Plasma Carotenoids as Biomarkers of Vegetable and Fruit Intake

Deborah R. Campbell, Myron D. Gross, Margaret C. Martini, Greg A. Grandits, Joanne I. Slavin, and John D. Potter

Division of Epidemiology, School of Public Health [M. D. G., J. D. P.]; Department of Food Science and Nutrition [D. R. C., M. C. M., J. I. S.]; and Division of Biostatistics, School of Public Health [G. A. G.], University of Minnesota

Abstract

Higher intakes of vegetables and fruits are associated with a lower risk of certain human cancers. A biomarker of vegetable and fruit intake would be a valuable research tool. A cross-sectional study assessed the association between plasma carotenoid concentrations and intakes of vegetables and fruits. Plasma carotenoids (α-carotene, β-carotene, lutein, β-cryptoxanthin, and lycopene) were measured in 50 male and 49 female participants, aged 18–37 years, with a wide range of habitual vegetable and fruit intakes. Dietary intakes were assessed via a food frequency questionnaire. Intake of vegetables and fruits and high carotenoid foods were measured. The sum of the plasma carotenoids (excluding lycopene) was highly correlated with intake of total vegetables and fruits (r = 0.59). Of the individual plasma carotenoids, plasma α-carotene had the highest correlation with intakes of both total vegetables (r = 0.50) and total fruits (r = 0.58). Intakes of foods with high carotenoid contents were correlated with their corresponding plasma concentrations as follows: high β-carotene foods (r = 0.41); high lutein foods (r = 0.46); and high lycopene foods (r = 0.11). Multiple regression analyses showed that intake of total vegetables and fruits was the most significant determinant of each plasma carotenoid except lycopene. The utility of combining the plasma carotenoids as biomarkers of vegetable and fruit intake was assessed by a stepwise regression of total vegetable and fruit intake on plasma carotenoids. Significant determinants of intake of total vegetables and fruits were α-carotene, β-cryptoxanthin, lutein, and energy intake (R² = 0.53). These results indicate that plasma carotenoids are useful biomarkers of vegetable and fruit intake, suggesting their potential for monitoring primary prevention trials involving dietary modification.

Introduction

Epidemiological studies have consistently found an inverse association between vegetable and fruit intake and risk of several human cancers. The association appears strongest for epithelial cancers, particularly those of the digestive tract, with a weaker to nonexistent association for hormone-dependent cancers (1).

A variety of plant constituents, including antioxidants, fiber, and other phytochemicals, may contribute to the apparent protective effects accompanying a diet rich in vegetables and fruits. Individually, these plant components have shown anticarcinogenic properties in experimental animal and cell culture systems (1–3).

An important step toward unraveling diet and cancer risk relationships in humans is the development of techniques for monitoring and characterizing dietary exposure. Traditionally, this has been accomplished using subjective diet assessment tools such as FFQs (1), 24-h recalls, and diet records (4, 5). However, all are subject to misclassification due to reporting errors, such as inaccurate serving size estimation, and instrument limitations, such as the inclusiveness of the data base list of foods and nutrients (6). As an alternative, a biomarker, e.g., a plasma or urinary metabolite, may provide an objective measure of dietary exposures such as vegetable and fruit intake (7, 8). A biomarker may bypass the inherent errors associated with traditional diet assessment tools, may be less subject to intr.individual variation, and can allow blinded assessment of compliance in dietary intervention trials where a true placebo arm is not feasible.

A single biomarker of vegetable and fruit intake is unlikely because of the diverse phytochemical composition of plant foods. The carotenoids are widely distributed phytochemicals in vegetables and fruits, a number of which are measurable in human plasma (9, 10). Major carotenoids circulating in human plasma include: lutein; zeaxanthin; lycopene; β-carotene; α-carotene; and β-cryptoxanthin (11). The latter three carotenoids can be converted to vitamin A; however, other functions of carotenoids in humans are not well determined, although their potential role as chemopreventive agents has been suggested (12).

Data from clinical trials and feeding studies in humans have suggested that plasma carotenoids may be potential biomarkers of both carotenoid and vegetable and fruit intake. Clinical trials have demonstrated dramatic plasma β-carotene responses to purified β-carotene supplementation (13, 14). Human feeding studies have shown less dramatic, yet still significant, plasma carotenoid responses to intake of carotenoid-rich foods, such as carrots and broccoli (15, 16). These data suggest the potential of plasma carotenoids as biomarkers of vegetable and fruit intake; however, plasma carotenoid concentrations among individuals consuming diets containing a variety of vegetables and fruits have not been sufficiently studied.

Received 1/4/94; revised 3/30/94; accepted 4/5/94.

1 This project was supported by the National Cancer Institute, Grant P01 CA50305.

2 To whom all requests for reprints should be addressed, at 1300 South 2nd Street, Division of Epidemiology, Minneapolis, MN 55454.

3 The abbreviations used are: FFQ, food frequency questionnaire; OC, oral contraceptive.
In this study, we measured plasma carotenoid concentrations in a free-living adult population reporting long-term daily intakes of either high or low amounts of vegetables and fruits. Two questions were posed to assess the utility of plasma carotenoids as biomarkers of vegetable and fruit intake. First, do plasma carotenoid concentrations differ between populations consuming high and low intakes of mixed vegetables and fruits? Second, if they do differ, can a plasma carotenoid determination serve as an adequate indicator of vegetable and fruit intake? In addition, plasma α-tocopherol concentrations were measured because of potential interactions which have been observed between high carotenoid intakes and plasma tocopherol concentrations (17, 18).

Materials and Methods
One hundred three volunteers were recruited from 295 respondents to posters, mailbox flyers, and student newspaper advertisements within the University of Minnesota community. Subject recruitment occurred over a 4-month period, from March through June 1992. Ninety-nine participants completed adequate data collection. Subjects were 18- to 37-year-old nonsmokers, who reported vegetable and fruit intakes of either ≥5 servings/day or ≤2 servings/day in an initial telephone interview. The study was designed to recruit equal numbers of men and women and equal numbers of “high” and “low” vegetable and fruit consumers. Of the 99 participants, 50 were men (25 “high” and 25 “low”) and 49 were women (25 “high” and 24 “low”). Participants were not informed of the purpose of the study and thus were unaware that their inclusion was based on usual vegetable and fruit intake.

Estimation of vegetable and fruit intake was accomplished by asking respondents to report, during the initial telephone interview, the total number of vegetable and fruit servings eaten per day and a description of an average day’s intake. One serving was defined as 120 ml (½ cup) cooked or raw fruit or vegetable, 240 ml (1 cup) raw lettuce, 60 ml (¼ cup) dried fruit, or 180 ml (6 fluid ounces) of real juice. For the purpose of this study, juice could account for no more than 750 mg lycopene/100 g food.

Exclusion criteria included: (a) medical history of gastrointestinal disorders; (b) food allergies; (c) weight loss or gain greater than 4.5 kg within the past year; (d) major changes in eating habits within the past year; (e) exercise regimens requiring significant short-term dietary changes; (f) antibiotic use within the past 3 months; (g) body weight greater than 150% of ideal; (h) current treatment for a diagnosed disease; and (i) alcohol intake greater than 2 drinks per day (2 drinks are equivalent to 720 ml of beer, 240 ml of wine, or 90 ml of hard liquor). Nutritional supplement use was an initial exclusion criterion; however, due to the large numbers of respondents reporting supplement use, this criterion was abandoned. Nutritional supplements included multivitamins, vitamin E, and β-carotene.

Participants completed a series of activities over the course of a 7-day period. On day 1 and day 7, a fasted blood was collected and body weight measured; height was measured on day 1. Diet assessment included diet records kept on day 2 through day 6 and completion of an FFQ. In addition, a demographic and medical questionnaire was completed. Information obtained from the questionnaire included: age; smoking habits; supplement use; alcohol intake; history of weight fluctuations; and ethnicity. Menstrual history and OC use was queried of women.

The study design was approved by the Institutional Review Board: Human Subjects Committee at the University of Minnesota, and informed written consent was obtained from all subjects prior to the start of the study.

Dietary Assessment. In addition to the initial telephone interview assessment of vegetable and fruit intake, all participants completed a self-administered 153-item Willett FFQ assessing dietary intake over the past year (19, 20). The FFQ was modified to include additional vegetable and fruit items. Vegetable and fruit servings were quantified by summing the frequency of consumption across all vegetable and fruit items at the portion sizes specified on the FFQ. Intake frequency was summarized as servings per week.

Servings of total vegetables, total fruits, and total vegetables and fruits were determined, as well as servings of high carotenoid vegetables and fruits. Excluded from total vegetable servings were “vegetable or noodle soups” and “chowder or cream soups,” each of which is included as an item on the FFQ. Selection of vegetables and fruits containing high concentrations of carotenoids was based on recently updated food carotenoid composition data (9). These data helped identify high vegetable and fruit contributors to carotenoid intake on the basis of median concentrations. Vegetables and fruits were categorized using median cutpoints for the 3 major dietary carotenoids, β-carotene, lutein/zeaxanthin (hereafter, referred to as lutein), and lycopene. A high β-carotene food source was defined as one that contained more than 2000 μg β-carotene/100 g food; a high lutein food source contained more than 1000 μg lutein/100 g food; and a high lycopene food source contained more than 750 μg lycopene/100 g food.

A limitation in using the FFQ when categorizing vegetables and fruits by carotenoid content is the preexisting groupings of vegetables and fruits within single food items on the FFQ. Within one item, only one or two of the fruits or vegetables may, in fact, be high contributors of a given carotenoid. In this study, if an FFQ item containing multiple vegetables or fruits included at least one high carotenoid contributor, this item was included in the calculations of high carotenoid vegetable and fruit intake. See Table 1 for a list of vegetables and fruits as categorized on the FFQ, and the vegetables and fruits contributing to high intake of each carotenoid.

Vitamin E intake was calculated from the questionnaire by multiplying the frequency of consumption of each food by the vitamin E content of the portion size specified on the questionnaire, and summing across all foods. Vitamin E content is as specified in the Nurses’ Health Study data base.

No participants were excluded because of missing items on the FFQ; no more than 6 items (4%) were missed by any one participant, except for one participant who missed 17 items (11%). Previous data indicate that fewer than 20% missing items on an FFQ is acceptable for two reasons: (a) there should be little effect on energy intake; and (b) missing items generally represent rarely consumed foods (20, 21). In this study, missed items appeared infrequently in the vegetable and fruit sections.

Plasma Carotenoids, α-Tocopherol, and Cholesterol Analysis. Following an overnight fast, 30 ml of blood were collected in red dye-coated ethylenediamine tetraacetate
The method of Bieri (22). Alpha-tocopherol was determined by high-performance liquid chromatography. The carotenoids, lutein, 3-cryptoxanthin, lycopene, a-carotene, and a-carotene were determined by high-performance liquid chromatography on a column coated with a C18 stationary phase. The carotenoids were eluted with a gradient of methanol-water-acetonitrile. The eluted compounds were detected at 495 nm.

<table>
<thead>
<tr>
<th>Veggies</th>
<th>Fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomatoes; tomato juice; vegetable juice or V-8; tomato sauce; red chili sauce; tofu or soybeans; string beans; broccoli; cabbage or coleslaw; cauliflower; Brussels sprouts; carrots, raw; carrots, cooked; corn; peas or lima beans; mixed vegetables; beans, lentils, chili beans, or garbanzos; bean, pea, or lentil soup; yellow (winter) squash; eggplant, zucchini, or other summer squash; yams or sweet potatoes; spinach, cooked; spinach, raw; kale, mustard or chard greens; iceberg or head lettuce; romaine or leaf lettuce; celery; beets; alfalfa sprouts; garlic, fresh or powdered; green or chili peppers; mushrooms; onions, raw; onions, cooked</td>
<td></td>
</tr>
<tr>
<td>Raisins; grapes; prunes; prune juice; bananas; cantaloupe; honeydew melon; watermelon; fresh apples; applesauce; fresh pears; apple juice or cider; oranges or mandarin oranges; orange juice; grapefruit; grapefruit juice; other fruit juices; strawberries, fresh, frozen or canned; blueberries, fresh, frozen or canned; peaches, nectarines, apricots, or plums; dried peaches, apricots, or nectarines; apricot, peach, or passion fruit nectar; pineapple, fresh or canned; other fruits, fresh, frozen, or canned</td>
<td></td>
</tr>
<tr>
<td>Fresh apricots, dried apricots, cantaloupe, raw carrots, cooked carrots, kale, mustard greens, dried peaches, red pepper, raw spinach, cooked spinach, sweet potatoes, Swiss chard, winter squash</td>
<td></td>
</tr>
<tr>
<td>Broccoli, Brussels sprouts, celery, kale, leeks, mustard greens, romaine lettuce, green peas, scallions, raw spinach, cooked spinach, summer squash</td>
<td></td>
</tr>
<tr>
<td>Dried apricots, pink grapefruit, guava, tomatoes, tomato juice, tomato sauce, watermelon, pizza</td>
<td></td>
</tr>
</tbody>
</table>

*Vegetables and fruits listed in the high carotenoid food categories include those which can be reported as additional foods on the FFQ.*

Control pools containing high and low concentrations of each analyte. In addition, our laboratory routinely analyzes National Institutes of Standards and Technology reference sera and is a participant in the National Institutes of Standards and Technology Fat-Soluble Vitamin Quality Assurance Group.

Plasma cholesterol concentrations were measured using a Kodak Ektachem cholesterol oxidase-based assay (24). Analysis was performed in a hospital laboratory that is accredited by the American College of Pathologists and maintains acceptable lipid comparisons against the University of Minnesota, which is a Centers for Disease Control lipid-testing laboratory.

**Statistical Analysis.** The recruitment of participants as “high” or “low” vegetable and fruit consumers by the telephone interview provided two relatively distinct vegetable and fruit intake populations based on data from the FFQ (Fig. 1); however, significant overlap did occur between the two groups. Given the overlap between groups and the wide range of vegetable and fruit intake across the entire population, dietary intake was analyzed as a continuous variable on the basis of FFQ data, as well as by the initially defined “high” versus “low” groups. Comparisons of “high” and “low” vegetable and fruit intake groups for plasma carotenoid concentrations were made using standard t-tests. Correlation analysis was used to assess the relationship between plasma carotenoids and vegetable and fruit intake derived from the FFQ. Simple and adjusted Pearson correlations were computed. The adjusted correlations were obtained by computing two sets of residuals: residuals from regressing each plasma carotenoid on plasma cholesterol, and residuals from regressing vegetable and fruit intake on total calories. The adjusted correlations were then computed as the simple correlations between these two sets of residuals.

Multiple regression was performed to assess the relationship of other variables in addition to vegetable and fruit intake on plasma carotenoids. Independent variables considered were: age; alcohol intake; plasma cholesterol; vegetable and fruit intake; BMI; energy intake; use of nutritional supplements; vitamin E intake; and OC use in women. To assess the relationship between vegetable and fruit intake and the combined effect of the plasma carotenoids, a stepwise multiple regression was performed against total vegetable and fruit intake as the dependent variable, with all the carotenoids and other variables described above as potential independent variables.

For all correlation and regression analyses each plasma carotenoid, except lycopene, was log-transformed to improve normality; lycopene was used in the raw scale. Total vegetable and fruit intake and the various components of vegetable and fruit intake were square-root transformed. Vitamin E intake was log transformed. Some analyses were done separately for men and women.

The averages of the two plasma values (day 1 and day 7) were used for each subject for statistical analysis. Pearson correlations between the two plasma sample concentrations were 0.93, 0.94, 0.94, 0.84, and 0.78 for a-carotene, beta-carotene, beta-cryptoxanthin, lutein, and alpha-tocopherol, respectively.

**Results**

Ninety-nine participants (96.1%) of the 103 initial recruits, 50 men and 49 women, completed this cross-sectional study.
Plasma Carotenoids as Biomarkers were used by 29% of the participants. Thirty-nine % of the current smokers, with current smoking status defined as ± 1 cigarette/week, had an average age of participants was 24.8 years. Ninety-one % of the participants was 52.9 servings per week, with fruit servings accounting for 42% and vegetable servings for 58% of the total. In addition, participants consumed on average 4.4 drinks/week. Nutritional supplements were used by 29% of the participants. Thirty-nine % of the female participants had taken OCs within the past 3 months. For almost all characteristics, “high” and “low” groups were similar, with the exception of alcohol intake; there was a higher proportion of drinkers in the “low” group.

Means and quartiles of intake of vegetables and fruits, high carotenoid foods, and vitamin E are given in Table 3. The mean intake of total vegetables and fruits for all participants was 52.9 servings per week, with fruit servings accounting for 42% and vegetable servings for 58% of the total. In addition, participants consumed on average 5.5, 7.0, and 6.0 servings of high lutein, high lycopene, and high β-carotene foods per week, respectively. Mean vitamin E intake was 36.5 mg tocopherol equivalents per week.

High carotenoid foods consumed by this population, for each food category, were as follows. High lutein food contributors, in descending order of intake were: broccoli; celery; peas or lima beans; romaine lettuce; cooked spinach; eggplant or zucchini; raw spinach; kale, mustard, or chard greens; and Brussels sprouts. (Scallions and leeks, which are high in lutein, were not consumed by this population). For lycopene, the major food contributors were: pizza, tomatoes, tomato sauce, grapefruit, watermelon, dried apricots or peaches, and tomato juice. (Guava, a source of lycopene, was not consumed by this population). Top food contributors to β-carotene intake were: raw carrots; cooked carrots; peaches, apricots, or plums; cantaloupe; cooked spinach; raw spinach; dried apricots or peaches; yellow (winter) squash; yams or sweet potatoes; and kale, mustard, or chard greens. (Intake of red pepper was not reported in this population).

Mean plasma concentrations for all subjects were 18.0 ± 6.2, 8.3 ± 4.7, 37.3 ± 14.7, 5.6 ± 4.3 and 15.7 ± 11.7 µg/dl for lutein, β-cryptoxanthin, lycopene, α-carotene, and β-carotene, respectively. The mean plasma concentration of α-tocopherol was 0.8 ± 0.2 mg/dl. Significant differences in plasma carotenoid concentrations were observed between the “high” and “low” vegetable and fruit intake groups (Table 4). Plasma lutein, sum of the carotenoids, β-carotene, β-cryptoxanthin, and α-carotene concentrations were approximately 33, 50, 51, 57, and 99% higher in the “high” versus the “low” group (P < 0.01). There were no significant differences in plasma α-tocopherol or lycopene concentrations. Mean analyte concentrations did not change significantly when nutritional supplement users were excluded and did not differ between men and women.

The results of correlation analyses between vegetable and fruit intake and plasma carotenoid concentrations are presented in Table 5. The simple and adjusted correlations were similar, therefore only adjusted values are discussed in the text. Significant correlations were found between several plasma carotenoids and the intake of both vegetables and fruits and specific carotenoid food groupings. Each plasma carotenoid except lycopene was significantly correlated with total vegetable and fruit intake with correlations ranging from 0.44 for lutein to 0.61 for α-carotene. The small inverse correlation between plasma lycopene and total vegetable and fruit intake (r = -0.14) was not statistically significant. Plasma lutein was more strongly correlated with total vegetable intake (r = 0.48) than total fruit intake (r = 0.24), whereas plasma β-cryptoxanthin was more strongly correlated with total fruit (r = 0.56) than total vegetable intake (r = 0.32).

Of the high carotenoid food categories, high lutein foods were most strongly correlated with plasma lutein and α-carotene (r = 0.46 and 0.47, respectively). High β-carotene foods had the highest correlation with plasma α-carotene (r = 0.69). Correlations of plasma carotenoids with high lycopene foods were smaller than for other dietary components, with the largest correlation being 0.28 for plasma β-cryptoxanthin.

Plasma β-carotene had the highest correlations with intake of total vegetables and fruits (r = 0.45), total fruits (r = 0.46), high β-carotene foods (r = 0.41), and vitamin E (r = 0.45). Plasma α-carotene was correlated also with intake of total fruits (r = 0.58) and total vegetables (r = 0.50). A sum of plasma carotenoids, including lutein, β-cryptoxanthin, α-carotene, and β-carotene, was most highly correlated with total vegetable and fruit intake (r = 0.59). Plasma α-tocopherol concentrations were significantly correlated with intake of vitamin E.
Table 3: Intake of vegetables, fruits, and high carotenoid foods

<table>
<thead>
<tr>
<th>Dietary components</th>
<th>Mean no. of servings/week (SD)</th>
<th>Percentiles of intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25th</td>
<td>50th</td>
</tr>
<tr>
<td>Total vegetables and fruits</td>
<td>52.9 (32.4)</td>
<td>29.5</td>
</tr>
<tr>
<td>Total vegetables</td>
<td>30.7 (20.7)</td>
<td>15.5</td>
</tr>
<tr>
<td>Total fruits</td>
<td>22.2 (15.8)</td>
<td>10.8</td>
</tr>
<tr>
<td>High lutein foods</td>
<td>5.5 (5.9)</td>
<td>2.0</td>
</tr>
<tr>
<td>High lycopene foods</td>
<td>7.0 (4.2)</td>
<td>4.0</td>
</tr>
<tr>
<td>High β-carotene foods</td>
<td>6.0 (6.5)</td>
<td>2.0</td>
</tr>
<tr>
<td>Vitamin E intake (mg TE)</td>
<td>36.5 (92.5)</td>
<td>7.5</td>
</tr>
</tbody>
</table>

* Intake based on the Willett FFQ.

Table 4: Plasma concentrations of biochemical indicators in "low" versus "high" vegetable and fruit consumers

<table>
<thead>
<tr>
<th>Biochemical indicator</th>
<th>&quot;Low&quot;</th>
<th>&quot;High&quot;</th>
<th>Difference</th>
<th>T-Stat</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutein (µg/dl)</td>
<td>15.45 (0.61)</td>
<td>20.61 (0.96)</td>
<td>5.16</td>
<td>4.54</td>
<td>0.0001</td>
</tr>
<tr>
<td>β-Cryptoxanthin (µg/dl)</td>
<td>6.51 (0.48)</td>
<td>10.19 (0.74)</td>
<td>3.69</td>
<td>4.21</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lycopene (µg/dl)</td>
<td>38.35 (2.15)</td>
<td>36.27 (2.04)</td>
<td>-2.09</td>
<td>-0.71</td>
<td>0.4823</td>
</tr>
<tr>
<td>α-Carotene (µg/dl)</td>
<td>3.73 (0.48)</td>
<td>7.43 (0.62)</td>
<td>3.70</td>
<td>4.73</td>
<td>0.0001</td>
</tr>
<tr>
<td>β-Carotene (µg/dl)</td>
<td>12.57 (1.64)</td>
<td>18.90 (1.56)</td>
<td>6.34</td>
<td>2.83</td>
<td>0.0056</td>
</tr>
<tr>
<td>Sum of carotenoids (µg/dl)</td>
<td>38.25 (2.15)</td>
<td>57.22 (2.62)</td>
<td>18.97</td>
<td>5.62</td>
<td>0.0001</td>
</tr>
<tr>
<td>α-Tocopherol (mg/dl)</td>
<td>0.76 (0.02)</td>
<td>0.82 (0.02)</td>
<td>0.06</td>
<td>1.91</td>
<td>0.0592</td>
</tr>
</tbody>
</table>

* "Low" and "High" intake status based on the initial telephone report.

Table 5: Pearson correlations between intake of dietary components and plasma carotenoids

<table>
<thead>
<tr>
<th>Dietary component</th>
<th>Plasma carotenoids</th>
<th>Lutein</th>
<th>β-Cryptoxanthin</th>
<th>Lycopene</th>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>Sum of carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total vegetables/fruits</td>
<td>0.39</td>
<td>0.44</td>
<td>0.44</td>
<td>0.47</td>
<td>-0.04</td>
<td>-0.14</td>
<td>0.54</td>
</tr>
<tr>
<td>Total vegetables</td>
<td>0.43</td>
<td>0.48</td>
<td>0.30</td>
<td>0.32</td>
<td>0.00</td>
<td>-0.08</td>
<td>0.45</td>
</tr>
<tr>
<td>Total fruits</td>
<td>0.21</td>
<td>0.24</td>
<td>0.34</td>
<td>0.36</td>
<td>-0.07</td>
<td>-0.11</td>
<td>0.46</td>
</tr>
<tr>
<td>High lutein foods</td>
<td>0.44</td>
<td>0.46</td>
<td>0.31</td>
<td>0.32</td>
<td>0.00</td>
<td>-0.08</td>
<td>0.45</td>
</tr>
<tr>
<td>High lycopene foods</td>
<td>0.17</td>
<td>0.19</td>
<td>0.27</td>
<td>0.28</td>
<td>0.20</td>
<td>0.11</td>
<td>0.14</td>
</tr>
<tr>
<td>High β-carotene foods</td>
<td>0.30</td>
<td>0.33</td>
<td>0.26</td>
<td>0.27</td>
<td>-0.12</td>
<td>-0.15</td>
<td>0.67</td>
</tr>
</tbody>
</table>

* Plasma carotenoids are the averages of two samples.

A summary of the regression of each plasma carotenoid on total vegetable and fruit intake and other factors is given in Table 6. Plasma α-tocopherol was not considered in the regression analysis because no interaction was observed between carotenoid intake and plasma α-tocopherol concentrations. Of all variables considered, total vegetable and fruit intake had the strongest relationship with each plasma carotenoid, with the exception of lycopene. The regression coefficient of vegetable and fruit intake for plasma lycopene was not statistically significant.

Several other factors, in addition to vegetable and fruit intake, were statistically significant predictors of plasma carotenoid concentrations, including age and energy intake. Plasma lutein, α-carotene, and the sum of carotenoid concentrations increased with age, whereas plasma lycopene decreased with age. Energy intake was inversely related to plasma lutein, α-carotene, β-carotene, and sum of the carotenoids.

Regression analyses were conducted separately for men and women and no significant differences in regression coefficients were observed, with the following exception. There was a slight differential effect of vegetable and fruit intake on plasma carotenoids by gender for plasma β-cryptoxanthin (P = 0.06) and plasma α-carotene (P = 0.09). For both carotenoids, the predicted plasma response to vegetable and fruit intake was greater in men than women.

A stepwise regression analysis involving plasma carotenoids and other variables was performed with vegetable and fruit intake as the dependent variable. The resultant
Plasma Carotenoids as Biomarkers

prediction equation is:\n\[ \text{vegetable and fruit intake} = 1.116 \times \text{plasma } \alpha\text{-carotene} + 0.083 \times \text{energy intake} + 1.308 \times \text{plasma lutein} + 0.644 \times \text{plasma } \beta\text{-cryptoxanthin}. \]

Energy intake and plasma concentrations of \( \alpha\text{-carotene}, \) lutein, and \( \beta\text{-cryptoxanthin} \) explained 53\% of the variability in the measurement of vegetable and fruit intake in this population.

Discussion

In this cross-sectional study, we assessed the relationship between vegetable and fruit intake and plasma concentrations of carotenoids and \( \alpha\text{-tocopherol} \). Participants consumed a wide range of vegetable and fruit intakes, which was appropriate for assessing the utility of plasma carotenoids as biomarkers of vegetable and fruit intake. The mean intake of vegetables and fruits in this population was about 53 servings per week, reflecting both the underestimate of intake that FFQs appear to provide (25) and the fact that the study group, by design, excluded those who self-reported intake of mixed dishes, such as broccoli, celery, green beans, green peas, and raw tomato (9). Whatever the cause, because our major interest was in identifying markers of total vegetable and fruit intake, this is not regarded as a drawback.

\( \alpha\text{-carotene} \) and \( \beta\text{-cryptoxanthin} \) intakes were difficult to estimate because of inadequate vegetable and fruit composition data. Notably, plasma \( \alpha\text{-carotene} \) was highly correlated with total vegetable intake, paralleling related with fruit intake (10). In addition, plasma lutein was highly correlated with intake of high \( \beta\text{-carotene} \) foods (9). Whatever the cause, because our major interest was in identifying markers of total vegetable and fruit intake, this is not regarded as a drawback.

Alpha-carotene and \( \beta\text{-cryptoxanthin} \) intakes were difficult to estimate because of inadequate vegetable and fruit composition data. Notably, plasma \( \alpha\text{-carotene} \) was highly correlated with intake of high \( \beta\text{-carotene} \) foods (9). Whatever the cause, because our major interest was in identifying markers of total vegetable and fruit intake, this is not regarded as a drawback.

The low correlation between plasma lycopene and \( \beta\text{-cryptoxanthin} \) indicates its predominance in fruits versus vegetables; plasma \( \beta\text{-cryptoxanthin} \) was highly correlated with fruit intake (10). In addition, plasma lutein was highly correlated with total vegetable intake, paralleling food composition data on lutein (9, 10).

The low correlation between plasma lycopene and high lycopene foods has multiple possible explanations. First, the high food contributors to lycopene intake defined here do not include mixed dishes (except pizza), which is how lycopene is most commonly consumed in the United States (26). Mixed dishes are not listed on the FFQ; thus, if participants failed to record intake of mixed dishes, such as spaghetti or tomato-based casseroles, as individual ingredients on the FFQ, reported lycopene intake may be inaccurate. Second, the range of lycopene intake in this population may be narrow, \textit{i.e.}, everyone may eat similar amounts.
of lycopene-rich foods. Table 2 shows that the range of high lycopene food intake is narrower than intakes of high \( \beta \)-carotene and lutein-foods. Third, lycopene appears to be distributed among fewer vegetables and fruits than the other carotenoids (Table 1) (9). This may be due to inadequate food composition data or, more likely, to a real distribution phenomenon. Fourth, studies in humans and animal models indicate that carotenoids may differ in absorption, metabolism, and excretion (27–29). Lycopene may possess unique characteristics limiting its usefulness as a biomarker of intake. Finally, lycopene was the only plasma carotenoid significantly associated with plasma cholesterol concentrations in the regression analyses. Because of the resistance of plasma lipid concentrations to dietary change, plasma lycopene concentrations may remain relatively stable as well.

This is the first study, as far as we are aware, to correlate intake of specific carotenoid-containing vegetables and fruits with an extensive panel of plasma carotenoids. Aoki et al. (30) examined the relationship between plasma carotenoids and intake of green-yellow vegetables, seaweeds, and some fruits, in a Japanese community. That study showed low correlations between plasma carotenoid concentrations and foods high in carotenoids \((r = 0.07–0.21);\) however, intake of these foods was significant in multiple regression analysis in predicting plasma \( \beta \)-carotene concentrations. Limitations of this Japanese study included an undefined method of dietary assessment and an ambiguous listing of the claimed high carotenoid foods.

Previous studies have examined associations between plasma carotenoid concentrations and various estimates of “carotene” intake, because intake of individual carotenoids cannot be calculated using traditional food tables. For example, two recent studies measuring dietary intake using an FFQ calculated carotene intake on the basis of provitamin A carotenoids. This estimate accounted for the total vitamin A activity from plants plus one-third the vitamin A activity in dairy fat. Jacques et al. (31) reported an adjusted correlation of 0.37 between carotene intake and an undefined measure of total plasma carotenoids. Ascherio et al. (32) reported correlations between total dietary carotene intake (adjusted for energy) and individual plasma carotenoids. These correlations were 0.53, 0.29, 0.36, 0.02, and 0.32, for \( \alpha \)-carotene, \( \beta \)-carotene, \( \alpha \)-plus \( \beta \)-carotene, lycopene, and lutein, respectively. Note the low lycopene correlation.

Recently, the updated carotenoid data base used in the present study for creation of the high carotenoid food groupings was applied to all carotenoid-containing food items on an FFQ. The resulting carotenoid intake was then correlated with individual plasma carotenoid concentrations in a cross-sectional male population. Adjusted correlations ranged from 0.29 to 0.46 between calculated FFQ carotenoid intakes and plasma concentrations of \( \alpha \)-carotene, \( \beta \)-carotene, \( \beta \)-cryptoxanthin, lutein, and lycopene (33). While application of the updated carotenoid values improved carotenoid intake assessment in that male population, it is evident that this data base is still limited by the amount and quality of the food composition data (9).

Correlations observed in the abovementioned studies are similar to each other and to those observed in this cross-sectional study of vegetable and fruit intake in men and women. However, correlations between plasma carotenoid concentrations and vegetable and fruit intakes are higher than those between plasma concentrations and estimated carotenoid contents of vegetables and fruits. Vegetables and fruits as dietary intake variables encompass the contribution of all dietary carotenoids; therefore, potential limitations of food composition data and the consideration of provitamin A carotenoids only are bypassed.

Regression analysis indicated that total vegetable and fruit intake was the most significant predictor of all plasma carotenoids except lycopene. Conversely, the stepwise multiple regression emphasized the advantage of measuring more than one plasma carotenoid to assess vegetable and fruit intake in a population. An equation including energy intake and plasma concentrations of \( \alpha \)-carotene, lutein, and \( \beta \)-cryptoxanthin explained 53% of the variability in vegetable and fruit intake in this population. This equation emphasizes the advantage of measuring the entire carotenoid profile as opposed to a single carotenoid, such as \( \alpha \)-carotene, to assess vegetable and fruit intake in a population or to monitor compliance in dietary intervention studies. However, the limitation of this regression equation may be its population specificity, which in this case included a limited sample of individuals consuming a wide range of vegetables and fruits. Its more general application needs examination.

Plasma carotenoid concentrations may be influenced by various host and dietary factors potentially affecting carotenoid absorption and metabolism including dietary fat intake, vitamin A status, food matrix, and food preparation (13, 27, 34, 35). The design of this study and the diet assessment tool used do not permit speculation on these issues. However, these questions warrant consideration in future studies addressing carotenoid-rich food intake in various forms and in diverse populations.

In summary, plasma carotenoids appeared to be valid biomarkers of vegetable and fruit intake in this cross-sectional study. Both correlation and regression analyses indicated that vegetable and fruit intake was a significant determinant of plasma carotenoids and that plasma carotenoids were good predictors of self-reported vegetable and fruit intake. Recent food composition data show a wide distribution of carotenoids in vegetables and fruits, with little contribution from other food sources. Thus, plasma carotenoids should prove to be useful in monitoring dietary compliance in intervention studies emphasizing vegetable and fruit intake. However, carotenoids are not found in all vegetables and fruits; identification of other biomarkers of vegetable and fruit intake may facilitate creation of a panel of biomarkers which would be useful in epidemiological studies clarifying diet and disease relationships. This is currently under study in our group.

Acknowledgments

We thank Michelle Pfeiffer and Christian Prouty for high performance liquid chromatography analysis of the plasma carotenoids; and Pat Elmer, Stephanie Smith, and Jeff Ambord for handling the FFQs.

References


Plasma carotenoids as biomarkers of vegetable and fruit intake.
D R Campbell, M D Gross, M C Martini, et al.

Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/3/6/493

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.