Serum β-carotene and vitamin C as biomarkers of vegetable and fruit intakes in a community-based sample of French adults

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ABSTRACT  Relative high intakes of vegetables and fruit and relatively low intakes of fat are associated with lower rates of heart disease and many types of cancer. Biomarkers for vegetable and fruit consumption are most useful when applicable across different ages, body weights, diets, and varying patterns of fat intake. This study examined two biomarkers, serum concentrations of β-carotene and vitamin C, as a function of anthropometric, dietary, and lifestyle factors in a community-based sample of French adults. The interview-based dietary-history method was used to assess dietary intakes of 361 males and 476 females aged 18–94 y resident in the Val-de-Marne district southeast of Paris. Serum β-carotene was quantified by HPLC and vitamin C was measured by using an automated method. Serum β-carotene and vitamin C concentrations were positively associated with vegetable and fruit intakes and were negatively linked to the consumption of energy, alcohol, and fat. Multiple-regression analyses showed that serum β-carotene concentration was predicted by fruit and vegetable intakes but was inversely associated with body mass, energy and alcohol intakes, and tobacco use. Serum vitamin C concentration was positively associated with fruit consumption but was negatively associated with age, body mass, and tobacco use. Serum β-carotene and vitamin C concentrations are useful biomarkers of vegetable and fruit consumption in the French diet. However, other dietary and lifestyle factors also have a significant effect on circulating concentrations of these antioxidant micronutrients.  *Am J Clin Nutr* 1997;65:1796–802.

KEY WORDS  Biomarkers, β-carotene, vitamin C, vegetable and fruit consumption, age, body mass, dietary fat, alcohol, tobacco, French paradox, adults, Val-de-Marne study

INTRODUCTION  The consumption of vegetables and fruit has been associated with reduced risk of heart disease and some forms of cancer (1–3). Among potential biomarkers for vegetable and fruit consumption are plasma concentrations of carotenoids and vitamin C (4–6). Clinical studies have shown an increase in circulating carotenoids in response to increased intake of vegetables (5, 7); conversely, carotenoid concentrations decline rapidly when vegetables and fruit are withdrawn from the diet (8). Similarly, plasma concentrations of vitamin C correlate directly with the consumption of fruit and vegetables that contain vitamin C, provided that the vitamin is consumed at physiologic doses (9).

Plasma biomarkers are most useful when the relation between dietary intakes and circulating nutrients holds across groups with diverse body weights, ages, lifestyles, and diet habits. Circulating concentrations of carotenoids and vitamin C are known to be influenced by multiple dietary and lifestyle factors, including body weight and alcohol and tobacco use (10, 11). Carotenoids are transported in the circulation by association with lipoproteins and are distributed among them in a manner similar to the distribution of cholesterol (12, 13). Plasma lipids are important determinants of plasma carotenoid concentrations when data over a wide range of values are compared (12, 13). Smoking is associated with a reduction in plasma vitamin C concentration (14) and may reduce plasma concentrations of carotenoids (15), regardless of dietary intake. However, a relation between smoking per se and plasma carotenoid concentrations has not been unequivocally established because the dietary carotenoid intake of smokers may be reduced compared with that of nonsmokers (16).

The relation between fat intakes and the consumption of vegetables and fruit in a population is often assumed to be reciprocal. Dietary patterns in the United States that are associated with high fat intakes are generally marked by low intakes of vegetables and fruit (17). However, this need not be the case in other nations. This study analyzed dietary patterns and serum β-carotene and vitamin C concentrations in a sample of French adults. The French diet is said to be characterized by high consumption of fats, including saturated fats, and by ample consumption of vegetables and fruit (18–20). Dietary factors, such as the use of olive oil and red wine, have been
cited as potential contributors to the French paradox, that is, lower than expected mortality from heart disease (19). The plant components of the Mediterranean diet are further thought to have chemopreventive effects that might outweigh those of tobacco use and the consumption of saturated fats (18).

A link between dietary intakes of carotenoids and vitamin C and serum concentrations of these micronutrients in the French population has been established (21). However, past studies have not specifically addressed the relation between fat consumption, intakes of vegetables and fruit, and biomarker status. Accordingly, this study examined the relation between vegetable and fruit intakes and serum \( \beta \)-carotene and vitamin C concentrations in a sample of French adults representing a wide range of ages, body weights, and fat intakes. To our knowledge, serum \( \beta \)-carotene concentration as a biomarker of vegetable and fruit intakes has not been examined in a large population sample stratified by fat consumption.

**SUBJECTS AND METHODS**

The Val-de-Marne study

The study was based on the results of a cross-sectional nutritional survey conducted by the Institut Scientifique et Technique de l’Alimentation (ISTNA) in Paris (22–24). The subject sample was selected by using a two-stage cluster design sampling procedure. First, 12 of the 47 districts in the Val-de-Marne department (population 1 192 692) were selected by probability sampling, where the probability of selection was proportional to district size. In the second stage 75 families per district were selected, again at random, from area telephone directories. Of 849 families contacted, 527 participated in the study (response rate: 62%). The present analyses were based on a subpopulation of 837 adults \( \geq \) 18 y of age (361 men and 476 women). Although representative of the Val-de-Marne area, the sample was not a national probability sample of the French population.

Dietary intakes were estimated by using a dietary-history-interview procedure (25), as adapted by the French National Institute for Science and Medical Research (INSERM) for use in France (26). Members of each household were interviewed at home by trained dietitians. The interviewers recorded typical daily intakes representative of the previous 6 mo. The questions addressed all eating episodes during the day, beginning with breakfast. The interviewer inquired about the type and the amount of foods habitually consumed at breakfast and asked for the estimated frequency of consumption of each food over the preceding week, month, or 6-mo period. This procedure was followed for foods consumed at lunch and dinner and for foods consumed as snacks between meals. For each food, the interviewer obtained the frequency of consumption and the average amount consumed at each eating occasion (estimated in grams or in household measures). Additional information about portion size for packaged foods and meals away from home was obtained from commercial food suppliers. Further questions addressed family grocery shopping patterns and seasonal adjustments in food intakes. Each interview lasted between 20 and 60 min. Because the dietary-history procedure recorded food consumption in terms of frequencies and quantities ingested, it was comparable with quantitative food-frequency methods (25).

The foods consumed were regrouped into 73 separate foods and food groups. The groups included meat and fish (10 items), milk and cheese (15 items), fats and oils (6 items), grains (10 items), fruit and vegetables (15 items), sweets and sugars (8 items), alcohol (5 items), and other beverages including water and mineral water (4 items). The category of fruit and vegetables included fresh fruit (apples, pears, and grapes), citrus fruit, bananas, raisins, dried fruit, nuts, canned fruit, fruit juices, fresh vegetables, leafy vegetables (cabbage, lettuce, and spinach), root vegetables (carrots, turnips, and parsnips), fruit vegetables (peppers, tomatoes, and zucchini), avocados, fresh peas and beans, and dried pulses. The amounts consumed were estimated in grams per day. Energy intakes and macronutrient contents of the diet were estimated by using nutrient composition tables developed by INSERM and modified by ISTNA (26). Self-reported measures of weight and height collected during the interview were used to calculate body mass index (BMI; in kg/m\(^2\)). The methodology and the principal results of the Val-de-Marne study, including a detailed description of sampling and interview techniques, were published previously (22, 23). The Ethics Committee of CNAM-Paris approved the study protocol.

**Serum micronutrients and cholesterol**

Venous blood was drawn from fasting subjects between 0700 and 1030 for the measurement of blood chemistry. Serum \( \beta \)-carotene was measured by normal-phase HPLC on Silicagel (Alltech, Deerfield, IL), isocratic elution with \( n \)-hexane:dioxane (900:10), and detection at 436 nm (27). Cholesterol was measured with the Cobas Bio centrifugal analyzer (28). Serum ascorbic acid was measured by using an automated method based on the continuous-flow principle (29).

**Statistical analyses**

Descriptive statistics were calculated for all variables. Correlations among the variables were examined by using Pearson product-moment correlations. Serum cholesterol values were used in the interpretation of \( \beta \)-carotene concentrations, which were corrected as a ratio of carotenoid to cholesterol concentration, the approach applied to tocopherols described previously (30). The correction did not substantially improve the correlations, which were significant whether or not the correction was applied. As reviewed previously by others (31–33), total energy intake is highly correlated with many dietary factors. Previous analyses (34, 35) indicate that the most stable estimates of association and the greatest power to detect associations are obtained by using either the nutrient density method or the residual method. This study used the nutrient density method of energy adjustment (31), with fat and vegetable and fruit intakes adjusted for energy for the purpose of all correlation and regression analyses.

On the basis of results from the univariate tests, stepwise-multiple-regression models were used to determine the relative influence of dietary and other variables on serum \( \beta \)-carotene and vitamin C concentrations. Each model was established by using forward and backward stepwise regression with the likelihood-ratio test to determine variables to be added or removed from the model. Independent variables considered were age, BMI, alcohol intake, serum cholesterol concentration, vegetable and fruit intakes, energy intake, and fat intake. The subject
TABLE 1
Subject characteristics, selected dietary intake data, and serum nutrient variables by age group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>18-30 y (n = 211)</th>
<th>30-40 y (n = 196)</th>
<th>40-50 y (n = 162)</th>
<th>50-65 y (n = 173)</th>
<th>&gt; 65 y (n = 95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>24.0 ± 0.2</td>
<td>34.6 ± 0.2</td>
<td>44.0 ± 0.2</td>
<td>56.2 ± 0.3</td>
<td>74.0 ± 0.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.6 ± 0.2</td>
<td>22.7 ± 0.2</td>
<td>24.0 ± 0.2</td>
<td>25.2 ± 0.2</td>
<td>24.5 ± 0.4</td>
</tr>
<tr>
<td>Dietary intakes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kJ/d)</td>
<td>9375 ± 260</td>
<td>8663 ± 209</td>
<td>8219 ± 201</td>
<td>8424 ± 222</td>
<td>7327 ± 243</td>
</tr>
<tr>
<td>Total fat (g/d)</td>
<td>92.4 ± 2.9</td>
<td>85.7 ± 2.1</td>
<td>81.6 ± 2.4</td>
<td>79.3 ± 2.4</td>
<td>69.0 ± 3.4</td>
</tr>
<tr>
<td>Alcohol (g/d)</td>
<td>2.8 ± 0.6</td>
<td>11.9 ± 1.5</td>
<td>14.5 ± 1.7</td>
<td>17.3 ± 2.2</td>
<td>11.2 ± 2.2</td>
</tr>
<tr>
<td>Total vegetables (g/d)</td>
<td>315 ± 11</td>
<td>358 ± 11</td>
<td>377 ± 13</td>
<td>394 ± 14</td>
<td>400 ± 17</td>
</tr>
<tr>
<td>Total fruit (g/d)</td>
<td>263 ± 17</td>
<td>244 ± 14</td>
<td>227 ± 12</td>
<td>288 ± 16</td>
<td>252 ± 14</td>
</tr>
<tr>
<td>Serum concentrations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.64 ± 0.07</td>
<td>5.13 ± 0.08</td>
<td>5.46 ± 0.09</td>
<td>5.86 ± 0.08</td>
<td>6.13 ± 0.12</td>
</tr>
<tr>
<td>β-Carotene (μmol/L)</td>
<td>0.74 ± 0.03</td>
<td>0.93 ± 0.04</td>
<td>0.81 ± 0.04</td>
<td>0.94 ± 0.04</td>
<td>0.87 ± 0.05</td>
</tr>
<tr>
<td>Vitamin C (μmol/L)</td>
<td>50 ± 29</td>
<td>49 ± 30</td>
<td>42 ± 19</td>
<td>51 ± 20</td>
<td>45 ± 43</td>
</tr>
</tbody>
</table>

All values are means ± SEM. Comparisons of intakes were performed by using programs available in SPSS PC+ (version 6.1; SPSS Inc., Chicago). The reported data are means ± SEMs.

RESULTS

Subjects by age

The mean age of the respondent sample was 42.5 y for men and 42.8 y for women. The mean BMI was 24.4 for men and 22.6 for women. As expected, BMI values increased with age up to age 65 y. Alcohol use was reported by 54.8% of men and 53.6% of women. Current tobacco use was reported by 23.1% of the women and 41.6% of the men.

Selected dietary intake variables are summarized by age group in Table 1. Whereas energy intakes (kJ) and the consumption of fat (g/d) both declined with age, the consumption of vegetables (g/d) increased. The consumption of fruit was independent of age. Consumption of alcohol (g/d) increased with age up to the age of 65 y. The effects of age group on the consumption of energy, fat, alcohol, and vegetables were significant by one-way analysis of variance (P < 0.01).

Serum cholesterol concentrations increased as a function of age (P < 0.01). In contrast, serum β-carotene and vitamin C concentrations did not vary systematically across age groups. Women had significantly higher serum β-carotene concentrations than men (0.97 μmol/L compared with 0.70 μmol/L) and also had higher serum vitamin C concentrations (53.0 μmol/L compared with 40.8 μmol/L).

Subjects by fat consumption quartiles

Subject characteristics and dietary and nutrient variables are summarized in Table 2 by quartiles of energy-adjusted fat intakes. A comparison of respondents in the top and bottom quartiles by fat intake showed that the highest consumers of fat were significantly younger and had BMIs that were lower than those of the lowest consumers of fat. Increased fat consumption

TABLE 2
Subject characteristics, selected dietary intake data, and serum nutrient variables by energy-adjusted fat intake quartiles

<table>
<thead>
<tr>
<th>Fat intake quartile</th>
<th>1 (n = 209)</th>
<th>2 (n = 209)</th>
<th>3 (n = 210)</th>
<th>4 (n = 209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (% of energy)</td>
<td>28.4 ± 0.2</td>
<td>34.4 ± 0.1</td>
<td>38.6 ± 0.1</td>
<td>45.4 ± 0.3</td>
</tr>
<tr>
<td>Age (y)</td>
<td>46.8 ± 1.3</td>
<td>42.6 ± 1.1</td>
<td>40.7 ± 1.1</td>
<td>40.7 ± 1.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.8 ± 0.3</td>
<td>23.6 ± 0.3</td>
<td>23.3 ± 0.3</td>
<td>22.9 ± 0.2</td>
</tr>
<tr>
<td>Dietary intakes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kJ/d)</td>
<td>8274 ± 230</td>
<td>8387 ± 184</td>
<td>8801 ± 201</td>
<td>8763 ± 234</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>62.2 ± 1.8</td>
<td>76.3 ± 1.6</td>
<td>89.9 ± 2.0</td>
<td>105.1 ± 3.0</td>
</tr>
<tr>
<td>Total vegetables (g/d)</td>
<td>356 ± 12</td>
<td>366 ± 12</td>
<td>362 ± 11</td>
<td>369 ± 11</td>
</tr>
<tr>
<td>Total fruit (g/d)</td>
<td>298 ± 16</td>
<td>259 ± 14</td>
<td>237 ± 12</td>
<td>228 ± 13</td>
</tr>
<tr>
<td>Serum concentrations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.6 ± 0.09</td>
<td>5.3 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td>β-Carotene (μmol/L)</td>
<td>0.89 ± 0.04</td>
<td>0.85 ± 0.04</td>
<td>0.84 ± 0.04</td>
<td>0.83 ± 0.03</td>
</tr>
<tr>
<td>Vitamin C (μmol/L)</td>
<td>53 ± 33</td>
<td>49 ± 30</td>
<td>45 ± 16</td>
<td>44 ± 16</td>
</tr>
</tbody>
</table>

All values are means ± SEM.
was associated with unchanged vegetable consumption and with a sharply lower consumption of fruit \((P < 0.05)\). Energy intakes did not vary as a function of fat consumption quartile. When analyzed by quartiles, fat consumption had no effect on serum β-carotene or vitamin C concentrations.

**Subjects by BMI**

Analysis of dietary intake data by BMI tertiles, conducted separately for men and women, showed that respondents in the highest tertile of BMI consumed the least energy and least fat and saturated fat, but tended to consume the most vegetables. Women in the highest tertile of BMI also consumed the most fruit. The inverse relation between energy intakes and BMIs, also observed in other studies, has sometimes been interpreted as showing that overeating and overweight are not necessarily linked. However, note that respondents in the top BMI tertile (mean age 50 y) were significantly older than respondents in the bottom BMI tertile (mean age 35 y). The inverse relation between BMI and energy intakes no longer held after age was controlled for.

As expected, the top BMI tertile was associated with the highest serum cholesterol concentration. Higher BMIs were also associated with lower serum β-carotene concentrations; this effect was significant for men \((P < 0.05)\). In contrast, serum vitamin C concentrations were not influenced by BMI.

**Correlation analyses**

Correlation analyses between serum β-carotene and vitamin C values and energy-adjusted vegetable and fruit intakes are shown in Table 3. β-Carotene values were corrected for serum cholesterol and were log transformed. Consistent with previous data, significant positive correlations were found between serum β-carotene concentration and estimated intakes of vegetables and fruit \((r = 0.36, P < 0.05)\), vegetables alone \((r = 0.30, P < 0.05)\), and fruit alone \((r = 0.29, P < 0.05)\). Conversely, serum β-carotene concentrations were negatively correlated with energy and fat intakes and with alcohol use. The correlations between vegetable and fruit intakes and serum β-carotene concentrations were significant within each quartile by fat consumption. Although correction of β-carotene values for serum cholesterol has been the convention in some studies, this correction did not result in a major improvement in correlation coefficients, which were significant both before and after the correction.

Serum concentrations of vitamin C were log transformed. Vitamin C concentration was positively associated with fruit consumption \((r = 0.36, P < 0.05)\), with vegetable and fruit intakes \((r = 0.29, P < 0.05)\), and less markedly with vegetable intake \((r = 0.15, P < 0.05)\). Serum vitamin C concentration was negatively associated with intakes of energy, fat, and alcohol.

**Regression analyses**

Factors that influence biomarker status may also include age, BMI, and tobacco use. Regression analyses were conducted to identify significant independent predictors of serum β-carotene and vitamin C concentrations. As shown in Table 4, among the dietary factors thought to influence biomarker status, vegetable intake and alcohol use had the strongest, but opposite, effects on circulating β-carotene concentrations. Serum β-carotene was also positively associated with fruit intake. Several other factors were also significant predictors. Serum β-carotene concentration was negatively associated with BMI, serum cholesterol concentrations, and tobacco use. Although the effect of age was not significant, age became a significant factor in β-carotene concentrations when serum cholesterol concentration was removed from the model. Dietary fat and saturated fat were not significant independent predictors of serum β-carotene concentrations.

Serum vitamin C concentrations showed the strongest relation with reported fruit intake and only a lesser relation with vegetable intakes. Age, BMI, and tobacco use were all negatively associated with serum vitamin C concentration.

**DISCUSSION**

As shown by this community-based study of French adults, serum β-carotene and vitamin C concentrations appear to be useful biomarkers of vegetable and fruit intakes. The magnitude of the relation was similar to that observed in studies of population samples in the United States (4–6, 16). Estimated consumption of vegetables and fruit in the Val-de-Marne sample, obtained by using the dietary-history method, was higher than the estimated consumption in the United States (33). Consistent with US data, there was an inverse correlation between the consumption of fats and the consumption of fruit. In contrast, vegetable consumption was constant across fat consumption quartiles.

In controlled clinical studies, dietary fat has been shown to increase plasma response after acute administration of β-carotene (37, 38). However, prolonged exposure to high-fat diets has been associated with adiposity and increased body mass, which cause plasma and tissue concentrations of carotenoids to be reduced (10, 11). In this study, neither the consumption of total fat nor the consumption of saturated fat were identified as independent predictors of serum β-carotene in the regression analyses. However, note that no systematic dose-response effect has been observed within the range of fat consumption provided by typical Western diets (9). The lack of a profound effect of dietary fats on carotenoid concentrations in affluent societies in which fat intakes are above a threshold may contrast with the effects of fat on carotenoid absorption in developing nations with endemic hypovitaminosis A.

We found BMI to be negatively associated with serum β-carotene concentration, consistent with previous results (10, 11). Age was less important as a determinant, and independent
effects of age were not observed in regression analysis. Suggestions in the literature that age may influence circulating antioxidant micronutrient concentrations were confirmed by the present results for vitamin C (9, 12, 13, 16, 39). For serum β-carotene concentrations, age became a factor only after confounding variables (eg, dietary intake, serum cholesterol, BMI) were considered in the analysis.

Further analyses addressed the effect of smoking and alcohol use. Smoking and alcohol intake have been inversely correlated with plasma carotenoids in US epidemiologic studies (10, 13, 15, 16). Studies conducted in France generally supported the notion that alcohol consumption had an adverse effect on serum β-carotene concentrations (40, 41), and similar observations have been reported for the Val-de-Marne data set (21). Similar results were observed in different surveys on various populations (42–45).

The relation between alcohol intake and circulating concentrations of carotenoids is likely to be complex. Epidemiologic studies have shown an adverse effect of alcohol intake (particularly among men) on plasma carotenoid concentrations in US samples (10, 43). However, Brady et al (16) found this relation to be explained entirely by differences in dietary intake that are not apparent when this confounding variable was adjusted for because alcohol consumption is nearly inversely related to intake of carotenoids in US diets. Results from one laboratory animal study indicate that alcohol ingestion may exert the opposite effect on plasma β-carotene concentration in a more acute situation, apparently by delaying its clearance after the administration of purified compound (46). In a controlled crossover feeding study, Forman et al (47) observed higher plasma α- and β-carotene concentrations but lower concentrations of other carotenoids (ie, lutein) associated with daily alcohol ingestion, an inconsistency that may be explained by alterations in the lipoprotein profile, which affects transport of these compounds in the plasma.

Consistent with previous data (10, 15, 48–50), the present study found that smoking was an independent determinant of serum concentrations of β-carotene. Studies of US samples have also shown that smoking may be inversely related to the plasma concentration of β-carotene at constant carotenoid intakes (10, 15, 39, 51, 52). However, Brady et al (16) observed that reduced carotenoid intake was the more important determinant of lower plasma concentrations among smokers. Smoking was also associated with reduced serum vitamin C concentration (14), which may be due to increased metabolic turnover (53). Regression analyses showed that smoking was found to be an independent determinant of vitamin C concentration, although this effect was significant only for women.

Serum vitamin C concentrations were better predictors of fruit than of vegetable intakes. Serum vitamin C concentrations are known to reflect dietary intake of this vitamin within the physiologic range that is provided by the typical Western diet (9). At very high intakes the linear relation disappears as a result of reduced efficiency of absorption from the gastrointestinal tract and increased urinary losses. The pharmacokinetic properties of vitamin C, including its absorption and metabolism, do not appear to be altered in association with aging per se (54). The negative correlation between age and circulating vitamin C concentrations, as observed in the present study, is likely to be explained by differences in dietary intakes across age groups. In US diets, intake of vitamin C has been observed to be inversely related to the percentage of energy contributed by fat, analogous to β-carotene intake (17).

The present study has some interesting implications for the French paradox (18, 21). As in the United States, serum β-carotene and vitamin C concentrations appear to correlate directly with the vegetable and fruit intakes of French adults. However, differing rates of smoking (21), higher per capita alcohol consumption in men (19), and lower average BMI may affect the interpretation of circulating antioxidant micronutrient concentrations in this population compared with the US population samples. Unraveling the complexity of the relation between recommended dietary behaviors, biological activities of protective dietary constituents, and risk for chronic disease will benefit by the identification and interpretation of useful and meaningful biomarkers.

REFERENCES


31. Willett W, Stamper MJ. Total energy intake: implications for epide


