

Increases in Plasma Carotenoid Concentrations in Response to a Major Dietary Change in the Women's Healthy Eating and Living Study

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Abstract

Background: Cohort studies suggest that higher circulating carotenoid concentrations through food sources may reduce breast cancer events. Other intervention studies have not achieved the level of change in circulating carotenoids required to properly test this hypothesis.

Methods: In a randomized trial of 2,922 breast cancer survivors, we examined blood and self-reported diet at baseline and 1 year. Intensive telephone counseling encouraged a plant-based diet in the intervention group. Diet was measured via 24-hour recalls, and a panel of plasma carotenoid concentrations was assessed at both time points. **Results:** The study intervention was associated with a 51% increase in total carotenoid concentration, from 2.272 ± 1.294 to 3.440 ± 2.320 $\mu\text{mol/L}$, achieved mainly by marked increases in targeted carotenoids: α -carotene, β -carotene, and lutein. For each of these targeted carotenoids, the proportion of the

intervention sample remaining below the cutpoint for the lowest baseline quartile decreased by one third to one half. After 1 year of study, half of the intervention group was in the highest baseline quartile. No change in distribution was observed in comparison group. Intervention participants achieved this change by both dietary pattern and vegetable juice consumption. Participants who chose to change dietary pattern without consuming significant quantities of vegetable juice achieved 75% of the level of change observed in other intervention participants.

Conclusions: Innovative telephone counseling intervention and dietary targets in the Women's Healthy Eating and Living study were associated with the level of change in circulating carotenoid concentration necessary to test the diet and breast cancer hypothesis suggested by cohort studies. (Cancer Epidemiol Biomarkers Prev 2006;15(10):1886–92)

Introduction

The Women's Healthy Eating and Living (WHEL) Study is a randomized trial designed to test the hypothesis that adopting a plant-based dietary pattern following diagnosis and treatment for early-stage breast cancer could delay or prevent further breast cancer events (1). As previous efforts have not identified a consistently strong effect from an individual nutrient or food on cancer risk or progression (2–8), this study will test whether the combined effect of consuming numerous weak anticarcinogenic phytochemicals may improve breast cancer-free survival. The study encouraged intervention group participants to make a major change in their dietary pattern to achieve a low-fat, high-fiber diet that emphasized carotenoid-rich vegetables.

Most studies of the relationship between nutritional factors and cancer risk measure dietary intake through self-report methods, which are prone to both random and systematic measurement error (9–12). The need for a biomarker of dietary

pattern was further emphasized when two separate cohort studies, the New York Women's Health Study [dietary data reported as part of a cohort pooling project (13) and biomarker data reported independently (14)], and the European Prospective Investigation into Cancer and Nutrition (15) reported different health outcome results depending on the dietary measure used. The best available biomarkers for vegetable and fruit intake are concentrations of major carotenoids, which are biologically active compounds that are pigments in plants but not synthesized in animals. Vegetable and fruit consumption provides the overwhelming source for carotenoids in the circulation and peripheral tissues, which reflect intake because uptake and tissue concentrations are not regulated (16).

Several studies have associated blood carotenoid concentration with reduced risk for cancer. Baseline serum concentrations of α - and β -carotene, and lutein, were inversely associated with incident breast cancer in the New York Women's Health Study (14), and we have reported that breast cancer survivors in the highest quartile of total plasma carotenoid concentration (examined an average of 2 years after diagnosis) were 43% less likely to have a second breast cancer over the next 5 years (16). These cohort studies suggest that encouraging a dietary pattern associated with increased circulating carotenoid concentrations should be among the goals of dietary interventions in breast cancer survivors. The WHEL Study had such goals and achieved the following relative changes (compared with comparison group) in self-reported dietary intake at 12 months: 60% increase in green

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vegetable servings, 75% increase in orange vegetable servings, a 50% increase in cruciferous vegetable consumption, 20% increase in consumption of fruit servings, along with a 16% reduction in energy from fat (17).

This report describes the baseline to 12-month changes in the distribution of plasma carotenoid concentrations in WHEL Study participants. The study intervention encouraged consumption of vegetable juice to maximize intake of carotenoid-rich vegetable nutrients without exceeding tolerances for volume and fiber. However, a number of participants chose to markedly increase their vegetable consumption with little or no added vegetable juice, and we examine how this dietary choice influenced carotenoid concentrations. Another potential source of carotenoids for study participants was dietary supplements. In the present analysis, we assess the relative contribution of different sources (e.g., food, juice, supplements) to 12-month plasma blood carotenoid concentrations observed in the study.

Materials and Methods

Study Design and Sample. The WHEL Study design has been described previously (1). Briefly, between 1995 and 2000, the study enrolled 3,088 women (85% Caucasian and equal distributions of African Americans, Hispanics, and Asians) ages 18 through 70 years who had been successfully treated for early-stage breast cancer [stages I (≥ 1 cm), II, or IIIA] that was diagnosed within the previous 4 years. Women were identified using letters of invitation from tumor registries, community outreach, and physician records. All potential participants completed a run-in period focused on compliance with study assessments before a stratified randomization to either an intensive dietary intervention or comparison group. Participants will be followed through the start of 2007, with a mean follow-up time of 8 years after enrollment.

The two study groups were highly comparable on cancer- and treatment-related variables, as well as on socioeconomic and estimated dietary intake variables (1). The primary measure of dietary intake was sets of repeated 24-hour recalls (four recalls within 3 weeks). Clinic visits at baseline and 1 year involved fasting blood collection and body mass index (BMI) calculation using measured height and weight. In this report, we consider all 2,922 participants who provided a baseline fasting blood sample and who had not had an additional breast cancer event in the first year on the study.

The Intervention. Using a personal trainer model, the study assigned each participant in the intervention group to a telephone counselor who contacted the participant by telephone on a preset schedule and completed a computer-assisted counseling interview. The telephone counseling protocol was developed specifically for the study and used motivational interviewing techniques (18), drawing on theories of how people make judgments (19) to self-regulate their behavior (20, 21). The intervention included three phases of varying intensity, and intervention group participants received an average of 18 one-on-one tailored-counseling calls during the first year. Counselors helped participants work toward a set of daily behavioral targets that included five vegetable servings from solid food and an additional four servings from juice (or equivalent vegetable servings), three fruit servings, 30 g fiber, and $<20\%$ energy from fat. In addition, intervention participants were invited to monthly cooking classes and then received a regular study newsletter containing both educational and motivational information on targeted foods. Details of this intensive intervention have been provided elsewhere (1, 22). The comparison group did not receive individualized dietary counseling, although they did receive educational print materials focused on national dietary guidelines—five servings of vegetables and fruits (U.S. Department of Agricul-

ture, National Cancer Institute, American Cancer Society). In addition, they were offered quarterly cooking classes and received bimonthly newsletters, both of which focused on topics other than vegetable consumption.

Assessment of Dietary Intake. Self-reported dietary intake was assessed using a set of four 24-hour dietary recalls over a 3-week period (1, 17) using a multipass software-driven recall protocol to maximize completeness of recalled intake (23). Participants were taught to estimate food portions accurately and to describe the specifics of the foods consumed. The Minnesota Nutritional Data System software was used to collect and estimate nutrient intakes, including dietary intake of carotenoids (Nutritional Data System version 4.01, 2001 University of Minnesota, Minneapolis, MN). Comprehensive information about dietary supplement use was also ascertained by the WHEL staff, as previously reported (1, 24). Although $\sim 80\%$ of participants reported taking at least one supplement, often this was a multivitamin or mineral, and fewer than 10% reported taking supplements that had significant concentrations of carotenoids. Supplement intake was neither encouraged nor discouraged in the study, and usage remained fairly stable over the study period.

Measurement of Plasma Carotenoids. Fasting blood samples were collected by venipuncture using a standardized protocol that included protection of the samples from light at clinic visits at baseline (at randomization), 12 months (range 11-18 months, mean 13 months), and at three subsequent time periods. Plasma and serum aliquots were stored at -80°C . For this analysis, we used a single plasma cryovial from both the baseline and 12-month stored samples for each participant and ensured that both samples were measured in the same laboratory batch run. Carotenoids were quantified using high-performance liquid chromatography with a Varian Star 9010, 9050 system as previously described (16, 25). This high-performance liquid chromatography assay separates and quantifies α -carotene, β -carotene, β -cryptoxanthin, lycopene, and lutein plus zeaxanthin. Lutein and zeaxanthin elute together with this method, so the variable lutein concentration includes zeaxanthin. Throughout this study, quality assurance procedures included the concurrent analysis of a pooled plasma reference sample, and the laboratory participates in the National Institute of Standards and Technology round robin quality assurance program to monitor precision and reliability of these carotenoid measurements. The mean day-to-day coefficient of variation was 5.9%, with individual coefficient of variations of 4% for β -carotene, 5.3% for α -carotene, 6% for lutein, 6.4% for lycopene, and 7.6% for β -cryptoxanthin. Total cholesterol was determined using the Kodak Ektachem Analyser kit (Johnson&Johnson, Rochester, NY; ref. 26) using reference materials from the manufacturer for quality assurance. The laboratory also participates in the American College of Pathologists quality assurance program to monitor precision and reliability for these lipid measures.

Statistical Analysis. We used an intent-to-treat analysis of change in plasma carotenoid concentration. If the 12-month blood sample was missing for an eligible participant, we first investigated whether a subsequent blood sample was available, which was true for 169 women. After confirming that the 12-month blood sample was the highest carotenoid concentration achieved for the intervention group, for those missing this sample, we imputed the 12-month concentration from the next available sample. However, post-baseline blood measurements were not available for 313 participants; for this group, we assumed no change and substituted the baseline carotenoid concentration. This "intent-to-treat" analysis should result in a more conservative estimate of the intervention group plasma concentrations at 12 months than one in which nonrespondents were excluded.

Plasma carotenoid data were log transformed to improve their fit to the Gaussian distributions assumed by standard statistical tests. Distributions for each carotenoid were plotted for the two time points. We also compared distribution shifts in the groups at 12 months by baseline quartile. For each individual carotenoid, ANOVA linear regression models were used to assess the association between 12-month concentrations and group status for each of the carotenoids and also for total carotenoid concentration (the sum of the individually measured carotenoids), after adjusting for possible confounders. Resampling methods (1,000 replicates) for missing data imputation (27) were applied to compute bootstrap SEs for regression coefficients.

To assess the relative contribution of different dietary sources (e.g., food, juice, supplements) to 12-month plasma carotenoid concentrations, we developed regression models for the 2,346 women who had complete baseline and 12-month data for plasma, smoking status, BMI, and diet. For total carotenoids and each individual carotenoid, we developed a basic regression model with the outcome variable as the log-transformed, 12-month plasma concentration. Covariates in this basic model included the log-transformed baseline plasma concentration of the carotenoid, and 12-month measurements of BMI, plasma-cholesterol concentration, smoking status, alcohol use (in tertiles), season when the blood was drawn, and clinical site. Race/ethnicity was not found to be a significant predictor of plasma carotenoid change and was omitted from the final models. We calculated the variance explained by this basic model (R^2). From this basic model, we ran a series of additional models: The food model included only estimated carotenoid intake from food; the juice model included only estimated carotenoid intake from juice; and the supplement model included only estimated carotenoid intake from supplements. We then ran models for each two-item combination (e.g., food + juice; food + supplement; juice + supplement). The full model included estimates of carotenoid intake from all three sources. We report relative increases in the explained variance to obtain an estimate of the importance of each source of carotenoids to the changes observed in this study.

To examine the level of carotenoid change that could be achieved if juice was not a major component of dietary change, we identified a separate subgroup of participants for each carotenoid analysis, using cutpoints that minimized consumption of the carotenoid from juice while maintaining a reasonable sample size for estimation purposes. We then examined the amount of change observed for these participants and noticed a bimodal pattern in which approximately half of the women had no change or a decrease from their baseline self-reported carotenoid intake and approximately half had a change of over 100%. We selected this latter subgroup to address the question. The cutpoints used and the sample size who reported a major increase in carotenoid intake were as follows: for α -carotene, <20% of intake from juice, 137 with major change; for β -carotene, <16% from juice, 133 with major change; for lutein, <16% from juice, 278 with major change; for lycopene and β -cryptoxanthin, no intake from juice, 154 and 413 with major change, respectively. Analyses were done using the R statistical package (Free Software Foundation GNU project, 2004) and SAS version 9.1 (Cary, NC, 2004).

Results

Change in Total Carotenoid Concentration. In the intervention group, the mean total carotenoid concentration increased from an average of 2.272 ± 1.294 $\mu\text{mol/L}$ at baseline to 3.440 ± 2.320 $\mu\text{mol/L}$ at 12 months, a 51% relative change (Table 1). Plasma cholesterol showed direct (coefficient = 0.273, F statistic = 61.2), and BMI inverse (coefficient = -0.0143, F statistic = 134.7), association with carotenoid

Table 1. Carotenoid concentrations in study groups at baseline and 12 months

Carotenoid	Intervention group, <i>n</i> = 1,445, mean (SD)	Comparison group, <i>n</i> = 1,477, mean (SD)
α -Carotene ($\mu\text{mol/L}$)		
Baseline	0.204 (0.230)	0.204 (0.213)
12 mo	0.597 (0.686)	0.203 (0.219)
β -Carotene ($\mu\text{mol/L}$)		
Baseline	0.865 (0.874)	0.914 (1.065)
12 mo	1.466 (1.416)	0.868 (0.937)
Lutein ($\mu\text{mol/L}$)		
Baseline	0.380 (0.200)	0.376 (0.204)
12 mo	0.459 (0.243)	0.381 (0.213)
Lycopene ($\mu\text{mol/L}$)		
Baseline	0.653 (0.345)	0.655 (0.344)
12 mo	0.739 (0.368)	0.650 (0.340)
β -Cryptoxanthin ($\mu\text{mol/L}$)		
Baseline	0.171 (0.155)	0.178 (0.175)
12 mo	0.179 (0.159)	0.177 (0.157)
Total carotenoids ($\mu\text{mol/L}$)		
Baseline	2.272 (1.294)	2.327 (1.470)
12 mo	3.440 (2.320)	2.279 (1.371)

NOTE: All 12-month (log-transformed) carotenoids (except β -cryptoxanthin) were significantly different between groups ($P < 0.0001$ for group effect) in linear models adjusted for age, clinical site, baseline carotenoid concentrations, BMI at 12 months, plasma cholesterol at 12 months, smoking status at 12 months, and alcohol usage at 12 months. There were no group differences in carotenoid concentrations at baseline.

concentrations, as expected. Although very few of the sample smoked (4.5%), smoking was nevertheless inversely associated with 12-month concentration. Supplements were not associated with changes in concentration as consumption levels did not change during the study, and thus are reflected in the baseline carotenoid concentration.

At 12 months, only 17% of intervention participants were still categorized below the cutpoint for the lowest baseline quartile, whereas half were above the cutpoint for the highest baseline quartile. In contrast, the comparison group experienced neither a change in the overall mean total carotenoid concentration nor a change in the proportion of participants who remained in each baseline quartile level at 12 months.

Changes in Individual Carotenoid Concentrations. Distributions of carotenoids did not differ between the intervention and comparison groups at baseline (Fig. 1A-D; Table 1), but major shifts were observed in the intervention group for α -carotene (mean: 0.204 $\mu\text{mol/L}$ base to 0.597 $\mu\text{mol/L}$ at 12 months; a 200% increase), and β -carotene (mean: 0.865 $\mu\text{mol/L}$ at base to 1.466 $\mu\text{mol/L}$ at 12 months; a 69% increase). Significant shifts were also observed in lutein (mean, 0.380-0.459 $\mu\text{mol/L}$; a 21% increase) and lycopene (0.653-0.739 $\mu\text{mol/L}$, a 13% increase). Univariately, there was no significant change in β -cryptoxanthin concentration in the intervention group, nor in any of the concentrations in the comparison group. In the multivariate model for the four individual carotenoids with univariate significance, the intervention group was highly significant ($P < 0.001$), with the following coefficients: 0.367 for α -carotene, 0.214 for β -carotene, 0.078 for lutein, and 0.063 for lycopene.

This intervention effect could also be seen in the amount of change observed in the quartile distributions of carotenoids at baseline. In each of the carotenoids, the comparison group maintained ~25% in each baseline quartile at 12 months. Major changes were observed in the intervention group for all carotenoids except β -cryptoxanthin. The proportion of participants in the lowest baseline quartile was reduced to 18% for lycopene, 17% for lutein, 16% for β -carotene, and 12% for α -carotene, whereas the proportion in the highest quartile increased to 34% for lycopene, 42% for lutein, 47% for β -carotene, and 59% for α -carotene.

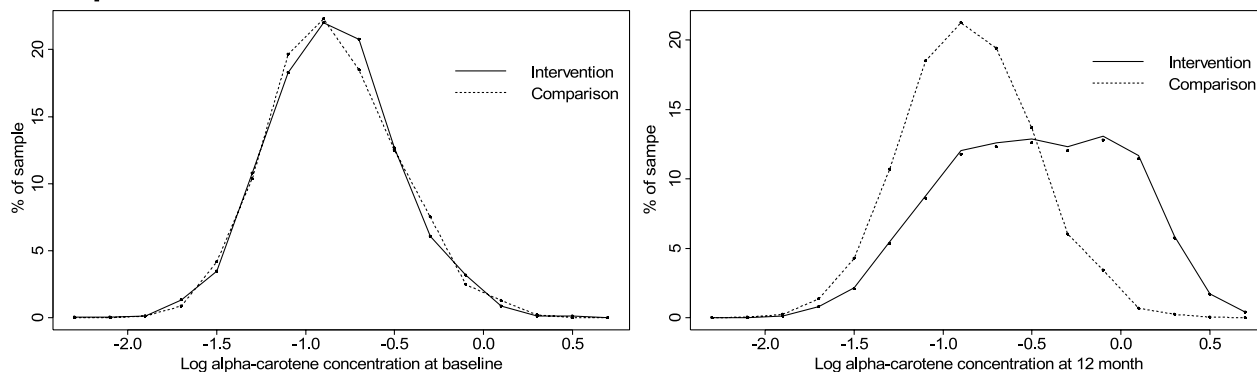
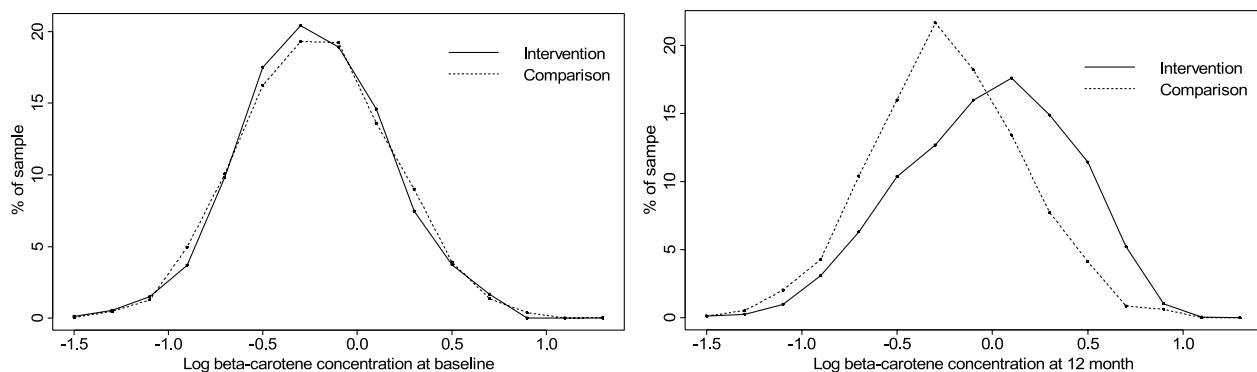
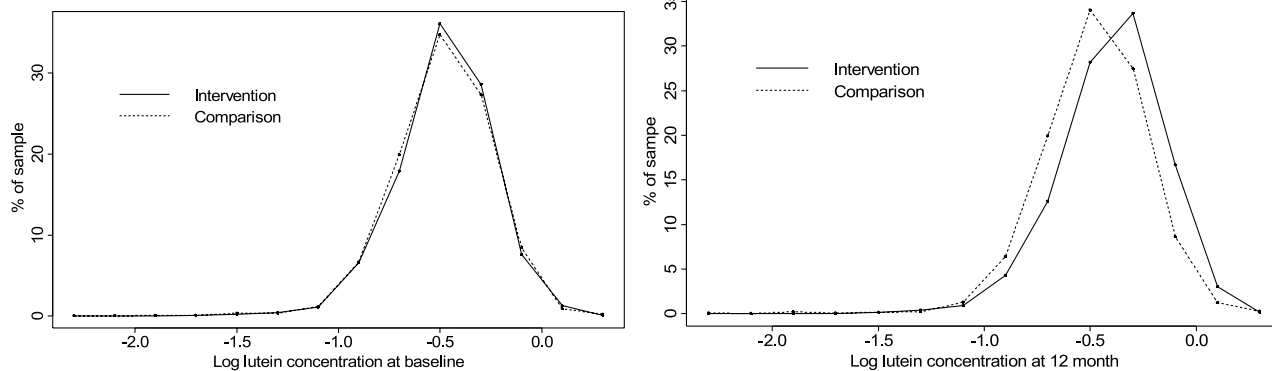
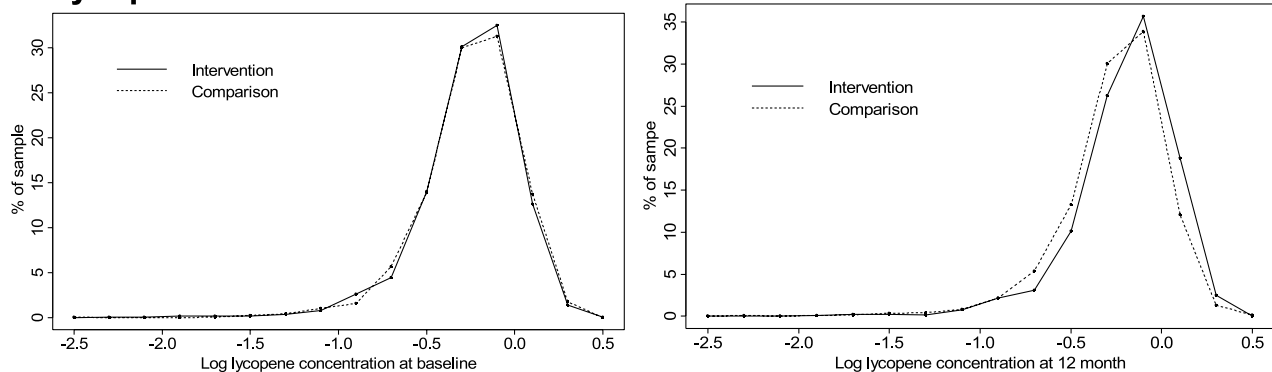
A alpha-carotene**B beta-carotene****C lutein****D lycopene**

Figure 1. Distributions of plasma carotenoid concentrations in logarithm-transformed micromoles per liter for α -carotene, β -carotene, lutein, and lycopene among WHEL Study intervention and comparison women at baseline and 12 months.

Relative Contributions of Food, Juice, and Supplements to Each 12-Month Carotenoid Concentration. The relative contributions of food, juice, and supplement consumption on each carotenoid concentration at 12 months can be assessed by studying changes in the explained variance (R^2) for a hierarchical set of models. The initial or base model predicts 12-month concentration from the baseline plasma concentration only, adjusted for covariates. The next added carotenoids from consumption of foods to the base model. A third model added only carotenoids consumed as supplements to the base model and the fourth model added only carotenoids consumed from juice sources to the base model. The final or full model includes carotenoids consumed from all three sources. Table 2 presents the variance explained (R^2) for each of these models for each of the four carotenoids that changed significantly with the intervention.

The full model for each of α -carotene, β -carotene, and lutein explained over 60% of the variance in the 12-month data with the model for lycopene explaining a lower 44% of the variance. Adding carotenoids from food sources increased the variance explained (over the base model) by 12% for α -carotene, 8% for β -carotene, 5% for lutein, and 4% for lycopene. Supplement usage did not increase the variance explained for any carotenoid. Consumption of carotenoid from juice, however, led to a major increase in the variance explained compared with the base model of 72% for α , 18% for β , 8% for lutein, and 9% for lycopene.

Plasma Carotenoid Changes for Participants Reporting Low Vegetable Juice Intake at 12 Months. There was a subsample of intervention participants who drank little or no vegetable juice, among whom the amount of self-reported change in carotenoid intake was bimodal, with one mode characterized by >100% dietary increase and the other mode characterized by no change. In the subsample that increased their carotenoids principally from foods, plasma concentrations of α -carotene increased by 100%, β -carotene increased by 53%, lutein increased by 34%, and lycopene increased by 13%. In this group, total carotenoids increased by 39% compared with the 51% achieved by the intervention group as a whole, which consumed an estimated daily average of 9 ounces of vegetable juice.

Discussion

Breast cancer survivors in the WHEL intervention group adopted a dietary pattern that significantly increased plasma

concentrations of carotenoids (17). The study intervention was associated with a major increase in the three carotenoids that have been associated with reducing risk for breast cancer events: α -carotene, β -carotene, and lutein (14). At 12 months, the intervention was associated with an approximate doubling of the proportion above the cutpoint for the highest baseline quartile group of carotenoid concentration. An intervention that minimizes the proportion of women below the cutpoint for the lowest baseline quartile of carotenoid concentration and maximizes those above the cutpoint for the highest quartile could potentially reduce additional breast cancer events, as suggested by our cohort study data (16).

Our results are in accordance with many other studies that have shown that blood carotenoid concentrations are responsive to self-reported dietary intake of carotenoids (25, 28-32). However, given the errors associated with self-reported dietary intake measuring instruments, lifestyle interventions may report substantial changes in self-reported dietary intake that are not reflected in biomarker change (33, 34). The WHEL Study achieved significant increases in carotenoid concentration at 12 months. This consistently large change in plasma carotenoid concentration was obtained by including vegetable juice as a major source of dietary carotenoids. However, large changes were also seen in those participants who chose to make major dietary changes without consuming much vegetable juice.

Our analysis defined the relative contribution of different dietary sources of carotenoids (vegetables and fruit consumed as foods, vegetable juices, or supplements) that were associated with increases in plasma carotenoid concentrations. To maximize plasma carotenoid concentrations, the WHEL Study intervention encouraged a carotenoid-rich diet that included vegetable juice in addition to at least five vegetable servings each day. The consumption of vegetable juice played the most significant role in increasing plasma concentrations of α -carotene, β -carotene, and lutein, but food sources also contributed significantly. There was no evidence to suggest that participants increased their supplement usage during the study as supplements had minimal influence on blood carotenoid concentrations over and above the baseline carotenoid concentration. The study intervention neither encouraged nor discouraged supplement usage and this lack of change may also reflect a secular trend against increasing consumption of β -carotene supplements following the negative health outcomes observed in studies examining high-dose β -carotene supplementation in heavy smokers (35, 36).

Table 2. Relative contributions of food, juice, and supplements to 12-month plasma carotenoids (n = 2,346)

Model	Variable	α -Carotene			β -Carotene			Lutein			Lycopene		
		β coefficient*	SE	R^2	β coefficient*	SE	R^2	β coefficient*	SE	R^2	β coefficient*	SE	R^2
Basic (B) [†]				0.361			0.549			0.552			0.395
Food + B	Food	0.166	0.013	0.406	0.248	0.016	0.593	0.144	0.011	0.581	0.040	0.006	0.412
Juice + B	Juice	0.087	0.002	0.620	0.046	0.002	0.649	0.021	0.001	0.594	0.019	0.002	0.432
Supp + B	Supplement				0.011	0.002	0.553	0.004	0.002	0.552			
Full	Baseline plasma	0.567 [‡]	0.019	0.628	0.635 [‡]	0.016	0.664	0.668 [‡]	0.015	0.606	0.511 [‡]	0.016	0.446
	Age	-0.001	0.001		0.000	0.001		0.000	0.000		-0.002 [‡]	0.001	
	Current smoker	-0.111 [‡]	0.031		-0.128 [‡]	0.027		-0.003	0.017		-0.033	0.023	
	Cholesterol	0.001 [‡]	0.000		0.001 [‡]	0.000		0.001 [‡]	0.000		0.001 [‡]	0.000	
	BMI	-0.012 [‡]	0.001		-0.012 [‡]	0.001		-0.005 [‡]	0.001		-0.004 [‡]	0.001	
	Juice	0.083 [‡]	0.002		0.011 [‡]	0.002		0.005 [§]	0.002		0.018 [‡]	0.001	
	Food	0.074 [‡]	0.010		0.135 [‡]	0.015		0.096 [‡]	0.012		0.034 [‡]	0.006	
	Supplement				0.040 [‡]	0.002		0.017 [‡]	0.001				

* β coefficients represent change in plasma carotenoids per unit change of independent variables. Food, juice, and supplement values were calculated as $\mu\text{g}/\text{d}$.

[†]Basic model includes baseline plasma carotenoid concentration, age, smoking status, 12-month plasma cholesterol, 12-month BMI, 12-month alcohol usage in tertiles, clinical site, season of the 12-month blood draw.

[‡] $P < 0.001$.

[§] $P < 0.05$.

The process of juicing breaks down the cellular matrix of foods, increasing carotenoid availability for absorption, which may help explain the marked influence of juice in increasing in carotenoid concentration observed in this study. It is important to compare the carotenoid increases achieved in this study with increases achieved with dietary β -carotene supplementation studies. Mayne et al. (37) reported on the effect of daily administration of one capsule containing 50 mg β -carotene in patients with a history of cancers of the oral cavity, pharynx, or larynx. The first difference of note between this study and the WHEL Study relates to baseline β -carotene concentrations. Participants in Mayne's study had a mean concentration of β -carotene of 0.2 $\mu\text{mol/L}$ compared with the 4-fold higher WHEL Study baseline mean of 0.8 $\mu\text{mol/L}$. However, at 12 months, high-dose supplementation was associated with a 10-fold increase in mean concentration to 2.7 $\mu\text{mol/L}$ in the head and neck cancer patients. The WHEL Study intervention increased mean β -carotene concentration to 1.5 $\mu\text{mol/L}$, which is half the concentration achieved in the high-dose supplement trials. Further, the WHEL intervention group at the 12-month time point had lower mean total carotenoid concentrations (3.44 versus 3.52 $\mu\text{mol/L}$) than women living in Ragusa and Naples in Southern Italy (38), suggesting that our intervention group reached carotenoid concentrations that are normal levels among populations that consume a diet rich in fruits and vegetables with little or no juice. Importantly, the WHEL Study was not focused solely on carotenoids but on nutrient-rich plant foods. Thus, participants in the WHEL Study likely achieved similarly high levels of changes in the intake of numerous other phytochemicals that have potential anticarcinogenic activity.

Although juice consumption strongly influenced the change in plasma carotenoid concentrations in this study, it does not seem to be essential; a number of participants doubled their carotenoid consumption from food sources with minimal additional juice. Although dietary counselors encouraged juice consumption and it was a behavioral target for intervention group participants, some participants preferred to increase their vegetable servings rather than drink juice. Even with minimal juice and supplement consumption, participants achieved significant increases in each of their carotenoid concentrations, changes that were considerably greater than those seen in the Polyp Prevention Trial (34). In addition, significant increases in plasma carotenoid concentrations were shown in our participants concurrent with reduced dietary fat intake, a secondary component of the WHEL intervention diet.

One issue to consider when interpreting carotenoid values from food and juice sources is the accuracy of the food content database for carotenoids. Although this database has improved (39, 40), the quality rating remains low for most foods. Several variables influence the actual amount of a carotenoid in a given food (e.g., genotype, plant maturity, climate, and other growing conditions), which results in substantial variability in the carotenoid content of the foods that participants report during their dietary assessment (41).

The dietary changes observed in the WHEL Study are significantly greater than have been reported in most dietary change studies among free-living individuals (42, 43) with the majority of the intervention group achieving a significant change in intakes and carotenoid concentrations. This was achieved with a novel behavior change intervention that used lay telephone counselors who were supervised in a central location (22). We had previously tested this intervention approach and showed success within a smoking cessation intervention (44, 45). The strategy focused on assisting participants when they needed to make important judgments (19) to optimize self-efficacy to maintain change (21, 46). Telephone dietary counselors completed an intensive training course that included motivational interviewing strategies (18),

which they used to encourage participants to focus on serial proximal goals to achieve long-term change (20). This counseling was reinforced by locally available registered dietitians who were involved in the initial phase of dietary counseling and monthly cooking classes; these dietitians were also available by telephone on an as-needed basis.

In their observational nested case-control study (270 breast cancer cases; 270 controls), Toniolo et al. (14) found an ~2-fold higher odds of breast cancer incidence in the lowest versus highest quartiles of serum concentration for α -carotene (odds ratio, 1.99; 95% confidence interval, 1.18-3.34), β -carotene (odds ratio, 2.21; 95% confidence interval, 1.29-3.79), lutein (odds ratio, 2.08; 95% confidence interval, 1.11-3.90), and total carotenoids (odds ratio, 2.31; 95% confidence interval, 1.35-3.96). The relative differences in mean serum concentrations between cases and controls in the Toniolo study were only 21% for serum α -carotene, 18% for β -carotene, 11% for lutein, and 11% for total carotenoids. Given that the WHEL Study achieved substantial increases in these biomarkers of dietary pattern, it is well positioned to assess the magnitude of the association between the recommended dietary pattern and risk of additional breast cancer events.

Appendix A. The Women's Healthy Eating and Living Study Group

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Increases in Plasma Carotenoid Concentrations in Response to a Major Dietary Change in the Women's Healthy Eating and Living Study

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