Effects of Passive Smoking on Serum Levels of Carotenoids and α-Tocopherol

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To evaluate the effects of passive smoking identified by urine cotinine on serum carotenoids and α-tocopherol, we categorized 124 residents in a rural city of Japan into 4 groups by their urine cotinine/creatinine (Cot/Cr) ratio (u.d. (undetectable); low (0-50 ng/mg); moderate (50-120 ng/mg); high (>120 ng/mg)) and compared the serum carotenoids and α-tocopherol levels among these groups. We identified passive smoking by low Cot/Cr ratio level. After controlling on related factors, men with low Cot/Cr ratio showed significantly lower serum zeaxanthin/lutein levels than men with u.d. Cot/Cr ratio. The difference was still marginally significant after excluding self-reported current smokers from the low Cot/Cr group. It is suggested that low level exposure to tobacco smoke, which has been reported to be equivalent to that for passive smokers, could be associated with decreased serum zeaxanthin/lutein levels in men.

Received November 28, 1997; accepted February 26, 1998.

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Analyses were performed by SAS Ver 6.04. In this study, previous studies indicated that the Cot/Cr levels for passive smokers were below 50 ng/mg or 120 ng/mg. We categorized the Cot/Cr ratio into the following four categories: undetectable; low = 0-50 ng/mg; moderate = 50-120 ng/mg; and high = >120 ng/mg.

One month after our visit, we conducted a self-administered questionnaire survey for the participants. We collected information on their birthday, height, and weight, past history of cancer, and smoking status, period from the start of habitual smoking, and number of cigarettes smoked per day using the questionnaire. Alcohol consumption per day in the last year and vitamin supplements use was also asked in the questionnaire. We converted alcohol consumption into amount of pure ethanol intake.

After excluding 68 persons with one or more missing values in their background information or with a history of cancer, we analyzed the data from 55 men (mean age = 58.1 years old; SD = 11.2) and 69 women (mean age = 54.4 years old; SD = 10.9). None of the subjects had 40 IU or over levels of γ-GTP which is a possible indicator of impaired liver function. Seventy three percent of the men were employees or employees in companies and eighty seven percent of the women were housewives.

In statistical analysis, we calculated Spearman rank correlation coefficients between urine Cot/Cr ratio and serum levels of carotenoids and α-tocopherol. Analysis of covariance was also employed to compare the serum carotenoids and α-tocopherol levels by Cot/Cr category after common log-transformation. Other variables which we included into the model were age (years old), body mass index (BMI (kg/m²)), common log-transformed values of serum triglycerides and cholesterol, common log-transformed value of serum triglycerides and vitamin supplements use (4 or more times per week for any kind of vitamin supplements = 1; others = 0). These variables were reported as influential factors on serum carotenoids and α-tocopherol in previous studies. We carried out the analysis by sex, since quintile category of ethanol intakes of men were different from that of women, i.e., the amount of ethanol intakes of men were much larger than that of women. All of the analyses were performed by SAS Ver 6.04.

**RESULTS**

No women indicated the Cot/Cr ratio of 120 ng/mg or over (Table 1). Men with moderate and high Cot/Cr ratio showed significantly lower mean values of serum β-carotene than men with u.d. Cot/Cr ratio. Women with high Cot/Cr ratio and women with moderate Cot/Cr ratio showed significantly lower levels of serum cryptoxanthin than subjects with u.d. Cot/Cr ratio.

Rank correlation coefficients between Cot/Cr ratio and serum levels of carotenoids and α-tocopherol were as follows: for β-carotene, -0.47 (P = 0.0003) and -0.13 (P = 0.30); for cryptoxanthin, -0.39 (P = 0.003) and -0.21 (P = 0.09); for zeaxanthin/lutein, -0.14 (P = 0.30) and -0.10 (P = 0.41); for α-tocopherol, -0.21 (P = 0.13) and -0.14 (P = 0.24), in men and women, respectively.

Analysis of covariance showed that men with high Cot/Cr ratio had significantly lower serum concentrations of β-carotene, zeaxanthin/lutein and α-tocopherol than men with u.d. Cot/Cr ratio (Table 2). Moreover, men with low Cot/Cr ratio had significantly lower serum concentrations of zeaxanthin/lutein and than men with u.d. Cot/Cr ratio. Serum concentrations of carotenoids and α-tocopherol for women were not statistically different among the groups by Cot/Cr ratio.

Out of 29 men and 62 women with Cot/Cr ratio below 50 ng/mg, 4 men (14%) and one woman (2%) reported to be current smokers in the self-administered questionnaires (Table 3). Even when we excluded the subjects who reported themselves as current smokers from the group with low Cot/Cr ratio, the group showed marginally lower concentration of serum zeaxanthin/lutein than men with u.d. Cot/Cr ratio (P = 0.06). No such findings were observed in other carotenoids and α-tocopherol.

**DISCUSSION**

We found possible effects of passive smoking on serum zeaxanthin/lutein in men. However, we couldn't find such effects of passive smoking on other carotenoids and α-tocopherol. Zeaxanthin/lutein is an oxygenated carotenoids. It is reported that it retards the lipid peroxidation more efficiently than β-carotene and is powerful antioxidant than β-carotene. Our finding suggest that serum zeaxanthin/lutein might be consumed to reduce oxidants faster than other antioxidant vitamins, being consistent with the in vitro study. Tribble et al. reported that female passive smokers indicated lower plasma ascorbic acid concentration than nonsmokers. Ascorbic acid is one of water-soluble antioxidant and is known to be consumed by regenerating reduced α-tocopherol. Furthermore, α-tocopherol is known to protect β-carotene from peroxidation. Ascorbic acid may be consumed faster by reducing oxidants and free radicals than carotenoids and α-
Table 1. Profile of the subjects in each urine Cot/Cr ratio for both sexes.

| Urine Cot/Cr ratio | u.d. | Low | Moderate | High |
|--------------------|------|-----|----------|------|
| Number             | 11   | 18  | 18       | 8    |
| Age (years)        | 66.9 | 55.8** | 55.2**   | 58.0** |
| (5.8)              | (12.9) | (11.2) | (7.2)    |
| BMI (kg/m²)        | 22.5 | 23.4 | 22.2     | 21.7 |
| (2.7)              | (2.1)  | (3.5)  | (3.3)    |
| Urine Cot/Cr (ng/mg)| 0.0  | 6.2  | 90.5     | 305.7 |
| (13.5)             | (19.3) | (166.9) |         |
| Serum β-carotene   | 0.73 | 0.51 | 0.29**   | 0.17** |
| (μ mol/l)          | (0.41-1.30) | (0.27-0.97) | (0.09-0.93) | (0.09-0.33) |
| Serum cryptoxanthin| 0.21 | 0.24 | 0.15     | 0.10** |
| (μ mol/l)          | (0.13-0.36) | (0.14-0.43) | (0.06-0.36) | (0.05-0.17) |
| Serum zeaxanthin/| 1.69 | 1.18 | 1.14     | 1.15 |
| lutein (μ mol/l)   | (0.95-3.00) | (0.71-1.97) | (0.66-1.96) | (0.89-1.48) |
| Serum α-tocopherol | 22.5 | 20.2 | 17.8     | 16.3 |
| (μ mol/l)          | (14.1-35.8) | (13.8-29.6) | (11.9-26.5) | (12.7-20.8) |
| Carotene intake    | 2.42 | 2.90 | 2.07     | 2.00 |
| (mg/day)           | (1.21-4.83) | (1.75-4.79) | (0.75-16.59) | (0.86-4.69) |
| Vitamin C intake   | 52.1 | 63.9 | 51.6     | 63.0 |
| (mg/day)           | (31.0-87.8) | (40.9-99.7) | (25.0-106.2) | (41.3-96.0) |
| Vitamin E intake   | 8.30 | 10.02 | 8.02     | 9.48 |
| (mg/day)           | (5.85-11.76) | (8.14-12.33) | (6.15-10.47) | (6.86-11.29) |
| Ethanol intake†    | 18.7 | 49.4 | 54.2     | 48.2* |
| (g/day)            | (14.9) | (63.3) | (45.4)  | (35.1) |
| Serum total        | 4.77 | 4.79 | 4.64     | 4.34 |
| cholesterol (mmol/l)| (0.35) | (1.00) | (1.23)  | (0.95) |
| Serum triglyceride | 12.0 | 17.0 | 15.4     | 15.6 |
| (mmol/l)           | (8.0-18.1) | (9.8-29.6) | (8.9-26.7) | (10.5-23.1) |
| Vitamin supplements| 9.1  | 5.6  | 5.6      | 0.0  |

* P<0.05, ** P<0.01 compared with u.d. (t test)
Common log-trans formed values for serum carotenoids, α-tocopherol, carotene intake, vitamin C intake, vitamin E intake and serum triglycerides.
S.D. or 95% confidence interval in parenthesis.
† Wilcoxon rank-sum test
‡ χ² test

Table 2. Comparison of serum levels of carotenoids and α-tocopherol by urine Cot/Cr ratio for both sexes.

| Urine Cot/Cr ratio | u.d. | Low | Moderate | High |
|--------------------|------|-----|----------|------|
| Serum β-carotene   | 0.83 | 0.82 | 0.52     | 0.33* |
| (μ mol/l)          | (0.59-1.16) | (0.60-1.13) | (0.38-0.69) | (0.22-0.49) |
| Serum cryptoxanthin| 0.26 | 0.31 | 0.21     | 0.15* |
| (μ mol/l)          | (0.20-0.34) | (0.25-0.40) | (0.17-0.27) | (0.11-0.20) |
| Serum zeaxanthin/| 1.65 | 1.09*| 1.27     | 0.99* |
| lutein (μ mol/l)   | (1.34-2.02) | (0.90-1.32) | (1.06-1.51) | (0.78-1.27) |
| Serum α-tocopherol | 26.0 | 21.5 | 20.8     | 19.0* |
| (μ mol/l)          | (22.9-29.5) | (19.1-24.2) | (18.7-23.3) | (16.4-22.2) |

* P<0.05 compared with u.d.  †P = 0.08 compared with u.d.
95% confidence interval in parenthesis.
§ Adjusted for age, BMI, carotene intake, vitamin C intake, vitamin E intake, ethanol intake, serum total cholesterol, serum triglycerides and vitamin supplements use (analysis of covariance).
We could not observe any statistically significant differences in the concentrations of serum carotenoids and α-tocopherol by Cot/Cr category in women. We must also consider sex differences in antioxidants metabolism. Ito et al. reported girls aged 7-19 years old had higher serum concentration of β-carotene and cryptoxanthin than same age boys although the differences were not larger than subjects aged 20-69 years.
Table 3. Relationship between reported number of cigarettes smoked per day in questionnaire and urine Cot/Cr ratio.

| Urine Cot/Cr (ng/mg) | Number of cigarettes smoked per day | Males | Females |
|----------------------|------------------------------------|-------|---------|
|                      | 1-10 | 11-20 | 21-   | none | 1-10 | 11-20 | 21-   |
| u.d.                 | 11   | 0     | 0     | 31   | 0     | 0     | 0     |
| 0-50                 | 14   | 1     | 2     | 30   | 1     | 0     | 0     |
| 50-120               | 1    | 4     | 8     | 4    | 3     | 0     | 0     |
| >120                 | 1    | 1     | 3     | -    | -     | -     | -     |

old 17. Furthermore, we observed lower mean value of Cot/Cr in women with low Cot/Cr ratio than that of men with low Cot/Cr ratio. Muramatsu et al. observed higher ambient nicotine concentration in several offices than that in households in Japan 22. Male nonsmokers most of whom are working in their office are probably exposed more to environmental tobacco smoke than female nonsmokers most of whom are housewives.

We attempted to evaluate the effects of passive smoking on serum carotenoids and tocopherols by using an index of urine Cot/Cr ratio, because self reported exposure to tobacco smoke might be incorrect due to response bias. Previous studies indicated that passive smokers had urine Cot/Cr ratio below 50 ng/mg 16 or below 120 ng/mg 18. We used the former criteria to evaluate lower exposure level of tobacco smoke. Comparison of Cot/Cr ratio and the reported number of cigarettes smoked per day indicated that 78-97% of those with low Cot/Cr ratio (0-50 ng/mg) reported themselves as nonsmokers, supporting our identification of passive smokers. Even when we limited the analysis to those who reported themselves as nonsmokers, men with low Cot/Cr ratio had lower levels of serum zeaxanthin/lutein than those not exposed to tobacco smoke (i.e. u.d. Cot/Cr group), although, the difference was not statistically significant (P = 0.06). However, we need more validated criteria or other indicators to identify passive smokers.

ACKNOWLEDGEMENT

We are grateful to the late Dr. Takeshi Hirayama for his valuable suggestions. This study was partly supported by a grant of 06280108 from the Ministry of Education, Culture and Science, Japan.

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