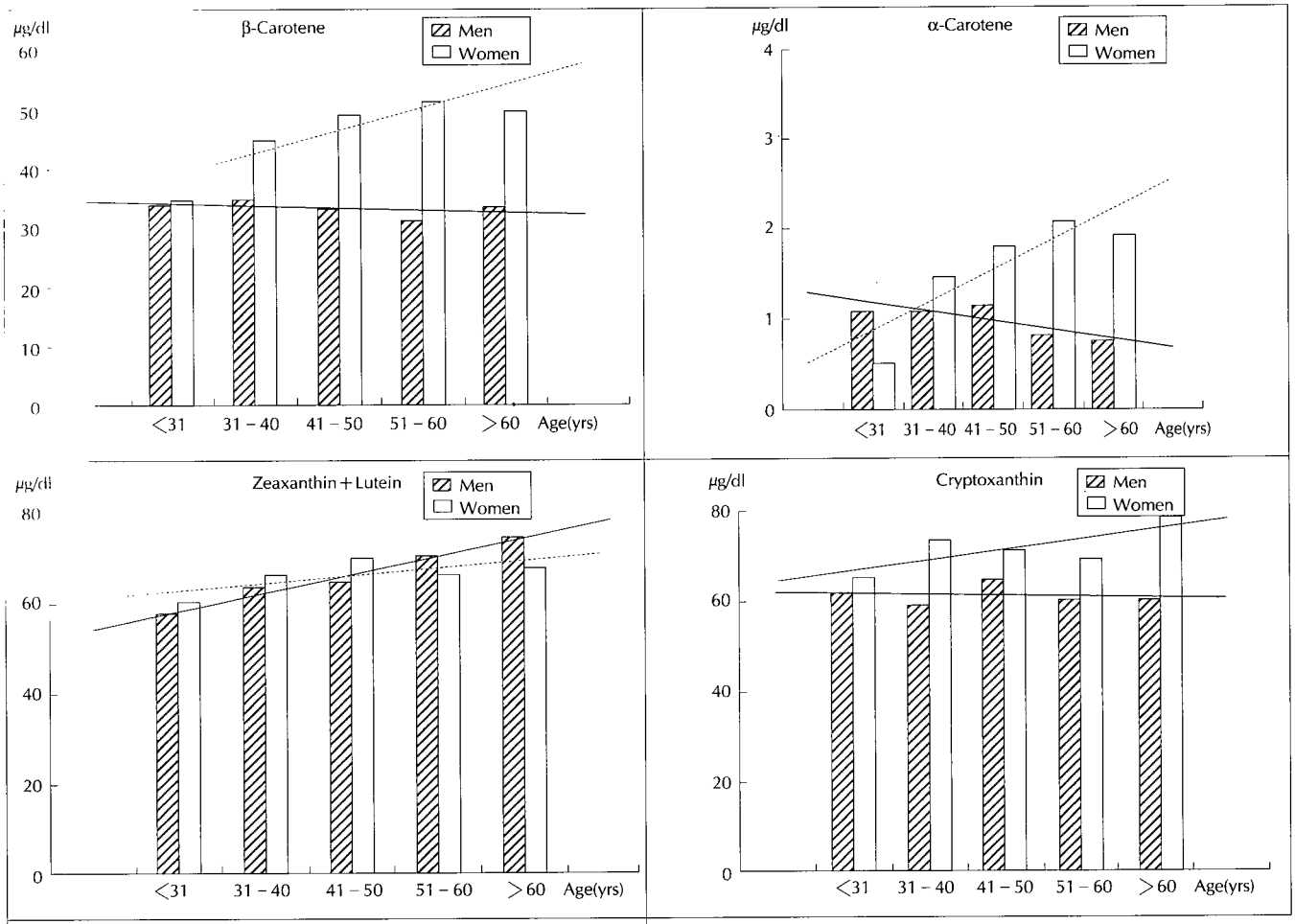

Fig. 3. Changes of cerum carotenoids concentrations by age.

peared when expressed on the basis of the serum lipid level.

DISCUSSION

The aim of the present study was to measure the normal average serum concentrations of α-tocopherol, β-carotene, α-carotene, zeaxanthin+lutein, cryptoxanthin, lycopene, and retinol among healthy Koreans by age and gender. The mean concentrations of α-tocopherol and carotenoids for women were higher than for men. On the other hand, the mean concentration of retinol for men was significantly higher than for women. The serum concentrations of α-tocopherol, retinol, and β-carotene based on the serum cholesterol and triglyceride levels for women were statistically different from those for men. The serum concentrations of zeaxanthin+lutein increased with the increase in age of men and those of α-carotene and β-carotene increased with the increase in age of women. The serum β-carotene concentrations for females were generally about twice as high as for males in Japan[31]. Comstock *et al.*[30] obtained similar results from males and females in Washington County, Maryland. In their study, females had higher levels of serum β-carotene, but lower levels of serum retinol than males when adjusted for age, marital status, smoking status, blood pressure medication and history of vitamin supplementation. Levels of α-tocopherol were only slightly higher among females.

Table 4. Serum vitamin concentrations by alcohol drinking habit

	Non-drinker	Ex-drinker	Current-drinker
Men	n=148	n=17	n=186
α-Tocopherol (μg/ml)	9.48± 0.60	11.3 ± 2.09	10.4 ± 0.57
Retinol (μg/dl)	81.1 ± 2.73	71.5 ± 6.83	83.7 ± 2.10
β-Carotene(μg/dl)	33.0 ± 1.96[b]	51.6 ±10.0[a]	31.5 ± 1.43[b]***
Lycopene(μg/dl)	8.20± 0.74	7.34± 1.65	7.93± 0.54
Zeaxanthin+Lutein(μg/dl)	65.1 ± 2.84[b]	82.1 ±10.3[a]	65.4 ± 2.42[b]*
Cryptoxanthin(μg/dl)	64.6 ± 5.63	80.7 ±10.4	59.4 ± 1.86
α-Carotene(μg/dl)	1.01± 0.15	1.63± 0.74	0.96± 0.11
α-Tocopherol/ (Chol+TG) (mg/mg)	29.3 ± 2.09	31.0 ±4.94	29.3 ± 1.71
Retinol/ (Chol+TG) (mg/mg)	251.5 ±10.9	223.2 ±25.1	254.8 ± 8.08
β-Carotene/ (Chol+TG) (mg/mg)	105.2 ± 6.97[b]	176.7 ±40.6[a]	100.0 ± 6.10[b]**
Women	n=182		n=41
α-Tocopherol (μg/ml)	11.0 ± 0.67		11.7 ± 2.84
Retinol (μg/dl)	64.2 ± 2.09		64.9 ± 5.54
β-Carotene(μg/dl)	49.1 ± 1.76		45.6 ± 3.94
Lycopene(μg/dl)	11.1 ± 0.53		10.3 ± 1.20
Zeaxanthin+Lutein(μg/dl)	66.8 ± 1.96		69.9 ± 4.89
Cryptoxanthin(μg/dl)	71.5 ± 2.21		74.1 ± 5.15
α-Carotene(μg/dl)	1.73± 0.15		1.41± 0.28
α-Tocopherol/ (Chol+TG) (mg/mg)	35.6 ± 2.17		26.5 ± 3.28*
Retinol/ (Chol+TG) (mg/mg)	223.9 ±11.2		201.2 ±20.5
β-Carotene/ (Chol+TG) (mg/mg)	173.9 ± 8.75		134.0 ±13.9*

Values are Mean±SEM.
Values with the different letters are significantly different from the others within the same row (*p<0.05, **p<0.01, ***p<0.001).

Table 5. Serum vitamin concentrations by cigarette smoking habit

	Non-smoker	Ex-smoker	Current-smoker
Men	n=186	n=56	n=109
α-Tocopherol (μg/ml)	10.2 ± 0.57	8.83± 0.60	10.5 ± 0.84
Retinol (μg/dl)	82.1 ± 2.33	81.7 ± 3.82	82.0 ± 2.86
β-Carotene (μg/dl)	33.3 ± 1.68[ab]	37.5 ± 3.63[a]	30.5 ± 2.03[b]*
Lycopene (μg/dl)	8.26± 0.61	8.78± 1.22	7.19± 0.66
Zeaxanthin+Lutein (μg/dl)	65.7 ± 2.39	70.0 ± 5.82	64.7 ± 3.03
Cryptoxanthin (μg/dl)	65.0 ± 4.61	63.1 ± 3.68	58.3 ± 2.43
α-Carotene (μg/dl)	1.07± 0.13[ab]	1.35± 0.29[a]	0.73± 0.14[b]*
α-Tocopherol/ (Chol+TG) (mg/mg)	29.6 ± 1.83	27.5 ± 2.03	30.1 ± 2.44
Retinol/ (Chol+TG) (mg/mg)	252.1 ±10.1	257.7 ±12.7	249.0 ±10.2
β-Carotene/ (Chol+TG) (mg/mg)	105.8 ± 6.50[ab]	125.4 ±15.6[a]	96.8 ± 7.41[b]*
Women	n=212		n=11
α-Tocopherol (μg/ml)	11.2 ± 0.79		8.99± 1.37
Retinol (μg/dl)	64.1 ± 2.03		64.4 ± 8.08
β-Carotene (μg/dl)	49.1 ± 1.66		31.8 ± 5.93**
Lycopene (μg/dl)	11.1 ± 0.51		9.65± 1.96
Zeaxanthin+Lutein (μg/dl)	67.8 ± 1.89		52.9 ± 6.25*
Cryptoxanthin (μg/dl)	71.7 ± 2.03		63.9 ±12.2*
α-Carotene (μg/dl)	1.71± 0.13		0.81± 0.46*
α-Tocopherol/ (Chol+TG) (mg/mg)	34.3 ± 2.00		27.6 ± 3.97
Retinol/ (Chol+TG) (mg/mg)	219.3 ±10.3		230.8 ±39.0
β-Carotene/ (Chol+TG) (mg/mg)	170.2 ± 8.09		107.3 ±23.4*

Valuesare Mean±SEM.
Values with the different letters are significantly different from the others within the same row (*p<0.05, **p<0.01).

Alcohol drinking affected the serum levels of antioxidant vitamins for men in this study. For men, current drinkers showed significantly lower serum concentrations of β-carotene and zeaxanthin+lutein than ex-drinkers. The mean concentration of cryptoxanthin in current drinkers was lower than in non-drinkers, but this difference was not statistically significant. For women, the mean concentrations of antioxidant vitamins did not differ bet-

Table 6. Serum vitamin concentrations by body mass index

	BMI (kg/m^2)		
	BMI<20	20≤BMI<24	BMI≥24
Men	n=48	n=191	n=112
α-Tocopherol (μg/ml)	11.6 ± 1.19	9.81± 0.63	9.84± 0.49
Retinol (μg/dl)	79.4 ± 4.08	81.9 ± 2.38	83.2 ± 2.60
β-Carotene (μg/dl)	42.2 ± 4.11[a]	33.8 ± 1.74[b]	28.2 ± 1.63[b]***
Lycopene (μg/dl)	9.27± 1.04[a]	8.48± 0.68[ab]	6.69± 0.53[b]*
Zeaxanthin+Lutein (μg/dl)	70.2 ± 5.40	66.0 ± 2.64	64.4 ± 2.68
Cryptoxanthin (μg/dl)	83.7 ±16.4[a]	61.2 ± 1.96[b]	56.1 ± 2.41[b]***
α-Carotene (μg/dl)	1.21± 0.31[a]	1.18± 0.14[a]	0.64± 1.63[b]*
α-Tocopherol/ (Chol+TG) (mg/mg)	37.9 ± 3.97[a]	29.3 ± 1.95[b]	26.5 ± 1.46[b]*
Retinol/ (Chol+TG) (mg/mg)	265.0 ±17.9	260.1 ± 9.14	233.8 ± 9.31
β-Carotene/ (Chol+TG) (mg/mg)	164.5 ±22.3[a]	108.1 ± 6.08[b]	81.1 ± 6.11[b]***
Women	n=53	n=114	n=60
α-Tocopherol (μg/ml)	10.5 ± 1.30	11.5 ± 1.23	10.9 ± 1.02
Retinol (μg/dl)	68.4 ± 4.85	63.7 ± 2.77	62.4 ± 2.99
β-Carotene (μg/dl)	43.1 ± 2.60	49.3 ± 2.23	50.4 ± 3.62
Lycopene (μg/dl)	10.2 ± 0.74	10.9 ± 0.61	11.7 ± 1.26
Zeaxanthin+Lutein (μg/dl)	68.7 ± 3.52	68.4 ± 2.51	65.3 ± 3.94
Cryptoxanthin (μg/dl)	72.7 ± 3.60	74.4 ± 2.80	66.2 ± 4.43
α-Carotene (μg/dl)	1.17± 0.21	1.77 ± 0.18	1.85± 0.28
α-Tocopherol/ (Chol+TG) (mg/mg)	32.6 ± 2.79	35.6 ± 2.71	32.9 ± 4.03
Retinol/ (Chol+TG) (mg/mg)	251.8 ±33.3	224.1 ±12.9	201.1 ±13.2
β-Carotene/ (Chol+TG) (mg/mg)	170.1 ±15.4	175.2 ±11.5	149.5 ±13.2

Values are Mean±SEM.
Values with the different letters are significantly different from the others within the same row (*$p<0.05$, ***$p<0.001$).

Table 7. Serum vitamin concentrations of the subjects by menopausal status

	Menopausal status	
	Premenopause (n=190)	Postmenopause (n=37)
α-Tocopherol (μg/ml)	10.3 ± 0.56	14.9 ± 3.46*
Retinol (μg/dl)	64.3 ± 2.01	65.5 ± 6.20
β-Carotene (μg/dl)	47.0 ± 1.71	53.8 ± 4.23
α-Carotene (μg/dl)	1.50± 0.10	2.41± 0.39*
Lycopene (μg/dl)	10.6 ± 0.49	12.9 ± 1.54*
Zeaxanthin+Lutein (μg/dl)	68.4 ± 2.05	63.8 ± 3.84
Cryptoxanthin (μg/dl)	71.0 ± 2.08	76.4 ± 6.32
α-Tocopherol/ (Chol+TG) (mg/mg)	34.2 ± 1.88	34.1 ± 8.97
Retinol/ (Chol+TG) (mg/mg)	226.9 ±10.8	184.4 ±21.7
β-Carotene/ (Chol+TG) (mg/mg)	168.5 ± 8.33	151.9 ±17.8

Values are Mean±SEM.
*Significantly different from premenopausal women at $p<0.05$.

ween subgroups of alcohol drinking. This result agrees with the results reported by Comstock *et al.*[30] and Shibata *et al.*[31]

The relation between alcohol consumption and plasma or serum carotenoids concentrations is likely to be complex. Epidemiologic studies have shown an adverse effect of alcohol intake, particularly in men, on plasma carotenoid concentrations.[32][33] In a controlled cross-over feeding study, Forman *et al.*[34] observed that higher plasma α-carotene and β-carotene concentrations, but lower concentrations of other carotenoids (e.g., lutein), were associated with daily alcohol ingestion. Alcohol ingestion may also impair nutritional status by interfering with gastrointestinal absorption of essential substances and by direct toxic effects on various organ systems[35]. Vitamins commonly deficient among those ingesting higher quantities of alcoholic beverages were folate, thiamin, β-carotene and pyridoxine.[36]

Epidemiologic evidence suggests that cigarette smoking is a major risk factor for chronic disease.[37] However, the precise mechanisms of these effects are not completely understood. Cigarette smoke is known to contain a plethora of potential reactive oxygen and nitrogen species.[38] Cigarette smoke can, therefore, induce some of the damaging effects caused by free radical mechanisms.[39][40] In this study, current male smokers showed significantly lower serum concentrations of β-carotene and α-carotene than ex-smokers. Although the mean concentrations of α-carotene, β-carotene, lycopene, and cryptoxanthin for current male smokers were not significantly different from non-smokers, the mean concentrations of α-carotene, β-carotene, and zeaxanthin+lutein for current female smokers were significantly lower than for non-smokers.

A previous study[41] showed that smokers had a higher energy intake and consumed larger quantities of saturated fats, more alcohol, smaller amounts of fruits and vegetables, and therefore had a smaller intake of vitamins A

and C than non-smokers. Some other studies[42] also emphasized that the recommended vitamin A and C levels for a healthy population were to be considered inadequate for smokers because these vitamins defend against the negative effects of cigarette smoke. Higher serum β-carotene concentrations have been observed among those who consumed green-yellow vegetables more frequently.[31] This association remained after adjusting for alcohol, smoking, age, and BMI.

Comstock *et al.*[30] reported that subjects taking medication for high blood pressure had lower serum β-carotene concentrations and also suggested that vitamin A supplements, estrogen, and obesity also play a role as modifiers of serum β-carotene levels. In the present study, men in the highest tertile of BMI (BMI≥24) showed significantly lower serum concentrations of β-carotene, α-carotene, lycopene, and cryptoxanthin than lean men (BMI<20). For women, there was no difference in serum antioxidant vitamins between BMI tertiles.

In conclusion, there were obvious differences in serum α-tocopherol, β-carotene, α-carotene, zeaxanthin+lutein, cryptoxanthin, and lycopene levels depending on gender, age, cigarette-smoking habits, alcohol consumption, BMI, and menopausal status. Further studies are needed to establish the normal levels of these vitamins in children and the elderly and to elucidate their roles in different disease states.

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