

Comparison of Serum Carotenoid Responses between Women Consuming Vegetable Juice and Women Consuming Raw or Cooked Vegetables¹

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Abstract

The objective of this study was to examine serum concentrations of α -carotene, β -carotene, lutein, lycopene, and β -cryptoxanthin due to consumption of vegetable juice versus raw or cooked vegetables. Subjects included female breast cancer patients who had undergone surgical resection and who were enrolled in a feasibility study for a trial examining the influence of diet on breast cancer recurrence. A high-vegetable, low-fat diet was the focus of the intervention, and some of the subjects were specifically encouraged to consume vegetable juice. At 12 months, blood samples were collected and analyzed for carotenoid concentrations via high-performance liquid chromatography methodology. Matched analysis and paired *t* test were conducted on two groups: those who consumed vegetable juice (the juice group) and those who consumed raw or cooked vegetables (no juice group). Serum concentrations of α -carotene and lutein were significantly higher in the vegetable juice group than in the raw or cooked vegetable group ($P < 0.05$ and $P = 0.05$, respectively). Paired *t* test analysis did not demonstrate a significant difference in serum values of β -carotene, lycopene, and β -cryptoxanthin between subjects consuming juice and those not consuming any juice. These results suggest that α -carotene and lutein appear to be more bioavailable in the juice form than in raw or cooked vegetables. Therefore, the food form consumed may contribute to the variability in serum carotenoid response to vegetable and fruit interventions in clinical studies.

Received 9/9/98; revised 1/4/99; accepted 1/17/99.

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¹ This research was supported in part by an award from the Walton Family Foundation.

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Introduction

The anticarcinogenic effects of various micronutrients and phytochemicals found in vegetables and fruits, such as carotenoids, have been demonstrated in laboratory studies (1). In addition to their possible biological role in cancer prevention, plasma carotenoids reflect fruit and vegetable consumption and appear to be reasonable biomarkers of vegetable and fruit intake (2–4). In several feeding studies, an increase in dietary intake of fruits and vegetables has been correlated with an increase in circulating plasma carotenoid concentrations (5, 6), although large interindividual variability in response is typically observed.

Plasma carotenoid response may be influenced by factors that affect the bioavailability of these compounds from foods. Absorption of carotenoids is dependent on the matrix of the food, presence of dietary fat, and various other factors (7–9). Consumption of tomato juice heated with oil was observed to increase serum lycopene concentration compared to unheated tomato juice (10). Mild heat treatment of carrots and pureeing or finely chopping vegetables also appears to enhance bioavailability of β -carotene (11–13). Components of vegetables and fruits, such as dietary fiber, may interfere with micelle formation and, therefore, reduce carotenoid response (14) and affect plasma carotenoid concentrations. Thus, differences in plasma carotenoid response to vegetable and fruit intake may be determined by the varying food forms in which the carotenoids are consumed (*i.e.*, vegetable juice versus raw or cooked vegetables).

The purpose of this study was to compare serum carotenoid response in a diet intervention study involving carotenoid-rich vegetables, in which carotenoids were consumed from vegetable juice and vegetables (raw or cooked). Identifying whether or not consumption of various forms of carotenoid-rich foods are associated with differing carotenoid response is relevant to the interpretation of plasma carotenoid concentrations as indicators of vegetable and fruit intake.

Materials and Methods

Subjects. This study was part of a larger project examining the feasibility of an epidemiological diet intervention trial to reduce the risk of breast cancer recurrence in women who had been diagnosed with primary breast cancer within the previous 4 years (the WHEL³ Study). Between May 1993 and October 1994, subjects were recruited from cancer registry lists and from community-based efforts. Inclusion criteria for the WHEL feasibility study and trial were: 18–70 years of age at time of diagnosis; a history of primary operable invasive breast carcinoma categorized as Stage I, Stage II, or Stage IIIA within the

³ The abbreviations used are: WHEL, Women's Healthy Eating and Living; BMI, body mass index; HPLC, high-performance liquid chromatography.

previous 4 years; treatment with total mastectomy and axillary dissection or breast-sparing surgical removal of cancer with clear macroscopic margins and axillary dissection, followed by adjuvant breast radiation; completion of any prescribed adjuvant chemotherapy; no evidence of recurrent disease or new breast cancer since completion of initial local treatment; good general health; geographical and telephone accessibility for participation and follow-up; and ability to communicate dietary data via 24-h food recall. WHEL Feasibility Study exclusion criteria were: current enrollment in another dietary clinical trial; diagnosis of a comorbidity requiring a specific diet or medication that contraindicated a high-fiber diet; estrogen replacement therapy; other primary or recurrent invasive cancer within the last 10 years; and inability to commit to the intervention schedule. In this study, we included all women ($n = 63$) for whom blood samples were available at the 12-month follow-up period.

The WHEL feasibility study involved an intervention in which one-half of the study participants were randomized to an intensive telephone counseling group that emphasized consumption of two 8-ounce portions of vegetable juice per day. Further details have been described previously (15). However, not all participants complied with the recommendations to drink the juices, and some of the nonintervention group consumed juices of their own volition. Accordingly, for this study, we ignored the study randomization and matched the participants on their self-reported dietary intakes. Dietary supplements were not a component of this trial, and participants were particularly discouraged from using high-dose micronutrient formulations that could interfere with the interpretation of the diet intervention results. Subjects in the matched analysis (described below) did not use β -carotene supplements or supplements of other carotenoids.

Participants provided fasting blood samples and other relevant study information at clinic visits. Weight and height were measured at enrollment and 12 months, and BMI [weight (kg)/height (m²); Ref. 16] was calculated. Procedures for this study were approved by the Human Subjects Committee of the University of California, San Diego School of Medicine.

Dietary Assessment. Dietary intake was assessed using trained telephone interviewers. Dietary assessment was based on four 24-h dietary recalls, collected on randomly selected days stratified for weekend *versus* weekdays over a 2-week period. Dietary data were collected and analyzed with the Nutrition Data System software (University of Minnesota, Minneapolis, MN) and nutrient analysis was conducted with the University of Minnesota Database (Version 2.8, 1995; University of Minnesota). Dietary intakes of carotenoids were also computed using the United States Department of Agriculture-National Cancer Institute carotenoid food composition database, which contains values for α - and β -carotene, β -cryptoxanthin, lycopene, and lutein plus zeaxanthin in >2240 fruits and vegetables and multi-ingredient foods containing fruits and vegetables (17).

Serum Measurements. Fasting blood samples were collected by venipuncture at 12 months postrandomization. Samples were protected from light throughout processing and handling. After collection by venipuncture, blood was allowed to clot and separated with refrigerated centrifugation at $2300 \times g$ at 4°C for 10 min. Samples were stored at -70°C until lipid extraction and HPLC analysis. Serum carotenoids were separated and quantified using the HPLC methods of Nierenberg *et al.* (18) and Peng *et al.* (19). With these methods, the peak designated lutein is assumed to also contain the isomerically related carotenoid, zeaxanthin. Accuracy was assessed by periodic anal-

ysis of National Institute of Standards and Technology Reference Material SRM 986: Fat-Soluble Vitamins, and both of the laboratories that provided the HPLC analyses for this study participated in the National Institute of Standards and Technology Micronutrients Measurement Quality Assurance Program. Determination of serum cholesterol was performed with the Kodak Ektachem Analyzer system (Johnson & Johnson, Rochester, NY; Ref. 20).

Statistical Analysis. Average daily dietary intakes of α -carotene, β -carotene, lutein, lycopene, and β -cryptoxanthin were calculated from the dietary recall data. On the basis of food content analysis data, total raw or cooked vegetable and vegetable juice intakes were calculated in servings in which one serving of raw or cooked vegetables was equivalent to 3 ounces of vegetable juice for the purpose of this study. The subjects were divided into two groups: those consuming juice (vegetable juice) and those replacing juice with vegetables (raw or cooked vegetables). Of the 63 subjects, the 30 with no vegetable juice intake comprised the "no juice" group. The 33 subjects with vegetable juice intake (2.6 ± 1.7 [Mean \pm SD] servings) made up the "juice" group.

Initially, we conducted a regression analysis on the entire group to examine the association between serum carotenoid concentrations and independent variables of interest (BMI, serum cholesterol concentration, vegetable, vegetable juice, and fruit intake). However, those consuming vegetable juice were also consuming a high amount of vegetables and the significant intercorrelation between the independent variables made the interpretation of the results very difficult. Therefore, to remove the joint effect of vegetable and vegetable juice consumption in the entire group, we separated the group on whether they consumed vegetable juice or vegetables only. To find comparable subjects for each carotenoid intake, the subjects not consuming any vegetable juice were matched with subjects consuming vegetable juice on total dietary intake for each carotenoid. Because dietary and serum carotenoid levels were highly skewed, all values were natural log transformed. Matching of the two groups was carried out by algorithm to minimize the squared difference for the carotenoid in log microgram units, with each subject being matched only one time for each carotenoid. However, subjects could be matched again for a different carotenoid. Before the matched analysis for each component was conducted, the no juice and juice groups were separately checked for outliers, as determined by residuals for the log serum measurement for the component in excess of 2.0 SDs from the fitted value for the component regressed on the log microgram of intake. The matched subjects for each carotenoid were compared for a successful match with a paired *t* test on log intake units, testing for no difference in dietary intake. Finally, the log serum concentration for the carotenoid was tested for a difference between the matched juice and no juice subjects with a paired *t* test. All statistical analysis was performed using the S-Plus system (Version 4.0, 1998; Seattle, WA).

Results

The ages of the matched participants ranged from 31 to 70 years. Self-reported ethnicity was 93% white, 4% Hispanic, and 3% Asian-American. Results from the regression analysis revealed that juice intake ($P = 0.0007$), vegetable intake ($P = 0.05$), fruit intake ($P = 0.03$), and BMI ($P = 0.008$) were significantly associated with α -carotene serum concentrations. No significant associations between the other serum carotenoid

Table 1 Dietary carotenoid consumption for juice vs. no juice subjects: median values and median ranges from matched analysis

Carotenoid	Vegetable juice ($\mu\text{g}/\text{day}$)		No juice (raw or cooked vegetables) ($\mu\text{g}/\text{day}$)		% difference between matched pairs		P^a
	Median	Range	Median	Range	Median	Range	
α -Carotene ($n = 9$)	647	12–6,207	716	12–6,215	0.3%	–1.5–0.0	0.55
β -Carotene ($n = 5$)	2,962	2,040–16,315	2,953	1,677–15,926	0.2%	0.0–2.6	0.24
Lutein ($n = 12$)	2,292	1,145–6,533	2,272	830–6,694	0.0%	–0.6–4.7	0.38
β -Cryptoxanthin ($n = 14$)	56	14–404	55	16–403	0.1%	–6.1–6.0	0.78
Lycopene ($n = 18$)	3,145	917–10,331	3,382	768–10,110	0.0%	–2.3–2.6	0.92

^a Significance of the difference between juice and no juice groups.

Table 2 Serum carotenoid concentrations ($\mu\text{mol}/\text{liter}$) between juice and no juice groups matched on dietary carotenoid consumption: median values and median ranges

Carotenoid	Vegetable juice ($\mu\text{mol}/\text{liter}$)		No juice (raw or cooked vegetables) ($\mu\text{mol}/\text{liter}$)		P^a
	Median	Range	Median	Range	
α -Carotene ($n = 9$)	0.46	0.10–1.6	0.15	0.07–0.29	<0.05
β -Carotene ($n = 5$)	0.68	0.19–2.9	0.98	0.25–5.3	0.74
Lutein ($n = 12$)	0.44	0.22–0.92	0.32	0.15–0.54	0.05
β -Cryptoxanthin ($n = 14$)	0.29	0.13–0.53	0.29	0.09–0.58	0.78
Lycopene ($n = 18$)	0.60	0.36–1.2	0.78	0.25–1.9	0.45

^a Significance of the difference between juice and no juice groups.

concentrations and vegetable or vegetable juice intake were present.

Matching subjects on dietary intake of α -carotene, β -carotene, lutein, β -cryptoxanthin, and lycopene allowed for appropriate comparisons of serum concentrations of carotenoids between juice and no juice subjects. Median values, ranges, and percentage median differences of dietary carotenoid consumption between the two matched groups (juice and no juice) are reported in Table 1. Matched analysis on dietary intake of each carotenoid between the juice group and no juice group produced 9 similar matches of intake for α -carotene, 5 similar matches for intake of β -carotene, 12 matches for lutein, 14 matches for β -cryptoxanthin, and 18 matches for lycopene. Median percentage difference between the matched pairs indicates the closeness of the matched analysis. Dietary carotenoid levels between the juice and no juice groups were not statistically significantly different, which allows for appropriate comparison of serum carotenoid concentrations between the two groups.

Table 2 reports median values of serum carotenoid concentrations for subjects consuming vegetable juice compared to raw or cooked vegetables (no juice group). These subjects were matched on dietary consumption of the respective carotenoid. Mean differences of serum concentration of α -carotene ($P < 0.05$) and lutein ($P = 0.05$) were significantly higher for the vegetable juice group compared to the no juice group. Mean differences of serum concentration of β -carotene, β -cryptoxanthin, and lycopene did not differ significantly between the two groups.

Discussion

In this study, serum concentrations of five carotenoids were examined in a study population consuming vegetable juice versus raw or cooked vegetables. The study population consisted of women at risk for breast cancer recurrence who were participating in a feasibility study for a trial examining the influence of diet on breast cancer recurrence. The daily dietary

prescription in the intervention group included consumption of two 8-ounce portions of vegetable juice and a diet high in vegetables, fruits, and fiber and low in fat. Because any combination of vegetables could be selected when preparing the vegetable juice, direct comparison of serum carotenoid concentrations between juice and no juice subjects could only be made by matching the two groups on dietary intake of the respective carotenoids. The matched analysis produced similar dietary consumption of carotenoids between the juice and no juice group. We found, for this study population, that women who were consuming vegetable juice had almost three times the serum concentration of α -carotene compared to those consuming the same amount of α -carotene from raw or cooked vegetables. We also found that women who were consuming vegetable juice had nearly 50% higher serum concentration of lutein compared to the women consuming equivalent amounts of raw or cooked vegetables. Serum concentrations for both lutein and α -carotene were significantly higher for the juice group compared to the no juice group. However, serum concentrations of β -carotene, β -cryptoxanthin, and lycopene were not statistically significantly different between the juice and no juice group.

The higher serum concentrations of α -carotene and lutein among the participants consuming juice may be attributed to several differences between the characteristics of vegetable juice compared to raw or cooked vegetables. The effect of particle size on bioavailability may influence carotenoid response. Pureeing vegetables results in smaller particle size and also mechanically disrupts the plant cells, so that the carotenoids are presumably more available in the intestinal lumen for absorption (12, 13). Dietary fiber, which is present in raw or cooked vegetables, may also limit bioavailability of carotenoids in humans and animals (14, 21, 22). Nutrient data from the United States Department of Agriculture reveal that raw apples contain almost four times the fiber contained in apple juice (0.77 and 0.21 g, respectively; Ref. 23). Mixed canned vegetables contain almost six times the fiber of a vegetable juice

cocktail (24). The inverse relationship between dietary fiber and carotenoid bioavailability may be due to the ability of soluble fiber to interfere with the formation of proper micelles (25).

Serum concentrations of β -carotene, β -cryptoxanthin, and lycopene were not significantly different between subjects who consumed vegetable juice and those who consumed cooked or raw vegetables. One limitation of this study is that the nature of the food choices and the diet intervention are likely to have influenced the ability to detect differences in responsiveness of these carotenoids. Early studies conducted by Erikson and Hoygaard (26) showed significantly greater absorption of carotene from cooked carrots and spinach than from raw carrots and spinach. Similar to the effects of β -carotene, heating tomato juice in the presence of oil was observed to slightly increase serum response (9). However, Zhou and colleagues (27) did not observe a difference in the tissue uptake of α -carotene and β -carotene from heated and nonheated carrot juice in ferrets. A very early study by Callison and Orent-Keiles (28) also reported no differences in availability of carotenoids based on heat processing. Another limitation in a comparison of this type is that levels of intake are based on the currently available carotenoid database which is of limited quality compared to other micronutrients (17).

β -Cryptoxanthin is more abundantly found in fruits than in vegetables (29). The women in this study were primarily consuming vegetable juice and very little, if any, fruit juice, which could explain the lack of difference in serum concentrations of β -cryptoxanthin between the juice and no juice group.

Serum concentrations of the carotenoids observed for this study population (both groups) were higher compared to those reported in previous descriptive studies and surveys of healthy subjects in the United States (30, 31). Subjects in the present study also had higher serum concentrations of β -carotene, α -carotene, and lycopene compared to those observed in women with breast cancer who were not enrolled in a dietary intervention study (32). Presumably, the high-vegetable diet prescribed for the participants may explain the higher concentrations of serum carotenoids for the subjects in this study compared to healthy subjects as well as women diagnosed with breast cancer in the general population.

Plasma concentrations of α -carotene, β -carotene, lutein, β -cryptoxanthin, and lycopene appear to be biomarkers of vegetable and fruit intake (4, 33). However, large interindividual variation in plasma response of these biomarkers and a low correlation between intakes and circulating plasma carotenoids are typically observed (34). Among subjects consuming identical doses of carotenoids, some individuals exhibit an increase in plasma concentrations, whereas others experience very little change (2). In a more recent study, intake of a high fruit and vegetable diet was significantly correlated with serum circulating carotenoids, although a wide range of serum response was observed (35). Variability in serum response of carotenoids could be explained by the food form consumed (vegetable juice *versus* raw or cooked vegetables). Here, subjects consumed similar amounts of the carotenoids in two different forms, vegetable juice and raw or cooked vegetables; however, identical doses of carotenoid intake were associated with differences in the serum carotenoid response. Although carotenoids are indicators of dietary consumption of fruits and vegetables, the matrix in which the fruits and vegetables are consumed should be considered in the interpretation of responsiveness of the carotenoids.

Results of this study suggest that consuming vegetable juice *versus* cooked or raw vegetables appears to increase

serum concentrations of lutein and α -carotene in a high-vegetable diet intervention study. Food form contributes to variability in serum response to vegetable and fruit intake in clinical studies. The importance of dietary carotenoids as reasonable indicators of fruit and vegetable intake and their potential role in disease prevention requires further investigation of factors influencing plasma response.

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Cancer Epidemiol Biomarkers Prev 1999;8:227-231.

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