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Differential Effects of Cigarette Smoking and Alcohol Consumption on the Plasma Levels of Carotenoids in Middle-aged Japanese Men

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Although several studies have reported that cigarette smoking and alcohol consumption are inversely associated with the plasma level of beta-carotene even after adjustment for dietary carotene intake, their effects on other carotenoids have not been examined extensively. The authors examined the associations of smoking and alcohol with plasma levels of the five principal carotenoids (beta-carotene, alpha-carotene, lutein, lycopene, and zeaxanthin). The subjects were 634 apparently healthy men aged 40–49 years who were sampled randomly from five areas in Japan with varying rates of mortality from gastric cancer. Multiple linear regression analysis controlling for age, serum total cholesterol, serum triglycerides, body mass index, and consumption frequencies of green vegetables, yellow vegetables and fruit, showed that both smoking and alcohol had a significant inverse association with the plasma levels of beta-carotene and alpha-carotene; only smoking reduced the level of lutein, and neither smoking nor alcohol significantly affected the level of lycopene or zeaxanthin. With regard to the reduction of beta-carotene and alpha-carotene, the effect of smoking was smaller in drinkers than in nondrinkers, and the effect of alcohol was smaller in smokers than in nonsmokers, and significant interactions between smoking and alcohol were observed ($P=0.034$ for beta-carotene and 0.026 for alpha-carotene). The results indicate that the differential effects of smoking and alcohol should be considered when the health effects of plasma carotenoids are examined.

Key words: Alcohol — Cigarette smoking — Plasma carotenoids

A number of epidemiologic studies have found an inverse relation of fruit and vegetable intake with risk of cancer.[1] Among many nutrients contained in fruit and vegetables, carotenoids, especially beta-carotene, have been considered the main contributors to the anti-cancer effect, and several researchers have reported that plasma levels of beta-carotene and total carotenes were associated with reduced risk of cancer.[2]

When the health effects of plasma carotenoids are examined, it is necessary to consider the influence of other lifestyle such as cigarette smoking and alcohol consumption. If the plasma levels are affected substantially by these factors, the true effects of plasma carotenoids may be over- or underestimated.

Previous studies have generally found that smoking and alcohol consumption are inversely correlated with the plasma level of beta-carotene, even after controlling for dietary intake.[3] However, their effects on other carotenoids have not been examined extensively. We therefore analyzed cross-sectional data obtained from 634 middle-aged Japanese men to assess how cigarette smoking and alcohol consumption affect the plasma levels of the five major carotenoids (beta-carotene, alpha-carotene, lutein, lycopene, and zeaxanthin) after controlling for fruit and vegetable intakes and other potentially confounding variables.

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MATERIALS AND METHODS

Study design and subjects The subjects were participants in a cross-sectional study that was conducted in five areas of Japan with varying rates of mortality from gastric cancer. Details of the study have been given elsewhere.[4–7] Briefly, 880 men aged 40–49 years were sampled randomly from the general populations residing in five Public Health Center districts and asked to participate. The response rate was 72% (n=634). The survey was conducted in the winters of 1989–1991, and included a questionnaire interview, anthropometric measurements, and blood collection.

Laboratory analyses Blood samples were obtained from all participants after fasting for at least 5 h. Plasma was separated immediately from whole blood by centrifugation, frozen within 30 min using a sufficient volume of dry ice, then stored in a deep-freeze at −80°C until subsequent assays. Plasma levels of the five major carotenoids (beta-carotene, alpha-carotene, lutein, lycopene, and zeaxanthin) were determined by high-performance liquid chromatography[8] in the laboratory of Hoffmann-La Roche, Ltd., Basel, Switzerland. Serum concentra-

tions of total cholesterol[9] and triglycerides[10] were determined by enzymatic methods in a commercial laboratory using a Hitachi 736 autoanalyzer (Special Reference Laboratory, Tokyo).

Collection of other data Trained public health nurses or dietitians interviewed the subjects on their smoking and drinking histories, and frequencies of fruit and vegetable intake. The subjects were classified as current cigarette smokers or nonsmokers (which included ex-smokers and those who had never smoked), as well as current drinkers or nondrinkers (which included ex-drinkers and lifetime abstainers). The alcohol information for current drinkers was converted to daily intake of absolute ethanol in grams.

Subjects were asked about the average number of days per week in which they had consumed fruit, yellow vegetables, and green vegetables during the past month. Fruit intake was assessed using a single question. In two questions for each type of vegetable, some examples were given as "yellow vegetables, such as carrot and pumpkin." Four frequency categories (and weights assigned to calculate weekly frequencies) were as follows; less often than once a week (0), 1–2 days/week (1.5), 3–4 days/week (3.5), and almost every day (6). Subjects' weight and height were measured, and body mass index (BMI) was computed as weight (kg) / height $(m)^2$.

Statistical analyses For descriptive purposes, untransformed mean values are presented for all variables assessed. The mean levels of plasma carotenoids are also presented according to smoking and drinking status. For subsequent correlation and regression analyses, smoking and alcohol intake were treated as continuous variables (number of cigarettes smoked per day and grams of alcohol consumed per day), and all variables except age were $\log_e$ transformed to improve normality. The univariate relationships between plasma carotenoids and other variables were assessed using Pearson correlation coefficients. Multiple linear regression models were used to examine the associations of plasma carotenoids with smoking and alcohol consumption after controlling for age, serum total cholesterol, serum triglycerides, BMI, and intake frequencies of green vegetables, yellow vegetables and fruit. Multivariate analyses were conducted for all subjects, and also for subjects stratified according to smoking or drinking status. Interaction between smoking and alcohol consumption was assessed using regression models that included a product term of the two variables.

Since the analyses with or without adjustment for the five study areas using four indicator variables revealed essentially the same findings, the results unadjusted for study areas are presented. All calculations were repeated employing carotenoid values that were standardized to serum total cholesterol and triglycerides using multiple regression models,[11] but the results remained essentially unchanged. Therefore the findings based on unadjusted values are given.

Table I. Characteristics of Subjects

Variable	Mean	SD
Age	44.4	3.0
Cigarette consumption (no./day)[a]	14.3	15.6
Current smoker (%)	57.7	
Alcohol intake (g/day)[b]	25.3	26.0
Current drinkers (%)	81.2	
Green vegetables intake (no. of days/week)[c]	3.9	2.0
Yellow vegetables intake (no. of days/week)[c]	2.6	1.9
Fruit intake (no. of days/week)[c]	3.4	2.2
Body mass index	23.9	2.9
Serum total cholesterol (mg/dl)	197.5	34.5
Serum triglycerides (mg/dl)	145.4	121.6
Beta-carotene (μmol/liter)	0.294	0.241
Alpha-carotene (μmol/liter)	0.066	0.050
Lutein (μmol/liter)	0.397	0.163
Lycopene (μmol/liter)	0.167	0.160
Zeaxanthin (μmol/liter)	0.072	0.045
Total carotenoids (μmol/liter)[d]	1.002	0.456

a) Including ex-smokers and nonsmokers.
b) Including ex-drinkers and nondrinkers.
c) Four frequency categories originally asked and weights assigned to calculate weekly frequencies are as follows; less than once a week (0), 1–2 days/week (1.5), 3–4 days/week (3.5), and almost everyday (6).
d) The sum of five carotenoids.

RESULTS

On average, the subjects smoked 14 cigarettes and consumed 25 g of alcohol per day (Table I). Sixty percent of the subjects smoked and 80% consumed alcohol currently. The subjects consumed green vegetables, yellow vegetables and fruit 3.9, 2.6 and 3.4 days/week, respectively. The mean values of BMI, serum total cholesterol and triglycerides were 24, 198 ml/dl and 145 ml/dl, respectively. The number of subjects taking supplements at least once a week was 15 (2.4%) for multiple vitamins, only one (0.2%) for vitamin A, and none for beta-carotene or other carotenoids. Exclusion of the supplement users did not change the findings materially, so they were included in subsequent analyses.

Figure 1 presents the proportional change in the mean levels of plasma carotenoids according to smoking and drinking status. The mean levels in nonsmoking nondrinkers were 0.45, 0.10, 0.41, 0.19, 0.07 and 1.22 μmol/liter for beta-carotene, alpha-carotene, lutein, lycopene, zeaxanthin and total carotenoids (the sum of the five carotenoids), respectively. For beta-carotene and alpha-carotene, the levels were highest in nonsmoking nondrinkers and lowest in smoking drinkers, who had only 60% of the concentrations relative to nonsmoking nondrinkers. For lutein and lycopene, the levels were higher in nonsmokers than in smokers but did not differ by drinking status. For zeaxanthin, the level did not vary with smoking or drinking status. The level of total carotenoids was higher in nonsmokers than in smokers, and also in nondrinkers than in drinkers.

Table II shows Pearson correlation coefficients between plasma carotenoids and other variables. The levels of beta-carotene, alpha-carotene, and total carotenoids were inversely correlated with both smoking and drinking. Lutein was negatively associated with smoking but not with alcohol consumption. The concentrations of lycopene and zeaxanthin were not correlated significantly with either smoking or drinking.

Table III presents standardized regression coefficients for smoking and alcohol in predicting plasma carotenoid levels. In analyses using all subjects, multivariate adjustments did not alter appreciably the associations of carotenoids with smoking and drinking that were observed

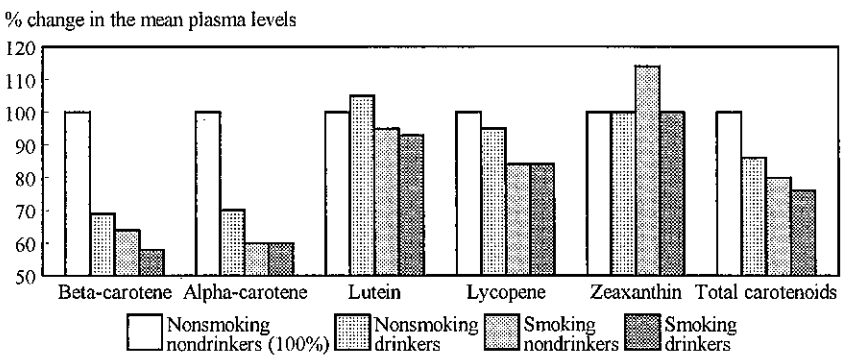

Fig. 1. Proportional change in the mean levels of plasma carotenoids according to smoking and drinking status.

Table II. Pearson Correlation Coefficients between Plasma Levels of Carotenoids and Other Variables

	Beta-carotene	Alpha-carotene	Lutein	Lycopene	Zeaxanthin	Total carotenoids
Smoking (no. of cigarettes/day)	−0.17****	−0.19****	−0.15***	−0.03	0.00	−0.16****
Alcohol (g/day)	−0.27****	−0.17****	0.01	−0.05	−0.01	−0.16****
Green vegetables (no. of days/week)	0.10*	0.15***	0.16****	−0.02	−0.01	0.12**
Yellow vegetables (no. of days/week)	0.15***	0.18****	0.15****	0.03	0.05	0.18****
Fruit (no. of days/week)	0.15****	0.05	0.12**	−0.10*	0.10*	0.11**
Age	0.17****	0.07	0.14***	−0.04	0.00	0.13***
Body mass index	−0.08*	0.00	−0.03	0.12**	0.00	−0.01
Serum total cholesterol (mg/dl)	0.13**	0.16****	0.25****	0.14***	0.14***	0.22****
Serum triglycerides (mg/dl)	−0.17****	−0.09*	0.03	0.07	0.12**	−0.04

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

with univariate Pearson correlations. In analyses that stratified the subjects by drinking or smoking status, differential effects of smoking and drinking were observed for beta-carotene and alpha-carotene; the inverse relations with smoking were smaller in drinkers than in nondrinkers, the inverse relations with alcohol were smaller in smokers than in nonsmokers, and significant interactions in an antagonistic direction were observed between smoking and drinking ($P=0.034$ for beta-carotene and 0.026 for alpha-carotene). For other carotenoids, no such interactions were detected.

DISCUSSION

Several epidemiologic studies have examined the effects of specific carotenoids (other than total carotene

Table III. Multivariate Standardized Regression Coefficients for Smoking and Alcohol in Predicting Plasma Levels of Carotenoids[a)]

	No. of subjects	Beta-carotene	Alpha-carotene	Lutein	Lycopene	Zeaxanthin	Total carotenoids
Standardized regression coefficients for smoking[b)]							
All subjects	634	−0.08*	−0.13***	−0.12**	−0.04	−0.01	−0.11**
Current drinkers	515	−0.08	−0.11*	−0.14**	−0.04	−0.05	−0.11*
Nondrinkers	119	−0.21*	−0.27**	−0.05	−0.08	0.07	−0.19*
Standardized regression coefficients for alcohol[c)]							
All subjects	634	−0.23****	−0.15****	0.04	−0.06	0.00	−0.14***
Current smokers	366	−0.18***	−0.07	0.01	−0.05	−0.07	−0.10*
Nonsmokers	268	−0.30****	−0.22***	0.07	−0.05	0.06	−0.17**
P value for interaction		0.034	0.026	0.351	0.823	0.089	0.231

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

a) Adjusted for age, serum total cholesterol, serum triglycerides, body mass index, intake frequency of green vegetables, yellow vegetables and fruit.

b) No. of cigarettes per day.

c) Grams of alcohol per day.

Table IV. Summary of Studies Examining the Relation of Smoking and Alcohol with Plasma Levels of Carotenoids[a)]

Author (ref.)	Subjects			Associations with smoking						Associations with alcohol					
	Sex	Age	n	Beta-carotene	Alpha-carotene	Lycopene	Lutein	Zea-xanthin	Crypto-xanthin	Beta-carotene	Alpha-carotene	Lycopene	Lutein	Zea-xanthin	Crypto-xanthin
Aoki *et al.* (16)	men	40–79	317	− m	− u	− u	•	•	•	− m	− u	(−) u	•	•	•
	women	40–79	532	− m	(−) u	(−) u	•	•	•	− m	(−) u	(−) u	•	•	•
Roidt *et al.* (17)	men and women	48–68	302	− m	− m	•	•	•	•	− m	(±) m	•	•	•	•
Stryker *et al.* (18)	men	18–79	137	− m	(−) u	(−) u	•	•	•	− m	(±) u	(±) u	•	•	•
	women	18–79	193	− m	(−) u	(−) u	•	•	•	(−) m	(±) u	(±) u	•	•	•
Ito *et al.* (19)	men and women	40–70	775	− m	•	•	− m[b)]	− m[b)]	− m	− m	•	•	(±) m[b)]	(±) m[b)]	− m
Ascherio *et al.* (20)	men	55.7 (10.6)[c)]	121	− u	•	•	•	•	•	(−) m	(−) m	(+) m	(+) m	(−) m	•
	women	52.7 (7.2)[c)]	186	− u	•	•	•	•	•	(−) m	(−) m	(−) m	(+) m	(+) m	•
Sanders *et al.* (21)	men and women	66–87	99	− m	(−) m	(−) m	− m	•	− m	•	•	•	•	•	•
Pamuk *et al.* (22)	women	30–69	91	− m	− m	− m	(−) m[b)]	(−) m[b)]	− m	•	•	•	•	•	•
The present study	men	40–49	634	− m	− m	(−) m	− m	(−) m	•	− m	− m	(−) m	(+) m	(−) m	•

a) −, inversely associated; +, positively associated; ±, no or inconsistent association; •, not reported. Without parenthesis, statistically significant. With parenthesis, statistically not significant or not tested. m, multivariate analysis. u, univariate analysis.

b) Lutein and zeaxanthin were not separated.

c) Mean (SD).

or beta-carotene) against cancer occurrence. For instance, Giovannucci *et al.* conducted a prospective study in 48,000 U. S. male health professionals to assess prostate cancer risk in relation to the intake of five carotenoids (beta- and alpha-carotenes, beta-cryptoxanthin, lycopene, and lutein), and found a lower risk of the disease with higher intake of lycopene.[12] Helzlsouer *et al.* undertook a nested case-control study which compared the prediagnostic serum levels of five carotenoids (beta- and alpha-carotenes, cryptoxanthin, lycopene, and lutein) in 35 ovarian cancer cases with those in 67 controls, and found no associations between the carotenoid levels and the risk.[13] International ecological studies have also examined the geographic correlations between the average intake of specific carotenoids and risk of lung cancer,[14] or cancers in several sites.[15] It is therefore important to clarify the roles of behavioral factors that may affect the plasma levels of specific carotenoids.

In this study of a population-based sample of middle-aged Japanese men, both smoking and alcohol were associated with reduced levels of beta-carotene, alpha-carotene, and total carotenoids. Smoking, but not drinking, was related to a decrease of plasma lutein. Neither smoking nor drinking affected significantly the levels of lycopene and zeaxanthin. Multivariate adjustment for potentially confounding variables did not change these associations.

Table IV summarizes the reports that have examined the effects of smoking and alcohol consumption on plasma levels of multiple carotenoids.[16–22] Comparability between these studies is limited, as they differed in subject characteristics (age, sex, ethnicity, and prevalence of smoking and drinking), extent of questionnaire estimates of dietary intake (fruit and vegetables, total carotenes, or specific carotenoids), method of data analysis (univariate or multivariate), and the sets of covariates adjusted for in multivariate analyses.

Nevertheless, all were quite consistent in showing a significant inverse association of beta-carotene with smoking and drinking. Inverse associations of alpha-carotene with smoking and drinking are also noted, although they were less consistent than the results attained with beta-carotene. Studies measuring lycopene found negative, mostly nonsignificant, correlations with smoking,[16, 18, 21, 22] which may suggest a small reduction in the plasma level due to smoking. Lutein, zeaxanthin and cryptoxanthin seemed to be correlated inversely with smoking, but their relations with alcohol consumption were inconsistent. These studies and our results indicate that the plasma levels of specific carotenoids are affected to different extents by smoking and alcohol, and therefore the influence of these two factors should be considered when the health effects of plasma carotenoids are examined.

We found interactions in an antagonistic direction between smoking and alcohol on the plasma levels of alpha- and beta-carotenes. This means that, with regard to the reduction of the two carotenoid levels, the effect of smoking was smaller in drinkers than in nondrinkers and the effect of alcohol was smaller in smokers than in nonsmokers. If there were no interactions, then the extent of the effect by smoking would have been the same in drinkers and in nondrinkers, and the extent of the effect by alcohol would have been the same in smokers and in nonsmokers. The observed interactions indicate that simple multivariate adjustment for smoking and alcohol may not suffice in order to reveal the unbiased effects of the two carotenoids against cancer, so that analysis stratifying the subjects according to smoking and alcohol status may be necessary.

The reasons why smoking and alcohol reduce the plasma levels of alpha- and beta-carotenes have not been well explored. Gastrointestinal absorption of the two carotenoids may be decreased among smokers and drinkers, or their utilization in cells and tissues may be increased owing to production of active oxygen and free radicals induced by smoking[19] and possibly by alcohol. Physiological mechanisms involving the reduction of plasma alpha- and beta-carotenes by smoking and alcohol warrant further investigation.

Various effects of smoking and drinking on plasma levels of specific carotenoids would restrict their utility as markers of dietary intake. Although plasma carotenoids have been used as reference standards to examine the validity of dietary intake estimated from food frequency questionnaires,[17, 18, 20, 23–27] some studies have in fact found that the correlation between plasma levels and dietary intakes of carotenoids was lower in smokers than in nonsmokers,[18, 26, 27] in current smokers than in former smokers,[17] and in drinkers than in nondrinkers.[18] Two recent studies found good agreement between plasma carotenoid levels and fruit and vegetable intake.[28, 29] However, the applicability of these findings is limited, since the studies excluded smokers and moderate to heavy drinkers,[28] or employed predominantly ex-smokers.[29] Therefore, when the plasma levels of carotenoids are compared with dietary intake, separate analyses based on smoking and drinking status would be necessary.

One limitation in this study is that the questionnaire estimates of fruit and vegetable consumption were crude and the intakes of specific carotenoids were not measured. Previous studies examining the relation of smoking and alcohol with plasma carotenoids also used the consumption frequency of fruit and vegetable[16, 19] or the intake of total carotene[18, 20] as proxy indicators for intakes of specific carotenoids, and only one study directly measured the intakes of specific carotenoids.[22] Although residual confounding due to such incomplete measure-

ments would not differ substantially with smoking or drinking status and the observed results should be comparable between these groups, further studies using more extensive methods of dietary assessment will be required.

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REFERENCES

1) Steinmetz, K. A. and Potter, J. D. Vegetables, fruits, and cancer. I. Epidemiology. *Cancer Causes Control,* **2,** 325–357 (1991).
2) van Poppel, G. Carotenoids and cancer: an update with emphasis on human intervention studies. *Eur. J. Cancer,* **29A,** 1335–1344 (1993).
3) Rimm, E. and Colditz, G. Smoking, alcohol, and plasma levels of carotenes and vitamin E. *Ann. N.Y. Acad. Sci.,* **686,** 323–333 (1993).
4) Tsugane, S., Gey, F., Ichinowatari, Y., Miyajima, Y., Ishibashi, T., Matsushima, S., Hirota, Y., Inami, T., Yamaguchi, M., Karita, K., Kabuto, M., Takashima, Y., Todoriki, H., Tsuda, M., Akabane, M., Furuichi, Y., Hamada, G. and Watanabe, S. Cross-sectional epidemiologic study for assessing cancer risks at the population level. I. Study design and participation rate. *J. Epidemiol.,* **2,** 75–81 (1992).
5) Tsugane, S., Gey, F., Ichinowatari, Y., Miyajima, Y., Ishibashi, T., Matsushima, S., Hirota, Y., Inami, T., Yamaguchi, M., Karita, K., Kabuto, M., Takashima, Y., Todoriki, H., Tsuda, M., Akabane, M., Furuichi, Y., Hamada, G. and Watanabe, S. Cross-sectional epidemiologic study for assessing cancer risks at the population level. II. Baseline data and correlation analysis. *J. Epidemiol.,* **2,** 83–89 (1992).
6) Tsugane, S., Tsuda, M., Gey, F. and Watanabe, S. Cross-sectional study with multiple measurements of biological markers for assessing stomach cancer risks at the population level. *Environ. Health Perspect.,* **98,** 207–210 (1992).
7) Tsugane, S., Tei, Y., Takahashi, T., Watanabe, S. and Sugano, K. Salty food intake and risk of *Helicobacter pylori* infection. *Jpn. J. Cancer Res.,* **85,** 474–478 (1994).
8) Hess, D., Keller, H. E., Oberlin, B., Bonfanti, R. and Schüep, W. Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. *Int. J. Vitam. Nutr. Res.,* **61,** 232–238 (1991).
9) Richmond, W. Preparation and properties of a cholesterol oxidase from *Nocardia* sp. and its application to the enzymatic assay of total cholesterol in serum. *Clin. Chem.,* **19,** 1350–1356 (1973).
10) Spayd, R. W., Bruschi, B., Burdick, B. A., Dappen, G. M., Eikenberry, J. N., Esders, T. W., Figuerus, J., Goodhue, C. T., LaRossa, D. D., Nelson, R. W., Rand, R. N. and Wu, T. W. Multilayer film elements for clinical analysis: applications to representative chemical determinants. *Clin. Chem.,* **24,** 1343–1350 (1978).
11) Jordan, P., Brubacher, D., Moser, U., Stähelin, H. B. and Gey, K. F. Adjustment of the plasma vitamin E and vitamin A levels to cholesterol and triglycerides by multiple regression. *Clin. Chem.,* **41,** 924–927 (1995).
12) Giovannucci, E., Ascherio, A., Rimm, E. B., Stampfer, M. J., Colditz, G. A. and Willett, W. C. Intake of carotenoids and retinol in relation to risk of prostate cancer. *J. Natl. Cancer Inst.,* **87,** 1767–1776 (1995).
13) Helzlsouer, K. J., Alberg, A. J., Norkus, E. P., Morris, J. S., Hoffman, S. C. and Comstock, G. W. Prospective study of serum micronutrients and ovarian cancer. *J. Natl. Cancer Inst.,* **88,** 32–37 (1996).
14) Le Marchand, L., Hankin, J. H., Bach, F., Kolonel, L. N., Wilkens, L. R., Stacewicz-Sapuntzakis, M., Bowen, P. E., Beecher, G. R., Laudon, F., Baqué, P., Daniel, R., Seruvatu, L. and Henderson, B. E. An ecological study of diet and lung cancer in the South Pacific. *Int. J. Cancer,* **63,** 18–23 (1995).
15) Ocké, M. C., Kromhout, D., Menotti, A., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., Jansen, A., Nedeljkovic, S., Nissinen, A., Pekkarinen, M. and Toshima, H. Average intake of anti-oxidant (pro)vitamins and subsequent cancer mortality in the 16 cohorts of the Seven Countries Study. *Int. J. Cancer,* **61,** 480–484 (1995).
16) Aoki, K., Ito, Y., Sasaki, R., Ohtani, M., Hamajima, N. and Asano, A. Smoking, alcohol drinking and serum carotenoids levels. *Jpn. J. Cancer Res.,* **78,** 1049–1056 (1987).
17) Roidt, L., White, E., Goodman, G. E., Wahl, P. W., Omenn, G. S., Rollins, B. and Karkeck, J. M. Association of food frequency questionnaire estimates of vitamin A intake with serum vitamin A levels. *Am. J. Epidemiol.,* **128,** 645–654 (1988).
18) Stryker, W. S., Kaplan, L. A., Stein, E. A., Stampfer, M. J., Sober, A. and Willett, W. C. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. *Am. J. Epidemiol.,* **127,** 283–296 (1988).

19) Ito, Y., Sasaki, R., Suzuki, S. and Aoki, K. Relationship between serum xanthophyll levels and the consumption of cigarettes, alcohol or foods in healthy inhabitants of Japan. *Int. J. Epidemiol.*, **20**, 615–620 (1991).
20) Ascherio, A., Stampfer, M. J., Colditz, G. A., Rimm, E. B., Litin, L. and Willett, W. C. Correlations of vitamin A and E intakes with the plasma concentrations of carotenoids and tocopherols among American men and women. *J. Nutr.*, **122**, 1792–1801 (1992).
21) Sanders, T. A. B., Haines, A. P., Wormald, R., Wright, L. A. and Obeid, O. Essential fatty acids, plasma cholesterol, and fat-soluble vitamins in subjects with age-related maculopathy and matched control subjects. *Am. J. Clin. Nutr.*, **57**, 428–433 (1993).
22) Pamuk, E. R., Byers, T., Coates, R. J., Vann, J. W., Sowell, A. L., Gunter, E. W. and Glass, D. Effect of smoking on serum nutrient concentrations in African-American women. *Am. J. Clin. Nutr.*, **59**, 891–895 (1994).
23) Willett, W. C., Stampfer, M. J., Underwood, B. A., Speizer, F. E., Rosner, B. and Hennekens, C. H. Validation of a dietary questionnaire with plasma carotenoid and alpha-tocopherol levels. *Am. J. Clin. Nutr.*, **38**, 631–639 (1983).
24) Russell-Briefel, R., Bates, M. W. and Kuller, L. H. The relationship of plasma carotenoids to health and biochemical factors in middle-aged men. *Am. J. Epidemiol.*, **122**, 741–749 (1985).
25) Nierenberg, D. W., Stukel, T. A., Baron, J. A., Dain, B. J., Greenberg, E. R. and The Skin Cancer Prevention Study Group. Determinants of plasma levels of beta-carotene and retinol. *Am. J. Epidemiol.*, **130**, 511–521 (1989).
26) Bolton-Smith, C., Casey, C. E., Gey, K. F., Smith, W. C. S. and Tunstall-Pedoe, H. Antioxidant vitamin intakes assessed using a food-frequency questionnaire: correlation with biochemical status in smokers and non-smokers. *Br. J. Nutr.*, **65**, 337–346 (1991).
27) Coates, R. J., Eley, J. W., Block, G., Gunter, E. W., Sowell, A. L., Grossman, C. and Greenberg, R. S. An evaluation of a food frequency questionnaire for assessing dietary intake of specific carotenoids and vitamin E among low-income black women. *Am. J. Epidemiol.*, **134**, 658–671 (1991).
28) Campbell, D. R., Gross, M. D., Martini, M. C., Grandits, G. A., Slavin, J. L. and Potter, J. D. Plasma carotenoids as biomarkers of vegetable and fruit intake. *Cancer Epidemiol. Biomarkers Prev.*, **3**, 493–500 (1994).
29) Le Marchand, L., Hankin, J. H., Carter, F. S., Essling, C., Luffey, D., Franke, A. A., Wilkens, L. R., Cooney, R. V. and Kolonel, L. N. A pilot study on the use of plasma carotenoids and ascorbic acid as markers of compliance to a high fruit and vegetable dietary intervention. *Cancer Epidemiol. Biomarkers Prev.*, **3**, 245–251 (1994).