

Relationship of Alcohol Use, Physical Activity and Dietary Habits with Serum Carotenoids, Retinol and Alpha-tocopherol among Male Japanese Smokers

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Background. Despite considerable interest in the anticarcinogenic and anti-atherosclerotic effects of carotenoids and alpha-tocopherol, little is known about determinants of these serum micronutrients.

Methods. The association of lifestyle factors including alcohol use, physical activity and dietary habits with serum levels of carotenoids (lycopene, lutein, cryptoxanthin and beta-carotene), retinol and alpha-tocopherol were studied in 194 healthy men aged 24-60 years who smoked >15 cigarettes/day. A self-administered questionnaire ascertained consumption frequency of 12 food items, alcohol consumption, levels of physical activity and the number of cigarettes smoked per day.

Results. Of the dietary items studied, total vegetable intake was significantly, positively associated with beta-carotene levels, as was fruit intake with serum levels of each carotenoid. *Tofu* intake was unexpectedly, but strongly related to decreased levels of cryptoxanthin and beta-carotene. None of the food items was materially related to serum levels of retinol and alpha-tocopherol. Alcohol consumption was most strongly and inversely associated with levels of all the carotenoids except lutein, whereas was positively associated with retinol level but not with alpha-tocopherol level. Frequency of participation in sports was significantly and positively associated with both retinol and alpha-tocopherol levels. The amount of cigarettes smoked per day was unrelated to each micronutrient level in this study of moderate or heavy smokers.

Conclusions. The consumption of vegetables and fruits is an important determinant of serum carotenoid levels even in smokers. Alcohol consumption is inversely associated with carotenoid levels, although the mechanism for this is not clear. *Tofu* and physical activity influence serum levels of antioxidative micronutrients, and these relationships need further studies.

Keywords: lifestyle, carotenoids, retinol, alpha-tocopherol, male smokers

Much attention has been drawn to possible beneficial effects of antioxidative micronutrients, such as carotenoids and alpha-tocopherol, in the prevention of cancer¹ and cardiovascular disease.^{2,3} These antioxidants are hypothesized to inhibit oxidative DNA damage leading to cancer by scavenging free radicals and also to inhibit the oxidation of low density lipoprotein (LDL)

cholesterol which initiates atherosclerosis. Although a recent intervention trial of beta-carotene and alpha-tocopherol supplementation in Finnish smokers has cast scepticism on the anticarcinogenic effects of these antioxidants,⁴ high intake of carotenoid-rich foods and high levels of serum beta-carotene or total carotene have fairly consistently been associated with reduced risk of certain cancers in man.⁵⁻⁷ Furthermore, supplementation with beta-carotene, alpha-tocopherol and selenium combined resulted in a moderate reduction in stomach cancer incidence in China.⁸ Epidemiological studies also have suggested that dietary or supplemental vitamin E may confer a reduced risk of coronary heart disease.⁹

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Despite considerable interest in the anticarcinogenic and anti-atherosclerotic effects of these micronutrients, little is known about determinants of serum micronutrient levels except for dietary or supplemental intake of carotenoid or alpha-tocopherol. Only cigarette smoking has consistently been associated with lower concentrations of serum beta-carotene or total carotenoids.¹⁰⁻¹² We examined the relation of alcohol consumption, physical activity and dietary habits to serum levels of carotenoids, retinol and alpha-tocopherol among male Japanese smokers. Because these micronutrients are lipid soluble, we also evaluated the association with serum lipids and lipoproteins.

MATERIALS AND METHODS

Study subjects were 194 male healthy smokers who participated in a randomized intervention study designed to investigate the effects of supplementation with beta-carotene and chlorophyll on cancer-associated biological markers including natural killer cell activity, micronuclei and sister-chromatid exchanges. A total of 428 men aged 20-60 years smoking ≥ 15 cigarettes per day were selected among clerical workers of eight private companies by one of the authors (HK), who had been in charge of health management of employees at these companies. Two hundred men agreed to participate in the study, and all of them were informed of the design and procedures of the study 2-8 weeks prior to the baseline survey. The baseline survey was carried out on the occasion of the biannual health examination in February and March, 1994. Six men were found afterwards to be users of supplement carotenoids, retinol or alpha-tocopherol; they were excluded from this cross-sectional analysis although they continued to participate in the randomized trial. Follow-up surveys were performed 3 months and 6 months after the baseline survey.

At each survey visit, the study participants were asked about their current lifestyle characteristics including the amount of cigarettes smoked per day, drinking habits, physical activity, and dietary habits, using an identical self-administered questionnaire. Supplementary interview was done by one of the authors (YK) and public health nurses to complete unanswered questions. For drinking habit, subjects were first asked whether they were lifelong non-drinkers, former drinkers or current drinkers, and then former and current drinkers answered a closed question about the average amount of alcohol consumed per day expressed as the amount of *sake* using a conventional unit of *go* (1 *go* = 180 ml). A conversion table for different types of alcoholic beverages was presented in the questionnaire,

and six levels of alcohol consumption were given (<1, 1, 2, 3, 4 and 5+ *go* per day). Two closed questions queried the average duration of walking per day (<30 min., 30 min.-1 h, 1-2 h or 2+ h) and the frequency of participating in sports (none, occasional or frequent). Dietary questions ascertained the usual frequency of consumption of 12 food items: green/yellow vegetables, fresh vegetables, total vegetables, fruits, *tofu* (soy bean curd), soy bean, pickles, milk, eggs, meats, fish, and seaweed; seven categories of consumption frequency were prepared for each food item (none, 1-3 times per month, 1-3 times per week, 4-6 times per week, once per day, twice per day and three times per day). Table 1 shows the rank correlation coefficients of the above questionnaire variables at baseline with the corresponding variables at subsequent surveys. The correlation coefficients ranged from 0.47 for green/yellow vegetables, fresh vegetables, or fish to 0.88 for the number of cigarettes smoked per day.

Venous blood was drawn after an overnight fast, and serum was separated within 3 h. Serum levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) and chylomicron were measured at an external laboratory (CRC, Fukuoka). Part of the sera was stored at -70°C temporarily and were transported on dry ice to Miyazaki University for the determination of levels of carotenoids (lycopene, lutein, cryptoxanthin and beta-carotene), retinol and alpha-tocopherol. These micronutrients were assayed using the modified Miller-Yang's method¹³ with high performance liquid chromatography (HPLC). These fat soluble vitamins and carotenoids were stable after several months' storage at -70°C , and coefficients of variation for standard serum sample were less than 5% in intra- and inter-assay.

In the present study, the baseline data were utilized to examine possible determinants of serum micronutrients. Statistical analysis was performed with the PC/SAS statistical package (SAS Institute, Cary/NC). Spearman's rank correlation coefficients (r) were used to examine the univariate relation between the factors of interest and serum micronutrients. Potential confounding effects of age and other factors were accommodated by calculating Spearman's partial rank correlation coefficients and by performing multiple regression analyses. Since the distributions of serum lycopene, cryptoxanthin, beta-carotene, triglyceride, VLDL and chylomicron were skewed to the right side, log-transformed values of these micronutrients, lipid and lipoproteins were used in calculating the means and 95% confidence intervals (CI) and in regression analyses. In regression models, physical activities were

TABLE 1 Rank correlation coefficients^a of questionnaire variables at baseline with the corresponding variables at subsequent surveys

| Variable | 3 months later (N = 194) ^b | 6 months later (N = 193) |
|--------------------------------------|--|-----------------------------|
| Cigarettes per day | 0.88 | 0.83 |
| Alcohol intake (go/day) | 0.82 | 0.84 |
| Walking time ^c | 0.63 | 0.55 |
| Sporting activity ^d | 0.74 | 0.65 |
| Green/yellow vegetables ^e | 0.47 | 0.48 |
| Fresh vegetables ^e | 0.47 | 0.48 |
| Total vegetables ^e | 0.61 | 0.61 |
| Fruits ^e | 0.69 | 0.56 |
| Tofu ^e | 0.64 | 0.58 |
| Soy bean ^e | 0.60 | 0.58 |
| Pickled vegetables ^e | 0.63 | 0.66 |
| Milk ^e | 0.82 | 0.82 |
| Egg ^e | 0.67 | 0.76 |
| Meat ^e | 0.57 | 0.60 |
| Fish ^e | 0.47 | 0.62 |
| Seaweeds ^e | 0.54 | 0.53 |

^a Spearman's rank correlation coefficients. All the coefficients shown are statistically significant with $P < 0.001$.

^b Number of subjects who attended the corresponding survey. Because of missing data, the actual number of subjects used in calculating the coefficient is slightly fewer for some variables.

^c Four levels per day (<30 min., 30 min.–1 h, 1–2 h or 2+ h)

^d Three levels of none, occasional and frequent.

^e Seven categories of weekly frequency (0, 0.5, 2, 5, 7, 14 and 21).

treated as ordinal variables; weekly frequency was used for each food item with the mid-point of a range assigned to the consumption category; and daily amounts (go) of alcohol consumed currently were used for alcohol consumption with values of 0 and 0.5 respectively assigned to non-drinkers (including former drinkers) and the consumption level of <1 go. Adjusted means were derived from the GLM procedure, and a test for the trend was based on statistical significance of the regression coefficient of an ordinal variable for the factor under consideration. All the P -values are two-sided, and the P -values < 0.05 were considered statistically significant.

RESULTS

Table 2 summarizes characteristics of the study subjects with respect to cigarette consumption, alcohol use, physical activity, dietary habits, serum micronutrients and serum lipids and lipoproteins. Serum levels of carotenoids, retinol and alpha-tocopherol were generally intercorrelated showing rank correlation coefficients from 0.15 ($P = 0.04$) between beta-carotene

TABLE 2 Characteristics of study subjects

| Variable | Mean | (95% confidence interval)/% |
|---|------|-----------------------------|
| Age (year), mean | 43.2 | (42.1–44.3) |
| Body mass index (kg / m ²), mean | 23.3 | (23.0–23.7) |
| Cigarettes per day, mean | 32.9 | (31.3–34.4) |
| Alcohol users, ≥ 1 go/day (%) | 68.4 | |
| Walking, ≥ 30 min./day (%) | 49.0 | |
| Sports, frequent (%) | 6.2 | |
| Daily user (%) | | |
| Green/yellow vegetables | 11.3 | |
| Fresh vegetables | 9.4 | |
| Total vegetables | 42.3 | |
| Fruits | 9.8 | |
| Tofu | 11.3 | |
| Soy bean | 5.2 | |
| Pickled vegetables | 33.2 | |
| Milk | 14.4 | |
| Egg | 20.6 | |
| Meat | 7.7 | |
| Fish | 6.2 | |
| Seaweeds | 5.7 | |
| Serum micronutrients, means | | |
| Lycopene ($\mu\text{g} / \text{dl}$)* | 10.0 | (8.7–11.5) |
| Lutein ($\mu\text{g}/\text{dl}$) [†] | 39.2 | (37.5–41.0) |
| Cryptoxanthin ($\mu\text{g}/\text{dl}$)* | 10.9 | (9.7–12.2) |
| Beta-carotene ($\mu\text{g}/\text{dl}$)* | 14.9 | (14.1–15.9) |
| Retinol ($\mu\text{g}/\text{dl}$) [†] | 69.2 | (66.1–72.2) |
| Alpha-tocopherol ($\mu\text{g}/\text{dl}$) [†] | 680 | (643–718) |
| Serum lipids and lipoproteins, means | | |
| Total cholesterol (mg/dl) [†] | 203 | (198–208) |
| HDL-cholesterol (mg/dl) ^{†a} | 52 | (50–54) |
| Triglyceride (mg/dl)* | 147 | (134–161) |
| LDL (mg/dl) ^{†b} | 552 | (527–578) |
| VLDL (mg/dl)* ^c | 150 | (132–171) |
| Chylomicron (mg/dl)* | 26 | (21–31) |

^a High density lipoprotein-cholesterol.

^b Low density lipoprotein.

^c Very low density lipoprotein.

* Geometric mean.

[†] Arithmetic mean.

and alpha-tocopherol to 0.66 ($P = 0.001$) between retinol and alpha-tocopherol; although beta-carotene was not correlated with retinol ($r = 0.05$, $P = 0.52$). Serum concentrations of all the micronutrients under study were progressively higher with advancing age except for beta-carotene (Table 3).

Table 4 presents age-adjusted correlation of behavioural and dietary variables with serum levels of carotenoids, retinol and alpha-tocopherol. Alcohol intake was negatively correlated with lycopene, cryptoxanthin and beta-carotene, and positively so with retinol. A negative correlation between alcohol intake and beta-carotene was most pronounced. Daily amount

TABLE 3 Mean concentrations and 95% confidence intervals of serum carotenoids, retinol, alpha-tocopherol, lipids and lipoproteins according to age groups

| | Age group | | | Trend <i>P</i> |
|--|-------------------|-------------------|-------------------|----------------|
| | 20–39 (N = 69) | 40–49 (N = 80) | 50–60 (N = 45) | |
| Serum micronutrients | | | | |
| Lycopene (µg/dl)* | 8.0 (6.3–10.1) | 9.8 (8.0–11.9) | 14.4 (10.4–20.1) | 0.002 |
| Lutein (µg/dl) [†] | 36.9 (34.3–39.3) | 39.0 (36.2–41.7) | 43.4 (39.2–47.6) | 0.006 |
| Cryptoxanthin (µg/dl)* | 8.8 (7.3–10.7) | 12.1 (10.5–13.9) | 12.5 (9.1–17.2) | 0.02 |
| Beta-carotene (µg/dl)* | 14.6 (13.1–16.2) | 14.2 (13.0–15.5) | 17.0 (14.8–19.6) | 0.09 |
| Retinol (µg/dl) [†] | 65.4 (60.4–70.4) | 70.6 (65.5–75.8) | 72.3 (66.8–77.9) | 0.07 |
| Alpha-tocopherol (µg/dl) [†] | 637 (571–702) | 686 (627–746) | 735 (663–807) | 0.05 |
| Serum lipids and lipoproteins | | | | |
| Total cholesterol (mg/dl) [†] | 193 (184–201) | 207 (199–216) | 211 (200–221) | 0.007 |
| HDL-cholesterol (mg/dl) ^{†a} | 51 (48–54) | 53 (50–56) | 53 (49–57) | 0.38 |
| Triglyceride (mg/dl)* | 144 (123–170) | 150 (130–173) | 147 (125–173) | 0.83 |
| LDL (mg/dl) ^{†b} | 506 (470–542) | 557 (518–596) | 615 (552–677) | 0.0 |
| VLDL (mg/dl)* ^c | 134 (108–168) | 159 (129–197) | 161 (125–207) | 0.27 |
| Chylomicron (mg/dl)* | 28 (20–40) | 30 (22–40) | 18 (11–27) | 0.11 |

^a High density lipoprotein-cholesterol.

^b Low density lipoprotein.

^c Very low density lipoprotein.

* Geometric mean.

[†] Arithmetic mean.

TABLE 4 Age-adjusted rank correlation coefficients^a of cigarette consumption, alcohol intake, physical activity and consumptions of foods with serum carotenoids, retinol and alpha-tocopherol among male smokers

| Variable | Lycopene | Lutein | Cryptoxanthin | Beta-carotene | Retinol | Alpha-tocopherol |
|--------------------------------------|----------|--------|---------------|---------------|---------|------------------|
| Body mass index | 0.00 | -0.12* | -0.06 | -0.02 | -0.02 | 0.05 |
| Cigarettes per day | -0.04 | -0.01 | -0.06 | -0.08 | 0.07 | 0.07 |
| Alcohol intake (go/day) | -0.32** | -0.03 | -0.27** | -0.51** | 0.15* | 0.04 |
| Walking time ^b | -0.06 | 0.01 | -0.05 | -0.01 | 0.09 | 0.06 |
| Sporting activity ^c | 0.06 | 0.12* | 0.16** | 0.03 | 0.17** | 0.13* |
| Green/yellow vegetables ^d | 0.11 | -0.01 | -0.07 | 0.14** | -0.03 | -0.10 |
| Fresh vegetables ^d | 0.04 | 0.02 | -0.04 | 0.13* | 0.01 | -0.07 |
| Total vegetables ^d | 0.08 | 0.05 | 0.05 | 0.21** | 0.03 | -0.05 |
| Fruits ^d | 0.16** | 0.11* | 0.21** | 0.21** | 0.02 | -0.06 |
| Tofu ^d | -0.09 | -0.06 | -0.22** | -0.20** | 0.07 | 0.01 |
| Soy bean ^d | 0.09 | 0.03 | -0.03 | 0.06 | -0.13* | -0.10 |
| Pickled vegetables ^d | 0.01 | -0.04 | -0.05 | 0.10 | -0.05 | -0.06 |
| Milk ^d | 0.06 | 0.00 | -0.03 | 0.04 | -0.00 | -0.01 |
| Egg ^d | -0.04 | 0.06 | -0.06 | 0.00 | -0.03 | -0.03 |
| Meat ^d | -0.09 | -0.03 | 0.01 | -0.18** | 0.09 | 0.02 |
| Fish ^d | -0.06 | 0.01 | 0.01 | 0.03 | 0.00 | 0.04 |
| Seaweeds ^d | -0.04 | 0.05 | -0.05 | 0.03 | -0.05 | -0.05 |

^a Spearman's partial correlation coefficients controlling for age.

^b Four levels per day (<30 min., 30 min.–1 h, 1–2 h or 2+ h).

^c Three levels of none, occasional and frequent.

^d Seven categories of weekly frequency (0, 0.5, 2, 5, 7, 14 and 21).

* $P < 0.05$, ** $P < 0.01$.

TABLE 5 Age-adjusted rank correlation coefficients^a of serum lipids and lipoproteins with serum carotenoids, retinol and alpha-tocopherol among male smokers

| Variable | Lycopene | Lutein | Cryptoxanthin | Beta-carotene | Retinol | Alpha-tocopherol |
|------------------------------|----------|--------|---------------|---------------|---------|------------------|
| Total cholesterol | 0.18** | 0.27** | 0.20** | 0.13* | 0.25** | 0.61** |
| HDL-cholesterol ^b | -0.13* | 0.12* | 0.00 | -0.10 | 0.17** | 0.02 |
| Triglyceride | 0.02 | 0.04 | -0.07 | -0.15** | 0.13 | 0.42** |
| LDL ^c | 0.23** | 0.13* | 0.20** | 0.22** | 0.09 | 0.32** |
| VLDL ^d | 0.02 | 0.02 | -0.06 | -0.13* | 0.11 | 0.37** |
| Chylomicron | 0.04 | 0.02 | -0.04 | 0.02 | 0.10 | 0.24** |

^a Spearman's partial correlation coefficients controlling for age.

^b High density lipoprotein-cholesterol.

^c Low density lipoprotein.

^d Very low density lipoprotein.

* $P < 0.05$, ** $P < 0.01$.

of cigarettes was not related to either carotenoids, retinol or alpha-tocopherol. While daily duration of walking was not materially related to any of the serum micronutrients, there were significant positive correlations of sports with lutein, cryptoxanthin, retinol and alpha-tocopherol levels. Consumption frequency of total vegetables was significantly, positively correlated with beta-carotene, but not with other carotenoids, retinol or alpha-tocopherol. Both green/yellow and fresh vegetables also was significantly positively correlated with beta-carotene. Fruit intake was positively and significantly correlated with all the carotenoids. Again, fruit intake was unrelated to retinol and alpha-tocopherol. While the consumption of soy bean was not correlated appreciably with any of the micronutrients studied, *tofu*, a product of soy bean, was significantly negatively correlated with cryptoxanthin and beta-carotene. There was a significant negative correlation between meat consumption and beta-carotene.

Age-adjusted correlation between serum lipids and lipoproteins and serum micronutrient levels is shown in Table 5. In general, total cholesterol and LDL were positively correlated with serum carotenoids, retinol, and especially alpha-tocopherol. Alpha-tocopherol levels were also positively correlated with other lipids and lipoproteins except HDL-cholesterol. There were negative correlations of triglyceride and VLDL with beta-carotene, whereas the former was almost unrelated to other carotenoids.

In order to examine the independent relation of behavioural and dietary variables to each of the micronutrients, multiple regression analysis was performed with age, amount of cigarettes smoked, alcohol intake, total vegetables and serum total cholesterol included always in the models as independent variables. (Tables

6 and 7.) Because consumptions of green/yellow, fresh and total vegetables were not mutually exclusive and because the consumption of total vegetables was most strongly correlated with serum beta-carotene (Table 4), green/yellow and fresh vegetables were omitted in the models. Likewise, because serum lipids and lipoproteins were intercorrelated to various degrees and because serum total cholesterol, rather than LDL, was correlated more uniformly with each of the micronutrients, only serum total cholesterol was selected as an independent variable among the lipid and lipoprotein parameters. Other independent variables were selected when they were significantly correlated with each micronutrient at the level of $P < 0.10$.

Results of the multiple regression analyses were almost the same as those noted in the age-adjusted analysis although the positive association between age and each micronutrient was much weaker or negligible. Alcohol intake remained the strongest correlate of lycopene, cryptoxanthin and beta-carotene in a negative direction; vegetable consumption was positively related to beta-carotene, but not to other carotenoids; fruit consumption was a significant correlate of most carotenoids except lycopene; a significant negative association of *tofu* with cryptoxanthin and beta-carotene remained (Table 6). A positive relation to sports remained significant for both retinol and alpha-tocopherol (Table 7). Serum total cholesterol also remained a strong correlate of carotenoids, retinol and alpha-tocopherol (Tables 6 and 7).

DISCUSSION

There are several methodological limitations in this study. Although we believe that information on the

TABLE 6 Results of multiple regression analysis between serum carotenoids and contributory factors among male smokers

| Independent variable | Dependent variable | | | | | | | |
|----------------------|--------------------|---------|--------|--------|---------------|---------|---------------|---------|
| | Lycopene | | Lutein | | Cryptoxanthin | | Beta-carotene | |
| | SRC ^a | t | SRC | t | SRC | t | SRC | t |
| Age | 0.18 | 2.59** | 0.11 | 1.52 | 0.12 | 1.75 | 0.05 | 0.79 |
| Smoking | -0.01 | -0.17 | -0.01 | -0.09 | 0.003 | 0.04 | 0.004 | 0.06 |
| Drinking | -0.28 | -4.08** | -0.06 | -0.86 | -0.26 | -3.88** | -0.45 | -7.45** |
| Sports | - | - | 0.07 | 1.07 | 0.14 | 2.15* | - | - |
| Total vegetables | 0.00 | 0.00 | -0.02 | -0.23 | -0.00 | -0.02 | 0.25 | 3.81** |
| Fruits | 0.08 | 1.15 | 0.15 | 2.00* | 0.22 | 3.04** | 0.14 | 2.13** |
| Tofu | - | - | - | - | -0.23 | -3.29** | -0.17 | -2.64** |
| Meat | - | - | - | - | - | - | -0.11 | -1.63 |
| Total cholesterol | 0.14 | 1.98** | 0.29 | 4.05** | 0.20 | 2.99** | 0.16 | 2.61** |
| R ² | 0.17 | | 0.15 | | 0.25 | | 0.37 | |

^a Standardized regression coefficient.

* $P < 0.05$, ** $P < 0.01$.

TABLE 7 Results of multiple regression analysis between serum retinol, alpha-tocopherol and contributory factors among male smokers

| Independent variable | Dependent variable | | | |
|----------------------|--------------------|--------|------------------|--------|
| | Retinol | | Alpha-tocopherol | |
| | SRC ^a | t | SRC | t |
| Age | 0.13 | 1.82 | 0.07 | 1.10 |
| Sports | 0.15 | 2.15* | 0.14 | 2.33* |
| Smoking | 0.03 | 0.39 | 0.06 | 1.03 |
| Drinking | 0.12 | 1.72 | 0.07 | 1.16 |
| Total cholesterol | 0.23 | 3.30** | 0.56 | 9.26** |
| R ² | 0.12 | | 0.36 | |

^a Standardized regression coefficient.

* $P < 0.05$, ** $P < 0.01$.

lifestyle factors examined was sufficiently reliable, as suggested by the moderate or good reproducibility (Table 1), physical activity and dietary habits were not quantitatively measured. In this sense, inaccuracy was unavoidable, and thus we may have failed to detect a real association. Analysis was somewhat explorative for some factors. This may have resulted in associations without biological significance due to multiple comparison. Because the present study was based on only male smokers, our results may not be applicable to females and non-smokers. However, homogeneity in smoking habit is rather an advantage in that the

confounding by smoking and its interaction with other factors could be minimized.

Age was strongly associated with elevated levels of most serum micronutrients studied in the univariate analysis. However, most of these associations were attenuated substantially or nullified in the multivariate analysis. Some studies reported a positive association between age and some carotenoids, retinol or alpha-tocopherol.¹⁴⁻¹⁷ Our findings suggest that positive associations between age and fat soluble micronutrients are mainly due to other factors rather than the effect of ageing itself. In fact, serum total cholesterol levels accounted largely for the associations of age with the micronutrient levels; after adjustment for total cholesterol, the Spearman's rank correlation coefficients with age decreased 15-31% for carotenoids, 34% for retinol and 89% for alpha-tocopherol.

We did not ascertain dietary intakes of carotenoids, retinol or alpha-tocopherol. Nevertheless frequent consumption of vegetables and fruits were found to be positively, although modestly, associated with serum beta-carotene levels. It is noteworthy that our observed association with vegetables or fruits was as strong as the correlation between dietary carotenoid intake and serum beta-carotene reported elsewhere.^{11,12,14,18} These findings indicate that the simple dietary advice to consume quantities of vegetables and fruits is reasonably valid to augment serum beta-carotene levels. While fruit consumption was associated significantly and positively with beta-carotene and other carotenoids, none of the three vegetable items was related to lycopene, lutein and cryptoxanthin. Lycopene is found in only a

limited number of vegetables such as tomatoes,^{19,20} and thus our broad questions of vegetables may have missed principal dietary sources of lycopene. However, high contents of lutein and cryptoxanthin are found in a wide variety of vegetables.^{19,20} We have no plausible explanation for the lack of association between vegetable consumption and serum levels of lutein or cryptoxanthin. It is possible that the response of serum levels to given carotenoid intakes may be less among smokers compared with non-smokers.^{10,11}

It is an unexpected finding that *tofu* consumption was negatively related to serum beta-carotene and cryptoxanthin. In this study, the significant correlates of *tofu* consumption were age ($r = 0.17$) and intakes of vegetables ($r = 0.32$), fruits ($r = 0.28$) and meat ($r = 0.20$); confounding effects by the first three variables that appear most important are not responsible for the observed inverse association between *tofu* and serum beta-carotene. *Tofu* is a traditional product of soy bean in Japan. Because the consumption of soy bean (including fermented or cooked soy bean) was virtually unrelated to serum levels of beta-carotene, cryptoxanthin and other carotenoids, the finding on *tofu* and specific carotenoids is likely to be a chance phenomenon. However, *tofu* was much more popular than other preparations of soy bean; median weekly frequency of consumption was two for *tofu* and 0.5 for soy bean. Unidentified components of soy bean may influence the absorption and metabolism of carotenoids with provitamin A activity.

Meat intake was also inversely related to serum beta-carotene. However, the multivariate analysis adjusting for all the significant correlates of meat (age, $r = -0.23$; vegetables, $r = 0.21$; *tofu*, $r = 0.20$; and alcohol intake, $r = 0.18$) revealed no significant association between meat intake and serum beta-carotene as shown in Table 6. Milk and eggs are foods rich in retinol, but neither was related to serum retinol levels in the present study. Some homeostatic mechanisms seem to play a role in maintaining serum retinol at a stable level regardless of retinol intake if individuals are not severely deficient. Vegetable oils, seeds and nuts are major dietary sources of alpha-tocopherol, but our dietary questions did not specifically ascertain the consumption of these foods.

Several studies have consistently observed lower levels of blood beta-carotene and total carotenoids among cigarette smokers, as compared with non-smokers.¹⁰⁻¹² The number of cigarettes smoked per day was not related to either beta-carotene or other carotenoids in the present study. However, our study subjects were current smokers consuming ≥ 15 cigarettes per day. In fact, 98% were those smoking ≥ 20 cigarettes

per day. Thus the lack of an inverse association between the numbers of cigarettes and carotenoid levels is not necessarily incompatible with previous findings elsewhere.¹⁰⁻¹²

Our findings on alcohol intake and serum beta-carotene are in agreement with previous observations.^{11,15,18} Not many studies have addressed the relation between alcohol consumption and other carotenoids in blood, and their findings are variable.^{10,12,14,15} These studies reported that alcohol consumption was insignificantly related to serum or plasma levels of lycopene and cryptoxanthin in either direction.^{10,12,15} The present study demonstrated an evident inverse relation of alcohol consumption to serum levels of lycopene and cryptoxanthin. The elevated concentration of serum retinol associated with alcohol intake is consistent with the limited available findings.^{12,16} Because beta-carotene and cryptoxanthin are principal precursors of retinol, it can be postulated that alcohol intake may accelerate the conversion of provitamin A to retinol. However, this idea cannot explain the significant inverse association between alcohol consumption and serum lycopene which is not provitamin A. Alcohol consumption not only increases serum concentrations of HDL-cholesterol²¹ but also may decrease serum concentrations of LDL which transports carotenoids in blood,^{21,22} thereby lowering serum carotenoid levels. It is also possible that alcohol consumption may affect the absorption of dietary carotenoids from mucous membranes of small intestine.²³

Frequent participation in sports was associated with elevated concentrations of serum retinol and alpha-tocopherol. To our knowledge, no other study has addressed the relation between physical activity and blood levels of retinol, alpha-tocopherol or carotenoids. We have no plausible explanation to our findings on sports. Since our reported association was not strong ($r = 0.14-0.17$), unknown confounding factors may be responsible. It was recently reported that alpha-tocopherol in serum and erythrocyte membranes increased after long-term strenuous exercise; alpha-tocopherol may be mobilized due to enhanced lipolysis after strenuous exercise.²⁴ Further studies are needed to corroborate our findings regarding physical activity and serum retinol or alpha-tocopherol.

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