

RESEARCH COMMUNICATION

Sex and Seasonal Variations of Plasma Retinol, α -Tocopherol, and Carotenoid Concentrations in Japanese Dietitians

Jin Xiang^{1a}, Teruo Nagaya², Xin-En Huang^{1b}, Kiyonori Kuriki³, Nahomi Imaeda⁴, Yuko Tokudome⁵, Juichi Sato⁶, Nakako Fujiwara⁷, Shinzo Maki⁸, Shinkan Tokudome^{2*}

Abstract

Aim: To clarify sex and seasonal variations of plasma antioxidant concentrations among middle-aged Japanese. **Subjects and Methods:** We investigated sex and seasonal variations of plasma antioxidant concentrations, including retinol, α -tocopherol, and carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lutein and lycopene), in 55 middle-aged dietitians (46 women and 9 men) in Aichi Prefecture, Central Japan, who took no supplements from autumn 1996 to summer 1997. Reversed-phase high performance liquid chromatography was used to measure plasma antioxidant concentrations in overnight-fasting blood samples. **Results:** Plasma levels of α -tocopherol, α -/ β -carotene, β -cryptoxanthin and lutein were significantly influenced by sex, being significantly higher for women than men in each corresponding season; retinol and lycopene, however, showed no such difference. For women, winter values of α -tocopherol, α -/ β -carotene, lutein and lycopene were significantly lower than corresponding summer values, and had reached their annual lowest. Retinol failed to show any significant seasonal variation, whereas the winter value of β -cryptoxanthin had reached its annual highest. For men, β -cryptoxanthin exhibited significant seasonal changes and was also highest in winter. Winter values of α -tocopherol, α -/ β -carotene and lycopene were lower compared with other seasons, but not statistically significant, probably due to the small sample size. **Conclusions:** The findings indicate that sex and seasonal variations of plasma antioxidant concentrations should be taken into account in nutritional epidemiologic studies.

Key Words: Sex - season - variation - plasma antioxidants - retinol - α -tocopherol - carotenoids

Asian Pacific J Cancer Prev, 9, 413-416

Introduction

According to epidemiologic observations, many antioxidants are inversely associated with the risk or prevalence of chronic diseases (Goodman, 1984; Cutler, 1991; Mobarhan et al., 1991; Byers et al., 1992). Antioxidants are thought to play an important role in disease prevention, in particular, such as vitamin E in the prevention of heart disease (Rimm et al., 1993; Stampfer et al., 1993), and retinoids, tocopherols and carotenoids in the prevention of cancer (Moon et al., 1976; Suda et al., 1986; Bertram et al., 1991; Knekt et al., 1991; Rimm et al., 1993).

Plasma concentrations of antioxidants may fluctuate according to host and environmental factors. Sex (Shibata et al., 1989), smoking/drinking habits (Stryker et al., 1988) and season (Cantilena et al., 1992) have also been observed to impact on plasma antioxidant levels. However, in the

Asian countries, including Japan, where host and environmental factors are known to be fundamentally different from those in the Western world, few studies have been conducted to investigate the effects of sex and seasonal changes on plasma antioxidant concentrations.

Here we examined the influence of sex (a host factor) and season (an environmental factor) on variations in plasma antioxidant levels in Japanese dietitians living in Aichi Prefecture, Central Japan who participated in our previous nutritional studies (Tokudome et al., 2001; Imaeda et al., 2002; Kuriki et al., 2002; Tokudome et al., 2002).

Materials and Methods

Study Subjects

One hundred and six middle-aged dietitians (85 women, 21 men), who were members of the Aichi

^{1a}Dept of Science Research, ^{1b}Dept of Chemotherapy, Jiangsu Cancer Hospital and Research Institute, Nanjing, China, ²Dept of Public Health, Nagoya City University Graduate School of Medical Sciences, Mizuho-ku, Nagoya 467-8601, ³Aichi Cancer Center, Chikusa-ku, Nagoya, ⁴Nagoya Women's University, Mizuho-ku, Nagoya, ⁵Nagoya University of Arts and Sciences, Nisshin, ⁶Dept of General Medicine, Nagoya University Hospital, Showa-ku, Nagoya, ⁷Aichi Kiwami College of Nursing, Ichinomiya, ⁸Aichi Prefectural Dietitians' Association, Kita-ku, Nagoya, Japan *For correspondence: tokudome@med.nagoya-cu.ac.jp

Prefectural Dietitians' Association residing in Aichi Prefecture, Central Japan voluntarily participated in this study, as reported elsewhere (Tokudome et al., 2001). In brief, 97 subjects were enrolled after excluding those with chronic diseases. Among them, 86 whose four-season blood samples (Autumn 1996 - Summer 1997) were available, entered the current investigation. Information on demographic characteristics, drinking and smoking habits, regular exercise, and intake of supplements, including vitamins, was obtained from a self-administered questionnaire. Finally, 55 subjects (46 women and 9 men) who regularly took no supplements were eligible for in-depth analysis.

This protocol was approved by the Ethics Committee of the Nagoya City University Graduate School of Medical Sciences, and written informed consent was secured from each study participant.

Methods

Overnight-fasting venous blood was sampled using a tube with EDTA-2Na, and separated into plasma, buffy coat, and RBC clot. The samples were stored at -80° C until analysis.

Seven plasma antioxidants, including retinol, α -tocopherol and five carotenoids (α -/ β -carotene, β -cryptoxanthin, lutein and lycopene) were assessed by reverse-phase high performance liquid chromatography (HPLC) according to a modification of Talwar's method (Talwar et al., 1998). At the time of analysis, samples were thawed at room temperature. As an internal standard, 0.1 ml retinol acetate and 0.1 ml tocopherol acetate were added to 0.1 ml plasma. The total volume was raised to 1.3 ml with distilled water. After protein was precipitated by adding 0.9 ml ethanol, fat-soluble substances were extracted twice with 1.2 ml hexane.

Samples were mixed and centrifuged for 15 min. The hexane layer was gathered and evaporated under a nitrogen atmosphere. The residue was reconstituted with tetrahydrofuran making up to 0.3 ml with a mobile phase, and 0.2 ml was injected into Shimadzu HPLC with a Nucleosil C18 column. The mobile phase, which consisted of methanol, acetonitrile and tetrahydrofuran in the ratio of 75 : 20 : 5, flowed at 0.6 ml/min. Using an SPD-M10 Avp Shimadzu diode array detector, retinol and retinol acetate were detected at 325 nm, α -tocopherol and tocopherol acetate at 292 nm, α -/ β -carotene, β -cryptoxanthin and lutein at 450 nm, along with lycopene at 473 nm.

Laboratory accuracy and precision in the measurements of seven plasma antioxidants (retinol, α -tocopherol, and five carotenoids) were maintained and assessed by using pooled plasma. Concentrations of antioxidants in the plasma were calculated with the aid of commercially available lyophilized serum from NIST (the National Institute of Standards and Technology). Two pooled plasma samples were analyzed at the beginning and the end of each run. Coefficients of variation for the antioxidants ranged from 3.3% (α -tocopherol) to 13.7% (β -cryptoxanthin) for the same day determination, and from 5.0% (retinol) to 14.6% (β -cryptoxanthin) for day-to-day reproducibility.

Table 1. Baseline Demographic and Lifestyle Characteristics of 55 Japanese Dietitians Not Taking Supplements

Characteristics	Women (n=46)	Men (n=9)	p value ^a
Age(y) mean(SD)	46.4 (7.8)	47.7 (10.2)	0.67
BMI(kg/m ²) ^b n (%)			0.11
<19.8	12 26.1	1 11.1	
19.8-24.2	30 65.2	5 55.6	
>24.2	4 8.7	3 33.3	
Smoking habit			<0.001
Current	1 2.2	2 22.2	
Ceased	1 2.2	3 33.3	
Never	44 95.7	4 44.4	
Drinking habit			<0.05
Current	9 19.6	6 66.7	
Ceased	1 2.2	0 0	
Never	36 78.3	3 33.3	
Regular exercise			0.3
With	29 63.0	4 44.4	
Without	17 37.0	5 55.6	

^aBy Chi-square test or Student t-test, ^bCategorization was made according to obesity assessment standards set by Japan Obesity Association.

Table 2. Plasma Concentrations of Retinol, α -Tocopherol, and Carotenoids in Japanese Dietitians Not Taking Supplements

		Women (n=46)	Men (n=9)
Retinol (umol/L)	Autumn	2.12 (1.18-3.60)	2.33 (1.47-2.86)
	Winter	2.25 (0.77-3.45)	2.41 (1.82-2.75)
	Spring	2.16 (1.23-4.18)	2.80 (1.86-2.97)
	Summer	2.25 (1.34-3.88)	2.37 (1.66-3.11)
α -Tocopherol (umol/L)	Autumn	31.8 (18.4-65.3) ^e	21.8 (18.2-39.1)
	Winter	28.2 (17.0-49.0) ^{c,b,e}	21.7 (18.3-41.6)
	Spring	33.3 (23.2-68.9) ^e	27.2 (19.2-42.9)
	Summer	33.1 (21.6-60.1) ^e	26.0 (18.4-41.9)
α -Carotene (umol/L)	Autumn	0.30 (0.11-0.71) ^b	0.20 (0.10-0.72)
	Winter	0.25 (0.09-0.58) ^{b,e}	0.13 (0.08-0.40)
	Spring	0.26 (0.11-0.63) ^{b,e}	0.14 (0.07-0.59)
	Summer	0.36 (0.12-1.17) ^e	0.16 (0.09-1.04)
β -Carotene (umol/L)	Autumn	1.33 (0.42-2.53) ^e	0.60 (0.27-2.08)
	Winter	1.10 (0.21-3.12) ^{c,b,e}	0.55 (0.26-1.60)
	Spring	1.27 (0.47-3.16) ^e	0.67 (0.13-1.61)
	Summer	1.40 (0.48-3.74) ^e	0.60 (0.30-1.92)
β -Cryptoxanthin (umol/L)	Autumn	0.55 (0.10-1.70) ^{a,b,e}	0.24 (0.11-0.56)
	Winter	1.06 (0.14-3.20) ^{c,b}	0.73 (0.13-2.10) ^d
	Spring	0.49 (0.10-2.02) ^{b,e}	0.18 (0.11-0.57)
	Summer	0.39 (0.08-1.07) ^e	0.22 (0.08-0.32)
Lutein (umol/L)	Autumn	0.85 (0.36-1.21) ^{a,e}	0.50 (0.32-0.74)
	Winter	0.73 (0.30-1.09) ^{c,b,e}	0.53 (0.31-0.70)
	Spring	0.81 (0.39-1.66) ^e	0.61 (0.31-0.76)
	Summer	0.87 (0.37-1.71) ^e	0.57 (0.26-1.50)
Lycopene (umol/L)	Autumn	0.49 (0.12-1.49) ^b	0.53 (0.10-1.08)
	Winter	0.32 (0.11-1.67) ^{c,b}	0.33 (0.10-0.71)
	Spring	0.51 (0.09-1.83) ^b	0.49 (0.11-0.70)
	Summer	0.71 (0.18-2.17)	0.55 (0.12-0.97)

^{*}Medians; ranges (minimum-maximum) in parentheses. Significantly different: ^afrom women in winter, p<0.01, ^bfrom women in summer, p<0.05, ^cfrom women in spring, p<0.05, ^dfrom men in summer, p<0.05, ^ebetween women and men in the same season, p<0.05

Statistical analysis

The normality of distribution of seasonal antioxidant measurements in each sex group was determined by the Shapiro-Wilk test. If not normally distributed, distribution-free (non-parametric) methods were adopted for further

statistical analyses. The Kruskal-Wallis test was adopted for an analysis of variance with the seasons by sex, and the Wilcoxon 2-sample test for variance between men and women. Procedures of *FREQ*, *UNIVARIATE*, or *NPAR1WAY* in the SAS package were used for statistical calculations (SAS Institute, Inc., 1990). Differences were considered to be statistically significant at $p < 0.05$.

Results

Baseline demographic and lifestyle characteristics of 55 Japanese dietitians (46 women and 9 men) who took no supplements are summarized in Table 1. There were no statistical differences between women and men in age, BMI or physical exercise. However, smoking and drinking rates were significantly higher in men.

The Shapiro-Wilk test showed that few distributions of plasma antioxidant values in each sex and season appeared normally distributed. Therefore, non-parametric methods were adopted for statistical analyses. Table 2 shows sex- and season-specific medians and ranges of respective antioxidants. Retinol and lycopene did not significantly differ between men and women. α -Tocopherol was significantly higher in women than men in all seasons, and reached its highest in spring for all subjects. The Kruskal-Wallis test showed that there were statistically significant seasonal variations in α -/ β -carotene, β -cryptoxanthin, lutein and lycopene in women, as well as β -cryptoxanthin in men. Both α -carotene and β -carotene were higher in women than men in each corresponding season. A higher level was noted in women in summer, and in men in autumn or spring. The concentrations of β -cryptoxanthin were higher in women than men in each corresponding season with the highest value appearing in winter for both groups. Lutein was highest in summer in women, while it was highest in spring in men. Women showed higher lutein than men, irrespective of the season.

Discussion

Plasma levels of α -tocopherol, α -/ β -carotene, β -cryptoxanthin and lutein were significantly higher for women than men in each corresponding season; but no sex difference was observed for retinol and lycopene. For women, winter concentrations of α -tocopherol, α -/ β -carotene, lutein and lycopene were significantly lower than their corresponding summer values, and were the lowest of the entire year. Retinol demonstrated no significant seasonal variation; however, the winter level of β -cryptoxanthin was found to be the highest of the whole year. For men, β -cryptoxanthin showed significant seasonal changes and was also highest in winter. Winter concentrations of α -tocopherol, α -/ β -carotene and lycopene tended to be lower compared with other seasons. Plasma concentrations of α -tocopherol, α -/ β -carotene, β -cryptoxanthin and lutein in women were higher than in men throughout the year.

The present findings are in harmony with the observations of healthy Japanese students, university staff and general local inhabitants (Ito et al., 1990), a Spanish

clinical staff (Olmedilla et al., 1994), and the US general population (Krasinski et al., 1989), as well as in a US hospital-based study (Stacewicz-Sauntzakis et al., 1987). That may be explained by the fact that Japanese women consume more fruit and vegetables (Inoue et al., 1997), smoke less and drink less alcohol than Japanese men (Tsubono et al., 1997; Health Promotion and Nutrition Division 1998). It has been proven that fruit and vegetables are major sources of these micronutrients (Resources Council 1982; Willett, 1990; Resources Council 1992; Tokudome et al., 1998), while smoking and drinking risked diminishing the reserves of antioxidants (Russell-Briefel et al., 1985; Stryker et al., 1988). Interestingly, however, our results are also consistent with the reports that the retinol level in men may be slightly higher than in women (Stacewicz-Sauntzakis et al., 1987; Krasinski et al., 1989; Ito et al., 1990; Olmedilla et al., 1994). Although it is still impossible to fully elucidate the mechanism underlying why plasma antioxidant concentrations differ by sex, this variation should be taken into account in the assessment as well as the planning of any nutritional epidemiologic research; otherwise it could impose a bias due to the sex predominance in the study population.

In the present study, the seasons did not significantly affect retinol in either men or women. However, there were statistically significant seasonal changes of α -tocopherol, α -/ β -carotene, β -cryptoxanthin, lutein and lycopene in women; but statistical power did not appear sufficient to detect such seasonal variations in men. For all study subjects, higher α -tocopherol occurred in spring, and higher β -cryptoxanthin in winter, whereas, α -/ β -carotene and lycopene tended to be higher in summer or in autumn. This could be attributable to dietary variations by season, since all study participants come from central Japan (longitude around 136°~137° E., latitude around 35° N.), where there are distinct seasonal changes in the temperature and hours of sunlight. It is noteworthy that from September to later October Japanese enjoy an abundance of agricultural products, including fruit, and green and yellow vegetables. Our previous study of the same dietitian cohort showed that the dietary intakes of selected vitamins and minerals measured by weighed diet records were greater in autumn and winter than those in spring and summer (Tokudome et al., 2002). Similar seasonal changes have also been observed by Olmedilla (Olmedilla et al., 1994), and in studies on α -carotene (Van Staveren et al., 1986; Ziegler, 1989; Rautalahti et al., 1993). In the case of retinol, seasonal variations are not as remarkable as with other micronutrients, compatible with other studies (Olmedilla et al., 1994; Cooney et al., 1995); however, the reason remains obscure, suggesting that adjustments for seasonal effects should be allowed at least for α -tocopherol and the above-mentioned carotenoids in nutritional epidemiologic studies.

In conclusion, although mechanisms of sex and seasonal variations in plasma retinol, α -tocopherol and five carotenoids need to be further elucidated in different ethnic groups and areas, such changes in blood antioxidant levels should also be taken into account to determine any associations with disease prevention and health promotion in nutritional epidemiologic research.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, while Dr. Xin-En Huang was supported by the Japan Society for the Promotion of Science and its Postdoctoral Fellowships for Foreign Scientists from April 2000 to March 2004, and a research grant from "Liu Da Gao Fen Ren Cai" project of Jiangsu Province, China. The authors are also grateful to Ms. Y. Kubo, Ms. Y. Ito, Ms. K. Higuchi and Dr. Malcolm A. Moore for their technical and language assistance in preparing the manuscript.

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