Jpn. J. Cancer Res. 81, 463–469, May 1990

# Changes in Serum Concentrations of β-Carotene and Changes in the Dietary Intake Frequency of Green-Yellow Vegetables among Healthy Male Inhabitants of Japan

Sadao Suzuki,[1] Ryuichiro Sasaki,[1] Yoshinori Ito,[2] Nobuyuki Hamajima,[1] Atsuko Shibata,[1] Akiko Tamakoshi,[1] Motohiko Otani[2] and Kunio Aoki[1]

[1]*Department of Preventive Medicine, Nagoya University School of Medicine, 65, Tsurumai-cho, Showa-ku, Nagoya 466 and* [2]*Department of Hygiene, Fujita-Gakuen Health University School of Medicine, Dengakugakubo 1-98, Kutsukake-cho, Toyoake 470-11*

**Serum levels of β-carotene among 147 healthy male inhabitants were measured twice with an interval of one year in order to determine the relationship between changes in serum β-carotene levels and changes in the dietary intake of green-yellow vegetables. A positive association was found to exist between changes in the intake frequency of green-yellow vegetables and changes in serum β-carotene levels, whereas changes in alcohol intake and smoking were discovered to be negatively associated with changes in serum β-carotene levels. The positive association between changes in the intake frequency of green-yellow vegetables and changes in serum β-carotene levels was preserved after adjustment for these negative factors.**

Key words: β-Carotene — Green-yellow vegetable — Alcohol drinking — Smoking — Male inhabitant

A great deal of interest has been expressed in the relationship between β-carotene and cancer. It has been suggested that carotene has a chemopreventive potential with respect to cancer. Case-control studies of lung cancer,[1] prostate cancer[2, 3] and laryngeal cancer[4] have shown increased risks of these cancers in people with lower dietary intake of carotene. A few epidemiological studies based on collected blood samples have shown lower levels of β-carotene to be positively associated with lung cancer,[5, 6] and a large-scale cohort study conducted in Japan showed lower risks of death due to lung and stomach cancers among those who ate green-yellow vegetables every day than among those who did not.[7]

There have been a number of reports concerning the relationship between serum levels of β-carotene and both amounts of dietary β-carotene and the intake of green-yellow vegetables.[8–13] As a result of these preclinical and epidemiological studies, numerous clinical trials of β-carotene in various dosages have been begun in order to test its power as a preventative of carcinogenesis. The administration of 15 to 30 mg of β-carotene per day has been shown to result in a significant increase in plasma carotene[14] and plasma β-carotene[15, 16] concentrations.

However, there have been very few studies of the association between changes in lifestyle habits, including dietary habits, and changes in β-carotene levels. The purpose of the present study is therefore to clarify the relationship between changes in the intake frequency of green-yellow vegetables and changes of serum β-carotene concentration.

## MATERIALS AND METHODS

**Subject** The subjects in this study were inhabitants of Yakumo, a rural area located on the Pacific coast of southern Hokkaido. The population of Yakumo is about 20,000 people, and the area's main industries are agriculture, dairy farming and fishing. A general health screening program is conducted in the area every August, and the subjects of this study were males who had participated in the program twice successively during the years 1986 and 1987, or 1987 and 1988. As for the individuals who took part in the program three times successively, the data in 1988 were not used in the analysis. Twenty-five participants afflicted with liver dysfunction (indicated by serum GOT or GPT greater than or equal to 30) were excluded from this study. A total of 147 adult males aged between 40 and 79 years were thus chosen as the subjects of this study.

**Serum β-carotene concentration** Blood specimens were taken from the subjects after fasting (before breakfast or at least five hours after breakfast). The serum was immediately preserved at a temperature of −80°C. Samples of this serum were prepared for high-performance liquid chromatography (HPLC), and concentrations of β-carotene were measured using a modification of Miller and Yang's method.[17] Measurements for all of the specimens were performed by two chemists in one laboratory within a six-month period.

**Changes in intake frequency of green-yellow vegetables and other epidemiological characteristics** The subjects were interviewed by trained public health nurses at the time of the program each year. Subjects covered by the questionnaire used in the interview included dietary habits, especially regarding the intake frequency of green-yellow vegetables (measured as average times per week), as well as alcohol drinking and smoking habits.

The subjects were scored each year according to their frequency of green-yellow vegetable intake. Subjects who ate green-yellow vegetables less than once per week were given a score of 1; those who ate green-yellow vegetables once or twice per week were assigned a score of 2; those who ate green-yellow vegetables three or four times each week were assigned a score of 3; and those whose green-yellow vegetables intake consumption amounted to five or more times per week were given a score of 4. Then the subjects were divided among three categories according to the changes in their scores over the year for which they were studied. Those whose scores increased by two or three points will be referred to as 'increased remarkably'; those whose scores decreased by one to three points will be referred to as 'decreased'; and all other subjects will be referred to as 'unchanged or slightly increased.'

The subjects were also assigned to one of three categories reflecting changes in their intake of alcohol over the period of the study. The categories were: those who 'started' drinking, those who 'quitted' drinking, and those whose drinking habits remained 'unchanged,' that is, those who remained drinkers and non-drinkers throughout the period. They were similarly divided between three categories reflecting their smoking habits: they were described as having 'started' or 'quitted' smoking, while the 'unchanged' group included both those who were smokers and those who were non-smokers throughout the study period.

**Statistical analysis** The mean values and standard deviations for changes in serum $\beta$-carotene over the period of one year were calculated for each category. The difference between the mean value for each category was tested using Student's $t$ test. The relationship between changes in $\beta$-carotene concentration and the changes in lifestyle mentioned above — changes in green-yellow vegetable intake, drinking and smoking habits — were evaluated and multivariate regression analysis was performed, using the SAS general linear model (GLM) procedure[18] to compensate for the mutual effect of these factors upon each other. Mean changes after adjustment (Y) could be calculated by using the following formula;

$$Y = a + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_6x_6 + b_7x_7 + b_8x_8 + b_9x_9.$$

$a$: Intercept. $b_i$: Regression coefficient.
$x_i$: Category. If true $x_i=1$, if false $x_i=0$.

Means adjusted for mutual factors were also computed by each category.

## RESULTS

Table I shows the age distribution of the subjects of the study compared with that of the general male population of Yakumo aged 40 and older. The ages of the subjects ranged between 40 and 79 years, with a mean age (standard deviation) of 59.1 (9.0) years. The mean age (SD) of the general male population was 55.8 (10.6) years. The number of subjects between 40 and 49 years of age was proportionally smaller than the general male population, while the number of subjects between 60 and 69 years of age was proportionally greater. Of the subjects, 12.2% and 15.6% were engaged in agriculture and fishing, while 13.2% and 17.8% of male adults of this town were engaged in them.

The mean (SD) levels of serum $\beta$-carotene were 31.9 (21.6) $\mu$g/dl before and 33.0 (19.8) $\mu$g/dl after the one-year study period. As the difference between these mean levels was only 1.1 (18.4) $\mu$g/dl, the change was not significant.

The frequency distribution of the change in the serum $\beta$-carotene levels is depicted in Fig. 1. The distribution was nearly symmetrical. The levels of 83 individuals (or 56.5% of the study group) showed changes of less than 10 $\mu$g/dl in either direction. The levels of 21 individuals

Table I. Age Distribution of Male Subjects (at Time of First Interview) and of Yakumo (1988)

| Age (years) | Number (%) | |
|---|---|---|
| | Subjects | Yakumo all |
| 40–49 | 27 (18.4) | 1252 (32.5) |
| 50–59 | 44 (29.9) | 1159 (30.0) |
| 60–69 | 61 (41.5) | 885 (22.9) |
| 70–79 | 15 (10.2) | 561 (14.5) |
| Total | 147 (100.0) | 3857 (100.0) |

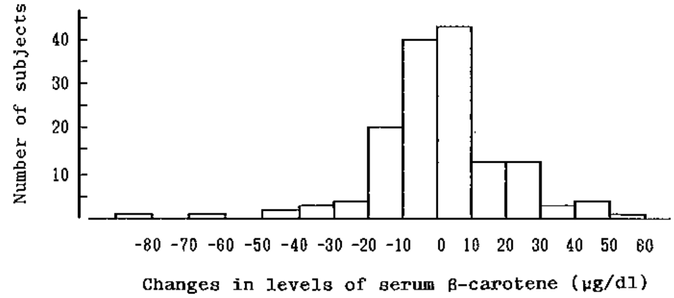


Fig. 1. Distribution of the changes in levels of serum $\beta$-carotene.

(14.2%) increased by more than 20 $\mu$g/dl, and those of 11 (7.5%) individuals decreased by more than 20 $\mu$g/dl.

Table II details the changes in dietary intake of green-yellow vegetables. The subjects were divided among the three categories mentioned previously. The green-yellow vegetables intake of 20 individuals (or 13.6% of the study group) was determined to have 'increased remarkably' during the period, while that of 30 individuals (20.4%) was judged to have 'decreased,' and that of the remaining 97 (66.0%) was 'unchanged or slightly increased.'

Table II. Intake Frequency of Green-Yellow Vegetables at Time of Firtst and Second Interviews and Change in the Frequency

| Intake frequency of green-yellow vegetables | Number (%) | |
|---|---|---|
| | First interview | Second interview |
| Less than 1/week | 17 (11.6) | 14 (9.5) |
| 1–2/week | 43 (29.3) | 22 (15.0) |
| 3–4/week | 20 (13.6) | 36 (24.5) |
| 5–/week | 67 (45.6) | 75 (51.0) |
| Change in intake frequency of green-yellow vegetables | | |
| Increased remarkably | 20 (13.6) | |
| Unchanged or slightly increased | 97 (66.0) | |
| Decreased | 30 (20.4) | |

Table III shows changes in alcohol drinking habits during the period of the study. The proportion of alcohol drinkers did not change significantly during the period: while 72.3% drank at the time of the first interview, this figure rose slightly, to 76.4%, at the time of second interview. With respect to changes in drinking habits, a total of 13 individuals (or 8.8% of the study) started drinking; 6 individuals (4.1%) quit drinking; and the drinking habits of the remaining 128 individuals (87.1%) were unchanged. This last category included 100 drinkers (68.0%) and 28 non-drinkers (19.0%).

Table IV shows changes in smoking habits during the interval between the first and second interviews. The proportion of smokers also did not change significantly

Table III. Drinking Habits at Time of First and Second Interviews

| | Second interview | | |
|---|---|---|---|
| | Drinker | Non-drinker | Total |
| First interview | | | |
| Drinker | 100 (68.1) | 6 (4.1) | 106 (72.1) |
| Non-drinker | 13 (8.8) | 28 (19.1) | 41 (27.9) |
| Total | 113 (76.9) | 34 (23.1) | 147 (100.0) |

Number (%)

Table IV. Smoking Habits at Time of First and Second Interviews

| | Second interview | | |
|---|---|---|---|
| | Smoker | Non-smoker | Total |
| First interview | | | |
| Smoker | 61 (41.5) | 3 (2.0) | 64 (43.5) |
| Non-smoker | 4 (2.7) | 79 (53.7) | 83 (56.5) |
| Total | 65 (44.2) | 82 (55.8) | 147 (100.0) |

Number (%)

Table V. Changes in Serum $\beta$-Carotene Concentration ($\mu$g/dl) According to Changes in Intake of Green-Yellow Vegetables and in Drinking and Smoking Habits

| Category | Mean (SD) | Adjacent comparison | Overall |
|---|---|---|---|
| Change in intake frequency of GYV | | | |
| Decreased | −5.3 (19.9) | NS | ** |
| Unchanged or slightly increased | 2.1 (19.4) | * | |
| Increased remarkably | 11.2 (24.4) | | |
| Change in drinking habit | | | |
| Quitted | 14.6 (21.0) | NS | ** |
| Unchanged | 1.7 (16.1) | * | |
| Started | −11.1 (30.4) | | |
| Change in smoking habit | | | |
| Quitted | 11.2 (22.1) | NS | ** |
| Unchanged | 1.6 (17.1) | ** | |
| Started | −27.5 (37.7) | | |

SD, Standard deviation; GYV, green-yellow vegetables; NS, not significant; *, $P<0.05$; **, $P<0.01$.

during the study period: it rose only very slightly, from 43.2% to 43.9%, during that time. A total of 4 individuals (or 2.7% of the study group) started smoking; 3 individuals (2.0%) quit smoking; and the smoking habits of the remaining 140 individuals (95.2%) did not change. The group of those whose smoking habits were unchanged included 61 smokers (41.5%) and 79 non-smokers (53.7%). Only two of the subjects changed both habits of drinking and smoking, one quit and the other started. As for those who 'started,' most of them had answered that they had quitted drinking/smoking at the first interview. The numbers of cases newly 'started' were small (drinking; one case, smoking; four cases).

Table V shows an analysis of the mean change in serum concentrations of β-carotene according to changes in intake frequency of green-yellow vegetables. The levels of serum β-carotene increased by 11.2 μg/dl among those subjects whose intake of green-yellow vegetables had 'increased remarkably'; they rose only 2.1 μg/dl among those subjects whose intake was 'unchanged or slightly increased'; and they decreased by 5.3 μg/dl among those whose intake of green-yellow vegetables 'decreased.' The differences of changes in serum β-carotene levels between 'increased remarkably' and 'decreased,' and between 'increased remarkably' and 'unchanged or slightly increased' were statistically significant ($P$=0.001 and 0.021 respectively). Changes in the intake frequency of other foods such as seaweed, oranges and other fruits had no significant effect on the concentration of serum β-carotene.

Table V also shows changes in serum β-carotene concentrations analyzed according to changes in drinking and smoking habits. The level for those who started drinking decreased by 11.1 μg/dl; that for those of the 'unchanged' group increased by 1.7 μg/dl; and the level of the group who quit drinking increased by 14.6 μg/dl. These differences of changes in serum β-carotene levels between 'started' and 'quitted,' and between 'started' and 'unchanged' were statistically significant ($P$=0.004 and 0.015 respectively).

As for the analysis according to changes in smoking habit, the level of the 'started' group decreased by 27.5 μg/dl; that of the 'unchanged' group increased by 1.6 μg/dl; and that of the 'quitted' group increased by 11.2 μg/dl. These differences of changes in serum β-carotene levels between 'started' and 'quitted,' and between 'started' and 'unchanged' were also statistically significant ($P$=0.005 and 0.001 respectively).

As for the effects of drinking and smoking habits on β-carotene levels, it should be noted that the 'unchanged' group in both cases contained subgroups of consumers and abstainers of these substances. However, as the difference between the changes in the β-carotene levels of drinkers (who showed an increase 0.8 μg/dl) as opposed to non-drinkers (an increase of 4.8 μg/dl) was not significant, it was determined that these subgroups could be grouped together for the purpose of comparison with the other two groups. Likewise, the difference between changes in the β-carotene levels of smokers (who showed a decrease of 0.1 μg/dl) as opposed to non-smokers (an increase of 4.4 μg/dl) was not significant, so these two subgroups were grouped together for comparison with the other groups.

The contribution of these factors to changes in serum β-carotene levels was evaluated using a linear regression

Table VI. Result of Multivariate Analysis of Changes in Serum β-Carotene Level (μg/dl), Intake Frequency of Green-Yellow Vegetables, and Drinking and Smoking Habits

| Category | Regression coefficient; $b_i$ (SE) | Adjusted mean (SE) | | |
|---|---|---|---|---|
| Change in intake frequency of GYV | | | | |
| $x_1$ Decreased | 0 | −9.2 (5.4) | NS | |
| $x_2$ Unchanged or slightly increased | 4.2 (3.7) | −5.0 (5.0) | | ** |
| $x_3$ Increased remarkably | 14.7 (5.1) | 5.4 (6.0) | * | |
| Change in drinking habit | | | | |
| $x_4$ Quitted | 0 | 7.3 (7.8) | NS | |
| $x_5$ Unchanged | −9.0 (7.4) | −1.6 (4.7) | | * |
| $x_6$ Started | −21.9 (8.7) | −14.5 (6.4) | * | |
| Change in smoking habit | | | | |
| $x_7$ Quitted | 0 | 9.0 (10.2) | NS | |
| $x_8$ Unchanged | −6.4 (10.3) | 2.6 (3.0) | | * |
| $x_9$ Started | −29.3 (13.6) | −20.3 (9.1) | * | |
| Intercept | 12.9 (11.6) | | | |
| R square | 0.172 | | | |

SE, Standard error; GYV, green-yellow vegetables; NS, not significant; *, $P<0.05$; **, $P<0.01$.

model which took into account changes in the intake frequency of green-yellow vegetables and changes in drinking and smoking habits. Other variables such as age, body mass index and changes in intake frequency of other foods were not taken into account in the model, because they did not have any appreciable effect on the changes in β-carotene levels.

Table VI shows the result of regression analysis. The *P*-value of this model was 0.0002. Adjusted means and standard errors were also calculated. The *P*-value for the parameter of changes in intake frequency of green-yellow vegetables was 0.0147, and it indicates that the serum β-carotene concentration changed positively with changes in the intake of green-yellow vegetables after adjustment for changes in alcohol drinking and smoking habits. The change in the serum β-carotene concentration was estimated to be 14.7 μg/dl lower in those whose green-yellow vegetable intake frequency 'decreased' than in individuals whose intake frequency 'increased remarkably,' and 4.2 μg/dl lower than those whose intake frequency was 'unchanged or slightly increased.' Adjusted means are also shown in Table VI. There was a 5.0 μg/dl decrease in those whose intake frequency of green-yellow vegetables was 'unchanged or slightly increased,' and in the 'increased remarkably' category, the concentration of serum β-carotene increased by 5.4 μg/dl after adjustment for changes in habits of alcohol drinking and smoking. The differences remained significant after adjustment.

The groups of those who started drinking and those whose drinking habits were unchanged during the study period decreased their serum β-carotene concentrations after adjustment compared with those who quit (21.9 μg/dl and 9.0 μg/dl respectively, $P=0.0164$). The adjusted means were 7.3 μg/dl in 'quitted' category, −1.6 μg/dl in 'unchanged' and −14.5 μg/dl in 'started' category. The differences remained significant after adjustment. In a like fashion, the group of those who started smoking and those whose smoking habits remained unchanged during the year decreased their serum β-carotene concentration levels after adjustment compared with those who quit smoking (21.9 μg/dl and 6.4 μg/dl respectively, $P=0.0345$). The adjusted means were 9.0 μg/dl in 'quitted' category, 2.6 μg/dl in 'unchanged' and −20.3 μg/dl in the 'started' category. The differences remained significant after adjustment.

## DISCUSSION

The subjects in this study were diagnosed as having normal health by the health screening program mentioned previously; however, they do not represent a random sample of the general population of the area. The proportion of subjects between 40 and 49 years of age was smaller and that of subjects between 60 and 69 years of age was larger than those of the general male population of Yakumo. The distribution of the subjects by residential area, however, was similar to that of the general population of the town; furthermore they were not clustered in specific occupational groups. Food intake pattern of the subjects was similar to that of the participants in the program. The percentages of smokers and drinkers among the subjects were about the same as those for all the participants in the program. Therefore, the subjects studied in this series were not considered much biased in the analyses above, though the number of examinees was small.

The same questionnaire was used throughout the present study, and the interviews were conducted by the same five trained public health nurses. At the time of the second interview, neither the subjects nor the interviewers were informed of the nature of the responses during the first interview.

Blood samples were collected in August each year, and the concentrations of β-carotene were measured by the same chemists using the same method within a six-month period thereafter. It was confirmed by one of the authors that serum β-carotene levels of the sampled sera remained stable after storage at −80°C during six months.[19] The laboratory's coefficient of variation for β-carotene was 4.3%; therefore, technical errors in measuring serum β-carotene were rather small.

It is obvious that dietary frequency data cannot replace quantitative data in the estimation of the exact intake of individual subjects. However, quantitative dietary methods require more time and cost for data collection in large-scale epidemiological research projects. Accordingly, the food frequency method was used in the current study. The intake frequency of foods containing carotene does have a positive association with the concentration of serum β-carotene.[8, 10, 13]

As the main concern of this study was β-carotene, which is considered to play a major role in carcinogenesis, we did not examine other carotenoids in detail. Changes in green-yellow vegetables correlated weakly with changes in serum levels of lycopene, but not with those of α-carotene.

In the present study, changes in serum β-carotene concentrations have been correlated positively with changes in the intake of green-yellow vegetables. The difference between changes in the serum levels of the group of those whose green-yellow vegetables intake frequency 'increased remarkably' and those whose intake frequency 'decreased' was 14.7 μg/dl after adjustment for changes in alcohol and smoking habits. This result suggests that an increase in serum β-carotene is due to an increased intake frequency of green-yellow vegetables. This study has thus shown a negative correlation between changes in alcohol drinking and smoking habits and changes in serum $\beta$-carotene levels. Many previous cross-sectional reports have shown evidence of association between both the habitual consumption of alcohol[9-13, 16] and smoking,[9-13, 20, 21] and relatively lower levels of serum $\beta$-carotene.

This study was not an interventional but an observational study, a so-called natural experiment. It is true that the number of subjects whose lifestyles changed was not large. The number of individuals who changed both drinking and smoking habits was particularly small, so it was impossible to analyze the interaction of the changes in the habits.

The amount of the changes in serum $\beta$-carotene concentrations explained by multiple regression analysis was 17.2% of the whole. Several factors might be considered in these associations. First, the data on dietary frequency obtained by the questionnaire in this study did not completely indicate the change in actual amount of green-yellow vegetables consumed. The influence of the amount of changes in alcohol and cigarette consumption, likewise, could not precisely be measured in this study. Other factors not taken into account here might also influence serum levels of $\beta$-carotene. A positive association between serum $\beta$-carotene levels and a high fat diet[14] has been reported, and negative associations between these serum levels and intake of medication for hypertension[20, 21] and between these levels and ultraviolet light exposure[22] were observed, though the influence of such factors was rather small.

If serum $\beta$-carotene level is closely associated with reduction of smoking- or alcohol-related cancer, dietary changes may be one of the most practical ways of cancer prevention.

ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid for Special Cancer Research, Monbusho International Scientific Research Program, and by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare.

*(Received January 5, 1990/Accepted March 2, 1990)*

REFERENCES

1) Shekelle, R. B., Lepper, M., Liu, S., Maliza, C., Raynor, W. J., Rossof, A. H., Paul, O., Shryoch, A. M. and Stamler, J. Dietary vitamin A and risk of cancer in the Western Electric Study. *Lancet*, **ii**, 1185–1190 (1981).
2) Ross, R. K., Shimizu, H., Paganini-Hill, A., Honda, G. and Henderson, B. E. Case-control studies of prostate cancer in blacks and whites in Southern California. *J. Natl. Cancer Inst.*, **78**, 869–874 (1987).
3) Ohno, Y., Yoshida, O., Oishi, K., Okada, K., Yamabe, H. and Schroeder, F. H. Dietary $\beta$-carotene and cancer of prostate: a case-control study in Kyoto, Japan. *Cancer Res.*, **48**, 1331–1336 (1988).
4) Mackerras, D., Buffler, P. A., Randall, D. E., Nichaman, M. Z., Pickel, L. W. and Mason, T. J. Carotene intake and the risk of laryngeal cancer in coastal Texas. *Am. J. Epidemiol.*, **128**, 980–988 (1988).
5) Staehlin, H. B., Roesel, F., Buess, E. and Brubacher, G. Cancer, vitamins, and plasma lipids: prospective Basel study. *J. Natl. Cancer Inst.*, **73**, 1463–1468 (1984).
6) Nomura, A. M. Y., Stemmermann, G. N., Heilbrun, L. K., Salkeld, R. M. and Vuilleumier, J. P. Serum vitamin levels and the risk of cancer of specific sites in men of Japanese ancestry in Hawaii. *Cancer Res.*, **45**, 2369–2372 (1985).
7) Hirayama, T. Diet and cancer. *Nutr. Cancer*, **1**, 67–81 (1979).
8) 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).
9) 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).
10) 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).
11) 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).
12) 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).
13) Shibata, A., Sasaki, R., Ito, Y., Hamajima, N., Suzuki, S., Ohtani M. and Aoki, K. Serum concentration of beta-carotene and intake frequency of green-yellow vegetables among healthy inhabitants of Japan. *Int. J. Cancer*, **44**, 48–52 (1989).
14) Willett, W. C., Stampfer, M. J., Underwood, B. A., Taylor, J. O. and Hennekens, C. H. Vitamin A, E, and carotene: effects of supplementation on their plasma levels. *Am. J. Clin. Nutr.*, **38**, 559–566 (1983).

15) Dimitrov, N. V., Meyer, C., Ullrey, D. E., Chenoweth, W., Michelakis, A., Malone, W., Boone, C. and Fink, G. Bioavailability of β-carotene in humans. *Am. J. Clin. Nutr.*, **48**, 298–304 (1988).
16) Costantino, J. P., Kuller, L. H., Begg, L., Redmond, C. K. and Bates, M. W. Serum level changes after administration of a pharmacologic dose of β-carotene. *Am. J. Clin. Nutr.*, **48**, 1277–1283 (1988).
17) Miller, K. W. and Yang, C. S. An isocratic high-performance liquid chromatography method for the simultaneous analysis of plasma retinol, α-tocopherol, and various carotenoids. *Anal. Biochem.*, **145**, 21–26 (1985).
18) SAS Institute. "User's Guide: Statistics (Version 5)" (1980). SAS Inst., Cary, NC.
19) Ito, Y., Sasaki, R., Mimohara, M., Ohtani, M. and Aoki, K. Quantitation of serum carotenoid concentrations in healthy inhabitants by high-performance liquid chromatography. *Clin. Chim. Acta*, **169**, 197–208 (1987).
20) Comstock, G. W., Menkes, M. S., Schober, S. E., Vuilleumier, J-P. and Helsing, K. J. Serum levels of retinol, beta-carotene, and alpha-tocopherol in older adults. *Am. J. Epidemiol.*, **127**, 114–123 (1987).
21) Nierenberg, D. W., Stukel, T. A., Baron, J. A., Dain, B. J., Greenberg, E. R. and The Skin Cancer Prevention Study Group. Determinations of plasma levels of beta-carotene and retinol. *Am. J. Epidemiol.*, **130**, 511–521 (1989).
22) White, W. S., Kim, C., Kalkwarf, H. J., Bustos, P. and Roe, D. A. Ultraviolet light-induced reductions in plasma carotenoid levels. *Am. J. Clin. Nutr.*, **47**, 879–883 (1988).