A Pilot Study on the Use of Plasma Carotenoids and Ascorbic Acid as Markers of Compliance to a High Fruit and Vegetable Dietary Intervention

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Abstract

The authors examined the feasibility of using plasma carotenoids and ascorbic acid as markers of compliance for dietary intervention trials aimed at increasing the quantity and variety of the fruit and vegetable intake of free-living individuals. Nineteen former cancer patients who had been successfully treated for a stage I or II squamous cell carcinoma of the mouth, pharynx, larynx, or lung were recruited. Subjects served as their own controls. However, in order to detect any seasonal trends, 4 individuals among the 19 were randomized to a nonintervention group. Subjects in the intervention group were counseled by dietitians with the goal of increasing their intake of fruits and vegetables to eight servings/day (1 serving of each of dark green vegetables, yellow-orange vegetables, tomato products, and other vegetables; 3 servings of vitamin C-rich foods; and 1 serving of other fruits). Subjects in the nonintervention group were advised to follow their usual diet. Three-day dietary recalls, documented an increase in mean daily fruit and vegetable consumption and may be useful as markers of compliance at the group level in intervention trials.

Introduction

Epidemiological studies on diet and cancer consistently have found inverse associations between fruit and vegetable consumption and risk for a number of epithelial cancers (1). Indeed, the data suggest that these foods contain a wide range of cancer inhibitors (2, 3) and that their consumption may provide a greater protection than that achieved with any single fruit or vegetable constituent alone, such as particular carotenoids, vitamin C, or folic acid (4, 5). Hence, there is a rationale for conducting intervention trials using diets rich in a variety of fruits and vegetables. In such trials, neither investigators nor participants would be blind to the nature of the intervention. Thus, of particular importance would be markers of fruit and vegetable consumption that would be sensitive to variations in the quantity and variety of intake and therefore would provide objective measures of compliance. In an attempt to identify such compliance markers, we monitored for 3 months the serial changes in plasma carotenoids and ascorbic acid among 15 former cancer patients who were instructed to consume a total of 8 servings/day from 6 fruit and vegetable food groups and among 4 controls who remained on their regular diet.

Methods

Subjects were individuals who had been successfully treated for squamous cell carcinoma of the lung (stage I) or the head and neck (stage I–II) in a single Honolulu medical center during 1981–1992 and who had not had any recurrences or other cancers. Nineteen individuals (12 men and 7 women), or 66% of all eligible patients, agreed to participate. The mean age of the participants was 63.6 years (range, 54–77). Only two of the subjects were currently smoking, and all but three had smoked in the past. Because of the small sample size and our primary interest in investigating the feasibility of the dietary intervention, each subject served as his/her own control. However, in order to be assured that any observed changes did not reflect seasonal changes in consumption patterns,2 we decided to randomly allocate a few subjects (four in total) to a nonintervention group. A 3-day measured food record was collected at base line and after 3

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months of intervention. Unannounced 24-h dietary recalls also were obtained at 1 month and 2 months. A fasting blood sample was obtained at base line, 2 months, and 3 months.

Subjects in the intervention group were asked to increase their consumption of fruits and vegetables to eight servings/day (1 serving each of dark green vegetables, yellow-orange vegetables, tomato products, and other vegetables; 3 serving of vitamin C-rich fruits; and 1 serving of other fruits). Our definition of a serving was the same as that used by the United States Department of Agriculture: for vegetables, 1 cup of raw or 1/2 cup yellow-orange servings/day (1 serving each of dank green vegetables, also were obtained at 1 month and 2 months. A fasting blood samples were stored at -70°C and analyzed in a single batch at the end of the study. Individual carotenoid levels were determined by high pressure liquid chromatography (8). Briefly, plasma proteins were precipitated with ethanol containing bis-hydroxy-toluene as antioxidant and three inter-

| Intake                              | Base line | 1 month | 2 months | 3 months | P  
|-------------------------------------|-----------|---------|----------|----------|---
| Dark green vegetables (1) (1)        | 0.2       | 0.8     | 0.7      | 0.5      | 0.01  
| Tomato products (1)                 | 0.1       | 1.2     | 0.9      | 0.9      | <0.01  
| Yellow orange vegetables (1)         | 0.4       | 0.7     | 0.9      | 0.7      | 0.14  
| Other vegetables (1)                | 1.3       | 1.8     | 2.0      | 2.1      | 0.01  
| Vitamin C fruits (3)                | 1.2       | 3.7     | 4.3      | 4.1      | <0.001  
| Other fruits (1)                    | 0.8       | 1.5     | 0.9      | 1.1      | 0.11  
| Total fruits and vegetables (8)      | 4.2       | 9.6     | 9.8      | 9.5      | <0.001  

Table 1 Mean intake (in servings/day) at base line and during the study in the intervention group (n = 15)

No recommendation was made about the use of vitamin supplements. Ten subjects in the intervention group and two individuals in the control group reported that they consumed vitamin supplements (multivitamins or vitamin C). Change in dosage was reported during the intervention for β-carotene and vitamin C supplementation by one and two subjects, respectively. They were excluded from analyses on the relevant plasma nutrient (Tables 3 and 4).

The dietary counseling was conducted by registered di-

Table 2 Mean daily nutrient intake at base line and at 3 months in the intervention group based on food records (n = 15)

- β-carotene (µg/d) 4,357 9,943 +128.2 0.01  
- a-carotene (µg/d) 1,398 1,598 +14.3 0.15  
- γ-carotene (µg/d) 1,637 5,126 +213.1 0.001  
- Lycopene (µg/d) 2,407 13,071 +443.0 0.002  
- Lutein (µg/d) 318 370 +16.4 0.24  
- Total carotenoids (µg/d) 10,117 30,108 +197.6 <0.001  
- Vitamin C (mg/d) 143 339 +137.1 <0.001  

Venous blood was collected from the 19 participants after a 12-h fast at base line, and at 2 and 3 months during the intervention period. All samples were protected from light and processed within 1 h after blood drawing. Plasma samples were stored at -70°C and analyzed in a single batch at the end of the study. Individual carotenoid levels were determined by high pressure liquid chromatography (8). Briefly, plasma proteins were precipitated with ethanol containing bis-hydroxy-toluene as antioxidant and three internal standards followed by repeated (3×) hexane extraction.

Previously substituted for carbonated beverages, and fruits were chosen in place of sweets for desserts and snacks. During the intervention period, the dietitians scheduled intermittent home visits and telephone calls to maintain enthusiasm and adherence to the diet among the subjects. Other activities included pamphlets and recipes on fruits and vegetables, a newsletter for the participants, and a pot-luck supper to encourage interaction among all of the subjects and the staff.

At the end of the 3 months, another 3-day measured food record was collected from the subjects. The 3-day food records and 24-h dietary recalls were analyzed for individual carotenoids, ascorbic acid, and other dietary components. The major sources for our food composition database are Mangels et al. (6) for carotenoids, and the United States Department of Agriculture Nutrient Database for Standard Reference (7) for ascorbic acid and other dietary components, along with data from Japan, China, the Philippines, Great Britain, and analyses of selected food items.
of the lipophilic micronutrients. The combined hexane layers were dried under nitrogen and redissolved in the high pressure liquid chromatography mobile phase consisting of methanol: dichloromethane : acetonitrile (65:25:10), bis-hydroxy-toluene (0.025%) as antioxidant, and aqueous bis-tris-propane (2 ml/l of 0.5 x, pH 7.0) as buffer to prevent column degradation. Twelve carotenoids, retinol, y- and 
\[\alpha\] -tocopherol were separated on a Spherex 5-\mu m C18 column (250 x 4.6 mm) (Phenomenex, Torrance, CA) and monitored by a dual multiple wavelength detector at each individual compound's absorption maximum. Levels were determined using peak areas and calibration curves of authentic standards. Analytical accuracy and reliability were verified by participation in the National Institute of Standards and Technology "round robin" for micronutrient analysis. With results consistently within 6% of the mean values reported for all "core" laboratories and coefficients of variation not greater than 4%, with the exception of \[\alpha\] -carotene for which the coefficient of variation was 6.8%.

Although 12 carotenoid fractions were used to calculate total plasma carotenoids, only the 5 major peaks (\[\alpha\] -carotene, \[\beta\] -carotene, lutein, lycopene, and \[\beta\] -cryptoxanthin) are reported in detail here. Plasma ascorbic acid was measured with the dichlorophenolindophenol method (9) in the Clinical Nutrition Laboratory of the University of New Mexico School of Medicine (P. Garry). Total plasma cholesterol was measured enzymatically in a cholesterol oxidase/peroxidase system using a diagnostics kit No. 352-50 from Sigma Chemical Co., (St. Louis, MO).

Summary statistics were computed for each dietary and plasma variable. The paired t test on the log-transformed data of changes in intake and changes in plasma levels. The changes were log transformed, after adding two times the SD of the change, which ensured that all values were positive. Other types of correlations were explored, such as that between serum change, y, and a power of the intake change, \[x^p\]. \[\beta\] was obtained as the slope in the linear regression of log y on log x. This allowed for nonlinear relationships between the variables. However, since the results were similar to those between the log-transformed changes, they are not shown here.

### Results

Table 1 presents the increase in mean fruit and vegetable intake achieved by the intervention subjects for each food group. Comparing the 3-day measured food records collected at base line and at 3 months, subjects increased their daily total fruit and vegetable intake from a mean of 4.2 servings (range, 1.0-11.3 servings) to a mean of 9.5 servings (range, 3.1-15.2 servings) \(P<0.001\). This increase had a high statistical significance for each fruit and vegetable group, except yellow-orange vegetables and other fruits, for which the \(P\) values were 0.1. The unannounced 24-h dietary recalls demonstrated similar increases in mean fruit and vegetable intake at 1 and 2 months (Table 1). There also was good agreement between the 8-A-DAY logs (results not shown) and the data in Table 1.

The change in mean nutrient intakes between base line and 3 months in the intervention group, as estimated from the 3-day food records, is shown in Table 2. The mean total carotenoid intake (taken as the sum of \[\beta\] -carotene, lutein, lycopene, \[\alpha\] -carotene, and \[\beta\] -cryptoxanthin intakes) increased 3-fold to 30 mg daily \(P<0.001\). The increase in mean intake of each specific carotenoid was statistically significant \(P<0.01\), except for \[\alpha\] -carotene \(P=0.15\) and \[\beta\] -cryptoxanthin \(P=0.24\). Mean vitamin C intake increased 2.4-fold to 339 mg daily \(P<0.001\). No significant change occurred in the mean fat intake of the subjects as the result of the intervention. The mean nutrient intakes computed from the 24-h recalls at 1 and 2 months (data not shown) were very similar to those found for 3 months using the 3-day food records (Table 2).

The changes in mean plasma levels of carotenoids and ascorbic acid are shown in Table 3 for the intervention group. We observed an increase over base line of 29.1% for total plasma carotenoids \(P=0.02\), with increases for specific carotenoids ranging from 8.6% for plasma \[\beta\] -cryptoxanthin \(P=0.16\) to 57.3% for plasma \[\alpha\] -carotene \(P=0.01\). Mean plasma levels of ascorbic acid increased by 27.1% \(P<0.001\). Other nutrients measured in the plasma (retinol, \[\alpha\] -tocopherol, y-tocopherol, cholesterol) did not change significantly over the intervention period. Changes in plasma levels were similar among supplement users and nonusers in the intervention group. No statistically significant change was observed in the mean plasma values of subjects in the nonintervention group over the 3-month
The changes in mean total carotenoids, β-carotene, and ascorbic acid levels in the controls were +0.0%, −0.6%, and +11.0%, respectively.

The changes in total fruit and vegetable intake and total plasma carotenoids are illustrated in Fig. 1 for each participant in the intervention group. The dietary intervention was unsuccessful in two individuals who did not increase their fruit and vegetable intake (Patients 4 and 14). Among participants who increased their fruit and vegetable intake, four (Patients 5, 8, 10, and 12) did not experience an increase in plasma carotenoids. Overall, a certain amount of interindividual variation in the plasma response was suggested by the data.

The correlations between the changes in dietary intake and plasma levels of carotenoids and ascorbic acid from baseline to 3 months among the intervention group are shown in Table 4. Reasonably good correlations (i.e., 0.4–0.7) were found for change in total plasma carotenoids and change in overall intake of fruits and vegetables, and for changes in specific plasma carotenoid levels and changes in intake of their main food sources (dark green vegetable for lutein, tomato products for lycopene, and yellow-orange vegetables for α-carotene). These correlations are underlined in Table 4. The correlation coefficient for change in plasma ascorbic acid and intake of vitamin C-rich fruits ($r = 0.2$) was lower than those observed for changes in plasma carotenoids and their food sources, possibly due to the water-soluble nature of vitamin C and its rapid urinary excretion.

Although we were more interested in the ability of plasma carotenoids and ascorbic acid levels to predict change in food intake (Table 4), we also examined the correlations between changes in micronutrient intake and plasma levels. Pearson correlation coefficients for these correlations were: β-carotene, 0.50; α-carotene, 0.25, lutein, 0.41; lycopene, 0.54; β-cryptoxanthin, −0.04; total carotenoids, 0.41; and ascorbic acid, 0.18.

**Discussion**

Because we wanted to mimic the conditions of a large-scale trial, we did not use a controlled diet in this intervention. Instead, subjects remained on a self-selected diet but were instructed to consume specified numbers of servings from six fruit and vegetable food groups. No other instructions were made with regard to other aspects of the subjects' diets. The participants were able to follow the intervention diet during the 12 weeks of the study and achieve a substantial increase in their daily fruit and vegetable intake (by an average of five servings) with minimal alteration to the rest of their diet or lifestyle.

This increase in fruit and vegetable intake was responsible for a significant rise (by an average of 29%) in their total plasma carotenoid levels. The data also showed that change in total plasma carotenoids correlated well ($r = 0.7$) with the overall change in fruit and vegetable intake. Such a correlation is usually thought to be adequate for a marker of compliance at the group level (10). Thus, during an intervention trial, change in the mean total plasma carotenoid level over time could be used to monitor adherence to a high fruit and vegetable diet in the study group, and, probably, to monitor drift in the control group.

Our intent in this study was to increase intake of a wide variety of fruits and vegetables in a standardized way. The emphasis was both on the quantity and variety of intake of fruits and vegetables known to contain suspected cancer inhibitors, including but not limited to carotenoids (2, 3). We took a practical approach and recommended groups of fruits and vegetables that were good food sources of specific carotenoids, with the hope that plasma levels of these carotenoids would increase accordingly.
noids would provide adequate markers of intake for the food groups and, hence, for the variety of the fruits and vegetables consumed.

Indeed, this study suggests that plasma lutein, lycopene, and a-carotene are appropriate markers of dark green vegetable, tomato product, and yellow-orange vegetable intake, respectively, since there were reasonably good correlations between the changes in intake and plasma levels. However, there appears to be some variation among carotenoids in the magnitude of the plasma response to a given increase in intake. At the two extremes were a-carotene, for which a small increase in intake (14%) resulted in a relatively large rise in plasma levels (57%), and lycopene, for which a large increase in intake (443%) resulted in only a moderate rise in plasma levels (25%). Some of these differences may reflect variation in intestinal absorption due to the quantity of fat ingested with the particular fruits and vegetables (11) or according to whether the foods were cooked or raw (12). A greater serum response for a-carotene already has been observed in past studies (13, 14).

Although the fasting plasma ascorbic acid levels of our subjects significantly increased as the result of more than a doubling of their vitamin C intake, the correlation between changes in plasma level and intake was low ($r = 0.2$). Thus, plasma ascorbic acid would not be a sensitive marker of compliance to a diet abundant in vitamin C-rich foods.

This study suggests that plasma carotenoids may constitute good markers of compliance to a high fruit and vegetable diet at the group level. However, they may not be sufficiently responsive to change in intake to be used at the individual level, for example, to identify noncompliers and attempt corrective action. This is consistent with previous observations of a large interindividual variation in plasma response to $\beta$-carotene ingested as a dietary component or as an oral supplement (11, 13, 15–17). Thus, other compliance monitoring methods, such as unannounced 24-h diet recalls, need to be used to monitor adherence at the individual level in dietary modification trials.

To our knowledge, this is the first published study examining the response of plasma carotenoids or vitamin C to a sustained high intake of fruits and/or vegetables using a structured self-selected diet. Micozzi et al. (13) have reported the effect on plasma carotenoid levels of consuming 30, 12, or 6 mg of carotenoids from single foods (broccoli, carrots, or tomato juice) or from purified $\beta$-carotene for 6 weeks. The 30 men in their study were fed an identical controlled diet that included a constant quantity of fat (40% of calories) and carotenoids (≤1.6 mg/d). Compared with baseline, plasma $\beta$-carotene increased in men receiving 272 g of carrots/day, although much less so than in men receiving the same amount of $\beta$-carotene in purified form (13). Carrots also raised the plasma levels of a-carotene in these men, while plasma lutein increased in men receiving 300 g of broccoli/day (13). Plasma lycopene remained unchanged in men receiving 180 g of tomato juice/day despite a decreased intake of other important sources of lycopene (13). These results are consistent with ours.

The present study also provides information on the extent to which plasma carotenoids and ascorbic acid could be expected to rise among participants in a high fruit and vegetable intervention trial. However, one should note that raising the plasma levels of some of these nutrients would not be the sole purpose of such a trial since, as mentioned above, much greater plasma responses are produced by purified supplements than by foods (13, 16). The purpose of feeding large quantities of fruits and vegetables would be to raise plasma and tissue levels of a large number of putative cancer inhibitors not available in purified form that may provide a greater protection than a single or few nutrient(s) (4, 5). It also may constitute a more practical approach for the prevention of certain cancers at the population level.

The rationale for such a dietary approach is strengthened by recent human and animal data suggesting that daily supplementation with a single nutrient may result within a few weeks in a decrease in the plasma level of other nutrients (e.g., lutein or $\alpha$-tocopherol with a $\beta$-carotene supplement of 12 or 15 mg, and total carotenoids with an $\alpha$-tocopherol supplement of 800 international units) due to negative interactions among antioxidants (13, 18–20). The present study suggests that this may not occur with a comparable amount of carotenoids ingested as food components, since no decrease in plasma level was detected for the antioxidants ($\alpha$- and $\gamma$-tocopherol) that were not included in the intervention diet. This is consistent with the observation by Micozzi et al. (13) of a decrease in plasma lutein levels only in groups receiving purified $\beta$-carotene and not in groups receiving similar amounts of carotenoids from single foods.

The participants in our study were former cancer patients and, as such, were highly motivated. It is unclear whether such a major dietary change could be achieved in other high risk but less motivated groups (e.g., smokers) and whether plasma carotenoids would be as clearly responsive to a smaller increase in fruit and vegetable intake.

Acknowledgments

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### Table 4

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<tr>
<th>Food intake</th>
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<tr>
<td></td>
<td>$\beta$-carotene</td>
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<td>Dark green vegetables</td>
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<td>Total fruits and vegetables</td>
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### References


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