

Associations of Plasma Carotenoid Concentrations and Dietary Intake of Specific Carotenoids in Samples of Two Prospective Cohort Studies Using a New Carotenoid Database¹

**Dominique S. Michaud,² Edward L. Giovannucci,
Alberto Ascherio, Eric B. Rimm, Michelle R. Forman,
Laura Sampson, and Walter C. Willett**

Departments of Epidemiology [D. S. M., A. A., E. B. R., W. C. W.] and Nutrition [D. S. M., A. A., E. L. G., E. B. R., L. S., W. C. W.], Harvard School of Public Health, Boston, Massachusetts 02115; Channing Laboratory, Department of Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115 [D. S. M., E. L. G., E. B. R., W. C. W.]; and Division of Cancer Prevention and Control, National Cancer Institute, NIH, Bethesda, Maryland 20892 [M. R. F.]

Abstract

Diet-plasma carotenoid associations were examined in samples of women and men from each cohort in the Nurses' Health Study and the Health Professionals Follow-Up Study. In each sample, participants completed two self-administered food frequency questionnaires with at least a 1-year interval and provided a blood specimen preceding the second food frequency questionnaire. Carotenoid intakes were estimated from values for the five major carotenoids found in human plasma, specifically, α - and β -carotene, β -cryptoxanthin, lutein, and lycopene, using the United States Department of Agriculture-National Cancer Institute Carotenoid Database, as well as updated values for tomato products. Pearson correlation coefficients were calculated to compare diet-plasma correlations over time by sex after adjustment for recognized covariates. Among nonsmoking women ($n = 162$), the adjusted diet-plasma carotenoid associations were 0.48 for α -carotene, 0.27 for β -carotene and lutein, 0.32 for β -cryptoxanthin, and 0.21 for lycopene. Among nonsmoking men ($n = 110$), diet-plasma correlations were 0.47 for α -carotene and lycopene, 0.35 for β -carotene, 0.43 for β -cryptoxanthin, and 0.40 for lutein. Correlations between total fruit or vegetable intake and each plasma carotenoid level were not as high as any of the calculated carotenoid intake using the new database values. The correlations observed in this study indicate that the new carotenoid database provides valuable information on specific carotenoid intake and may be useful in epidemiological studies that attempt to account for associations between fruit or vegetable intake and disease.

Introduction

High intake of fruits and vegetables has been associated with decreased risk of various cancers and coronary heart disease (1-4). The beneficial effect of fruits and vegetables could be partly due to carotenoids, many of which are strong singlet oxygen quenchers or antioxidants (5) and, thus, might protect biological structures (including DNA) from oxidative damage and reduce lipid peroxidation.

Over 600 different types of carotenoids are found in nature. α -Carotene, β -carotene, lycopene, lutein, and β -cryptoxanthin are among the major plasma carotenoids (6). β -Carotene, which can function both as an antioxidant and as a provitamin A, has been the focus of many studies. However, in the beta-Carotene and Retinol Efficacy Trial and Alpha-Tocopherol, Beta-Carotene randomized trials, β -carotene supplementation had no beneficial effect and may have had an adverse effect on the incidence of lung cancer in smokers (7, 8). In the Physicians' Health Study, a randomized trial with a 12-year follow-up period, β -carotene supplementation did not influence incidence of total malignant neoplasms (9). The null results for β -carotene suggest that other carotenoids or phytochemicals account for the apparent benefits of fruit and vegetables in cancer prevention (10). In fact, lycopene is a considerably stronger scavenger of singlet oxygen than β -carotene (5) and is more highly concentrated in some tissues. Recent epidemiological studies have found inverse associations between plasma lycopene levels or lycopene intake and prostate cancer risk (11, 12).

Until recently, no comprehensive food composition database for individual carotenoids has been available. This precluded the assessment of the relation of specific carotenoid intakes to their levels in blood or to the risk of disease. The USDA-NCI³ carotenoid database recently developed by Chugh-Ahuja *et al.* (13) and Mangels *et al.* (14) includes over 2400 different fruits, vegetables, and multicomponent foods containing fruits and vegetables. Recent studies examining diet-plasma relationships have applied the new carotenoid database to Block's FFQ (15-18). Subjects in these previous studies were volunteers for a clinical nutrition study (15, 16), selective subsamples of various studies (17), or an elderly cohort (18).

Here, we examine the association between plasma concentrations of individual carotenoids and intakes, as assessed by our semiquantitative FFQ, administered to samples of 186 women in the Nurses' Health Study and 121 men in the HPFS. Prior to the availability of the USDA-NCI database, we had examined the association between intake of total carotenoids containing vitamin A activity with plasma concentrations of

Received 9/22/97; revised 12/19/97; accepted 1/8/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by NIH Grants CA-40356, CA-55075, and HL-35464.

² To whom requests for reprints should be addressed, at Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115.

³ The abbreviations used are: USDA, United States Department of Agriculture; NCI, National Cancer Institute; FFQ, food frequency questionnaire; HPFS, Health Professionals Follow-Up Study; BMI, body mass index; LDL, low-density lipoprotein.

Table 1 Intake and plasma concentration of carotenoids and characteristics of the study population

Variable	Men (n = 121)		Women (n = 186)	
	Mean	SD	Mean	SD
Age (yr)	55.4	10.5	52.7	7.2
BMI (kg/m ²)	24.9	2.9	24.3	4.9
Alcohol (g/day)	12.3	15.8	6.9	10.1
Current smokers (%)	9.0		13.0	
Plasma concentrations (mg/dl)				
α-Carotene	6.12	5.54	6.71	5.24
β-Carotene	24.67	15.58	30.87	20.14
β-Cryptoxanthin	13.29	6.54	12.26	6.27
Lutein	15.68	5.62	15.38	6.18
Lycopene	43.90	20.17	40.77	17.11
Cholesterol	205.05	36.72	214.24	36.50
Triglycerides	109.13	58.33	95.36	48.44
Intakes				
FFQ1 ^a (not energy-adjusted)				
Energy (kcal/day)	2,080	590	1,846	549
α-Carotene (mg/day)	1,011	1,019	919	712
β-Carotene (mg/day)	5,004	3,313	5,100	2,997
β-Cryptoxanthin (mg/day)	94	82	75	72
Lutein (mg/day)	3,784	2,382	4,438	3,674
Lycopene (mg/day)	11,119	6,221	11,270	7,212
FFQ2 (not energy-adjusted)				
Energy (kcal/day)	2,005	619	1,810	525
α-Carotene (mg/day)	910	771	884	675
β-Carotene (mg/day)	4,888	2,873	4,755	2,574
β-Cryptoxanthin (mg/day)	77	56	67	68
Lutein (mg/day)	3,803	2,038	3,984	2,690
Lycopene (mg/day)	10,497	6,177	10,405	7,278

^a FFQ1, FFQ 1 year before blood samples; FFQ2, FFQ completed after collection of blood samples.

α-carotene, β-carotene, lutein, and lycopene (19). The objectives of this study were to incorporate the new data from the USDA-NCI database and examine associations between plasma and dietary intake of α-carotene, β-carotene, lutein, lycopene, and β-cryptoxanthin within each cohort and across sex and to compare the specific diet-plasma carotenoid correlations with the fruit and vegetable intake-plasma carotenoid correlations.

Subjects and Methods

Study Population. The HPFS is a cohort of 51,529 United States male health professionals enrolled in a prospective study of dietary etiologies of heart disease and cancer (20). The Nurses' Health Study is a prospective study of 121,700 female registered nurses (21). Cohort members completed a mailed self-administered FFQ in 1986. During the following year, a random sample of cohort members living in the Boston area (323 men and 346 women) were invited to participate in a dietary validation study. Of these, 157 men and 197 women agreed to participate. Those participants were asked to collect two 1-week diet records over a one-year period, followed by a second FFQ. We excluded one man for having more than 70 items left blank and seven more for reporting a total energy intake outside the range of 800-4200 kcal in either of the two questionnaires. Similarly, we excluded women for having more than 70 items left blank or being outside the range of 600-3500 kcal. The participants provided blood samples before completing the second FFQ. One hundred twenty-one men and 186 women provided complete dietary information and blood samples. Participants in this study had similar means for caloric and

main nutrient intakes as their parent cohort (22).⁴ The mean intakes of the individual carotenoids in the entire HPFS cohort were similar to the mean intakes in the subset of men in this study [means (in mg/day): α-carotene, 942; β-carotene, 5,135; β-cryptoxanthin, 81; lutein, 3,837; and lycopene, 10,369, compared to Table 1 values]. For the women, the mean intakes of the individual carotenoids were slightly but consistently lower in the entire cohort than in the subset of women in this study [means (in mg/day): α-carotene, 768; β-carotene, 4,378; β-cryptoxanthin, 62; lutein, 3,540; and lycopene, 9,661, compared to Table 1 values].

Food Frequency Assessment. Both questionnaires (131-item questionnaire completed by men and 126-item questionnaire completed by women) are refined and expanded versions of a previously described FFQ (22-24). The two FFQs for the men contained 15 questions on fruit and juice intake and 31 questions on vegetable intake, and those for the women contained 21 questions on fruit and juice intake and 32 questions on vegetable intake. Participants were asked how often they consumed each food listed over the past year. Each item has a specified portion size and nine possible responses, which ranged from never or less than once per month to six or more times a week. An open-ended section for unlisted foods that the participant ate at least once per week was included. We computed nutrient intakes and total energy intake based primarily on food composition tables from the USDA publications (25) and dietary carotenoid intakes, including α-carotene, β-carotene,

⁴ L. Sampson, E. B. Rimm, G. Colditz, M. J. Stampfer, B. Rosner, and W. C. Willett, unpublished data.

tene, lycopene, lutein, and β -cryptoxanthin, using the new carotenoid database of more than 2400 fruits, vegetables, and selected multicomponent foods (13, 14). The carotenoid content of tomato-based food products was updated with values from the USDA, which were recently derived from reversed-phase high-performance liquid chromatography (26). Although "lutein" intake represents both lutein and zeaxanthin intake, the main contributors of lutein intake (including green vegetables, green fruits, winter squash, pumpkin, and carrots) contain essentially no zeaxanthin, and, therefore, lutein and zeaxanthin values for these foods represent primarily lutein (14). We calculated nutrient intakes by multiplying the frequency of use for each food by the nutrient composition of the portion size specified on the FFQ and summing across all foods to obtain a total nutrient intake for each individual.

Dietary Records Assessment. All substudy participants completed 2 weeks of diet records approximately 6 months apart during the year following the first FFQ. For this, each participant was given a dietetic scale for weighing portion sizes and was provided with detailed instructions for weighing and recording. In two previous studies, the dietary records from this subset of men were analyzed and compared to the FFQ (22, 23). In our previous study comparing the reporting of foods based on FFQ and diet records (23), 1565 unique diet-record food codes were matched to one or more of the FFQ items to make the daily food intake measurements from the diet records comparable with those from the FFQ. Here, we used the data obtained from the diet records in the food comparison study (23); however, such data were not available for the women because no similar study has been completed in this subset of women. Specific carotenoid intake values were not calculated using the USDA-NCI database for the dietary records because the main objective of this study was to determine the association between FFQ estimated dietary intake of carotenoids and plasma carotenoid concentrations. We included the diet records in the fruit and vegetable analysis for comparison purposes.

Other Data. Participants reported their age, smoking habits, height, and weight. BMI, used as a measure of obesity, was computed as weight (in kg) divided by height (in m) squared. This data were collected at baseline.

Laboratory Analyses. Blood specimens were collected in EDTA-treated tubes between 8:00 a.m. and 12:00 p.m. from nonfasting participants. The tubes were immediately covered with aluminum foil and stored in the dark on ice for up to 3 h until the plasma was separated. Plasma was stored at -70°C for periods up to 15 months until shipment on dry ice for laboratory analyses. Plasma carotenoids and retinol were measured by reversed-phase high-performance liquid chromatography at Hoffman-La Roche (Basel, Switzerland; Ref. 27). Reported coefficients of variation from this laboratory, for the carotenoids, ranged between 2.1 and 9.4% for same-day determinations and between 5.8 and 10% for day-to-day reproducibility. Lutein plasma levels reported here do not include zeaxanthin plasma levels. Plasma cholesterol and triglyceride concentrations were determined using Roche kits based on the methods of Richmond (28) and Bucolo and David (29), respectively.

Statistical Analyses. We logarithmically (natural) transformed all variables, except age, to improve normality before correlation and regression analyses. Because a few carotenoid values for dietary intake were zero and the logarithm of zero is undefined, a constant of 100 $\mu\text{g}/\text{day}$ was added to lycopene (for the nurses), and a constant of 10 $\mu\text{g}/\text{day}$ was added to β -cryptoxanthin, before logarithmic transformation. Dietary nutrient values were adjusted for total caloric intake to remove variation

due to energy intake and its associated measurement error (30). Energy-adjusted nutrients were calculated as the residuals after regressing each specific nutrient on total energy intake using linear regression (30, 31). An analogous procedure was used to adjust plasma concentrations of carotenoids for age, plasma cholesterol, plasma triglycerides, and BMI.

Pearson correlations were used to compare plasma levels of individual carotenoids with their respective dietary intake. Because the first FFQ measured diet in the preceding year, correlation coefficients relating the first questionnaire to the plasma concentrations may underestimate the validity of the questionnaire. However, the completion of two 7-day dietary records may have heightened dietary awareness and artificially increased the accuracy of the second FFQ (22). Therefore, we report correlations for both FFQs. The average of intake using the two FFQs was calculated to get a more precise estimate of long-term intake.

Multivariate regression models were fitted to predict the different plasma carotenoids using calculated carotenoid intakes. Most carotenoids are transported in LDL, and consequently, LDL variability contributes to extraneous variation of the plasma carotenoids. Because LDL was not available for this study, total cholesterol and triglycerides were added to the models to control for some of the plasma lipid variation.

Using foods that contribute at least 2% of the individual carotenoid intakes (using the distribution from each cohort), stepwise regressions were performed to find the best food predictors of individual plasma carotenoids. A food entered the model if it was individually predictive ($P \leq 0.15$) of the plasma level but stayed only when the P remained below 0.10 in the model with other food variables. Blood lipid levels were included in these models to account for variation in plasma carotenoids. All statistical procedures were carried out using SAS (SAS Institute, Cary, NC).

Results

Means and SDs of plasma concentrations and carotenoid intakes among men and women in the study are shown in Table 1. Only 9% of the men and 13% of the women were current smokers. Because plasma β -carotene in current smokers was significantly lower than that of past and nonsmokers (for more details, see Ref. 19), main analyses are reported for current nonsmokers only.

Intakes of specific carotenoids did not vary substantially between the FFQs for either of the two populations. In nonsmoking women, intraclass correlations for energy-adjusted carotenoids between the two FFQs were as follows: α -carotene, 0.68; β -carotene, 0.70; β -cryptoxanthin, 0.59; lycopene, 0.53; and lutein, 0.73. In nonsmoking men, the intraclass correlations were also high: α -carotene, 0.58; β -carotene, 0.57; β -cryptoxanthin, 0.63; lycopene, 0.54; and lutein, 0.47. Intraclass correlations were similar when the nutrients were not energy adjusted (data not shown).

Pearson correlations between dietary carotenoids and plasma concentrations are shown in Table 2 for FFQ1, FFQ2, and their average. Correlations ranged from 0.18 to 0.48 for nonsmoking women and from 0.25 to 0.50 for nonsmoking men (with energy-adjusted dietary values). Adjusting the plasma concentrations for age, BMI, and plasma lipids did not improve the correlations appreciably for most carotenoids. In the men, correlations were similar or slightly attenuated when crude dietary carotenoids were compared with the energy-adjusted values. In the women overall, correlations using the dietary energy-adjusted values were similar to the crude correlations.

Table 2 Pearson correlations between dietary carotenoids and plasma concentrations of carotenoids^a

	FFQ1		FFQ2		Average FFQ	
	Crude	Adjusted ^b	Crude	Adjusted ^b	Crude	Adjusted ^b
Nonsmoking women (n = 162)						
Crude plasma level						
α-Carotene	0.35	0.38	0.45	0.48	0.44	0.47
β-Carotene	0.18	0.20	0.28	0.31	0.24	0.27
β-Cryptoxanthin	0.37	0.36	0.41	0.39	0.43	0.41
Lycopene	0.19	0.19	0.19	0.19	0.22	0.22
Lutein	0.29	0.25	0.23	0.22	0.28	0.26
Adjusted ^c plasma level						
α-Carotene	0.38	0.40	0.44	0.47	0.45	0.48
β-Carotene	0.20	0.21	0.28	0.30	0.30	0.27
β-Cryptoxanthin	0.32	0.29	0.34	0.31	0.35	0.32
Lycopene	0.16	0.18	0.17	0.18	0.19	0.21
Lutein	0.29	0.27	0.22	0.23	0.27	0.27
Nonsmoking men (n = 110)						
Crude plasma level						
α-Carotene	0.36	0.36	0.50	0.50	0.50	0.48
β-Carotene	0.21	0.25	0.21	0.25	0.24	0.28
β-Cryptoxanthin	0.27	0.31	0.30	0.35	0.31	0.37
Lycopene	0.29	0.31	0.39	0.39	0.38	0.40
Lutein	0.28	0.28	0.31	0.33	0.34	0.36
Adjusted ^c plasma level						
α-Carotene	0.37	0.36	0.47	0.48	0.47	0.47
β-Carotene	0.28	0.30	0.29	0.31	0.32	0.35
β-Cryptoxanthin	0.35	0.39	0.35	0.40	0.39	0.43
Lycopene	0.33	0.35	0.46	0.46	0.45	0.47
Lutein	0.30	0.30	0.36	0.38	0.38	0.40

^a Correlations of 0.20 for men and 0.16 for women were significant at the 0.05 level. FFQ1 and FFQ2 were logarithmically transformed.

^b Adjusted for total energy intake.

^c Adjusted for age, BMI, plasma cholesterol, and plasma triglycerides.

With the exception of α-carotene, correlations were higher in the men than in the women.

With the exception of β-carotene, the specific carotenoid intakes predicted their respective plasma levels better than did other plasma carotenoid levels. For lycopene and β-cryptoxanthin, correlations between intake and their respective plasma levels were at least 2-fold larger than correlations between each of those intakes and the other carotenoid plasma levels (data not shown).

In both sexes, intake of each carotenoid was significantly predictive of its respective plasma carotenoid level, controlling for age, blood lipid levels, BMI, calorie intake, alcohol, and menopausal status and postmenopausal hormone use among women only ($P < 0.05$; Table 3). All plasma carotenoid levels among women were negatively associated with plasma triglycerides and positively associated with plasma cholesterol. Plasma cholesterol in men was also positively associated with plasma carotenoids, but plasma carotenoids were only weakly inversely associated with plasma triglycerides.

In multivariate analysis, premenopausal women had elevated carotenoid plasma levels compared to postmenopausal women not currently on postmenopausal hormone use. However, this association was only significant in the lycopene model (Table 3). Similarly, women who used postmenopausal hormones had higher carotenoid plasma levels compared to postmenopausal women not using exogenous hormones, but this difference was only statistically significant for lutein ($P < 0.05$).

Table 4 shows the results of the correlation analyses between total vegetable and fruit intake and plasma carotenoid levels, using the average fruit and vegetable intake of the two FFQs and the average of the two 1-week diet records. Plasma

carotenoid levels were more highly correlated with their corresponding estimates of carotenoid intake (Table 2) than with the sum of fruits and vegetables (Table 4). Plasma lycopene level was poorly correlated with fruit intake (correlation = -0.02 for women and men) because lycopene is found primarily in tomatoes and is only present in one commonly eaten fruit in the United States, namely, watermelon. All other correlations between plasma carotenoids and fruit or vegetable intake ranged between 0.12 and 0.43 for men and between -0.02 and 0.23 for women. Correlations between plasma carotenoids and total fruit and vegetable intake obtained from the diet records ranged between 0.17 and 0.47.

We used stepwise regression analysis to determine which foods are most predictive of the plasma carotenoid levels, using only those foods known to contribute at least 2% of each carotenoid (Table 5). Carrots were the most significant predictor of α-carotene plasma levels for both the men and the women and for each FFQ. Carrots were also the main predictor of β-carotene. Tomato sauce was the most significant predictor of lycopene plasma levels, for both the women and the men (FFQ1 and FFQ2). Tomato consumption was the second most important predictor of lycopene plasma levels ($P < 0.05$ in the men). Orange juice and oranges were the main predictors of β-cryptoxanthin in the men and women. Lettuce and spinach intake were the most consistent predictors of lutein plasma levels. Using diet records obtained from the men, food models for α-carotene and lycopene plasma levels were similar to those models using foods from the FFQs [carrots alone predicted α-carotene plasma (partial $r = 0.46$) and tomato sauce alone predicted lycopene plasma (partial $r = 0.35$)]. For β-carotene, β-cryptoxanthin, and lutein plasma, the foods from the diet records were more predictive of their respective carotenoid

Table 3 Predictors of plasma carotenoids ($\mu\text{g}/\text{dl}$) in multivariate analyses among nonsmokers^a

Independent variable	α -Carotene		β -Carotene		Lutein		Lycopene		β -Cryptoxanthin	
	Coefficient	SE	Coefficient	SE	Coefficient	SE	Coefficient	SE	Coefficient	SE
Women (n = 162)										
Intercept	-2.53	1.70	-0.28	1.54	0.73	0.96	1.31	1.27	0.31	1.07
Age (yr) ^b	0.15	0.12	0.15	0.10	0.11	0.07	0.03	0.08	0.17 ^c	0.08
Plasma cholesterol (mg/dl) ^d	0.15	0.16	0.42 ^c	0.14	0.16	0.09	0.31 ^c	0.11	0.13	0.11
Plasma triglycerides (mg/dl) ^d	-0.24 ^c	0.07	-0.33 ^f	0.06	-0.12 ^c	0.04	-0.04	0.05	-0.09 ^c	0.05
BMI (kg/m ²)	-0.04	0.19	-0.16	0.17	-0.16	0.11	0.10	0.13	-0.23	0.13
Alcohol intake (g/day)	-0.02	0.02	-0.01	0.02	0.01	0.01	0.02	0.01	-0.01	0.01
Energy intake (kcal/day)	-0.13	0.19	-0.10	0.16	0.06	0.10	-0.01	0.13	0.12	0.13
Premenopausal (1) ^g	0.10	0.18	0.10	0.15	0.04	0.10	0.27 ^c	0.12	0.04	0.12
Postmenopause on PMH ^h (1) ^g	0.25	0.19	0.12	0.16	0.22 ^c	0.11	0.16	0.12	0.23	0.13
Dubious menopause (1) ^g	-0.27	0.26	-0.20	0.22	-0.07	0.14	0.00	0.17	-0.41 ^c	0.17
α -Carotene intake ($\mu\text{g}/\text{day}$) ⁱ	0.63 ^e	0.09								
β -Carotene intake ($\mu\text{g}/\text{day}$) ⁱ			0.38 ^f	0.10						
Lutein intake ($\mu\text{g}/\text{day}$) ⁱ					0.14 ^c	0.05				
Lycopene intake ($\mu\text{g}/\text{day}$) ⁱ							0.14 ^c	0.07		
Cryptoxanthin intake ($\mu\text{g}/\text{day}$) ⁱ									0.19 ^c	0.05
Model R ²	0.33		0.33		0.20		0.16		0.31	
Men (n = 110)										
Intercept	3.66	2.60	5.01	2.32	2.10	1.37	1.35	1.71	5.28	1.80
Age (yr) ^b	-0.03	0.06	-0.08	0.06	-0.06	0.03	-0.23 ^f	0.04	-0.04	0.05
Plasma cholesterol (mg/dl) ^d	0.05	0.21	0.68 ^c	0.18	0.27 ^c	0.10	0.85 ^f	0.13	0.34 ^c	0.15
Plasma triglycerides (mg/dl) ^d	-0.03	0.07	-0.09	0.07	0.01	0.04	0.01	0.05	0.03	0.05
BMI (kg/m ²)	-1.32 ^c	0.58	-1.31 ^c	0.50	-0.55	0.30	-0.45	0.37	-1.02 ^c	0.41
Alcohol intake (g/day)	-0.03	0.05	-0.02	0.04	0.03	0.02	-0.01	0.03	0.00	0.03
Energy intake (kcal/day)	-0.04	0.22	-0.10	0.19	0.02	0.11	0.13	0.14	-0.15	0.16
α -Carotene intake ($\mu\text{g}/\text{day}$) ⁱ	0.40 ^f	0.07								
β -Carotene intake ($\mu\text{g}/\text{day}$) ⁱ			0.31 ^c	0.09						
Lutein intake ($\mu\text{g}/\text{day}$) ⁱ					0.27 ^f	0.06				
Lycopene intake ($\mu\text{g}/\text{day}$) ⁱ							0.31 ^f	0.06		
Cryptoxanthin intake ($\mu\text{g}/\text{day}$) ⁱ									0.32 ^f	0.07
Model R ²	0.31		0.25		0.22		0.50		0.23	

^a All variables are log-transformed, except age; intake from FFQ2.

^b 10-year increments.

^c $P < 0.05$.

^d 50-mg/dl increments.

^e $P < 0.002$.

^f $P < 0.0001$.

^g Reference category is postmenopausal women not on postmenopausal hormones.

^h PMH, postmenopausal hormones.

ⁱ Energy-adjusted intake from FFQ2.

plasma level than the models obtained using FFQ data, but the main foods predicting the plasma carotenoid levels were similar (carrots, lettuce, and cantaloupe predicted plasma β -carotene levels; orange juice, oranges, and peaches/apricots predicted plasma β -cryptoxanthin levels; and lettuce, broccoli, and peas/lima beans predicted plasma lutein levels).

Discussion

In a sample of the Nurses' Health Study and the HPFS, correlations between plasma levels and α -carotene, β -cryptoxanthin, lutein, and lycopene intake were similar or higher than comparable correlations reported in other studies (15–18, 32, 33). In this sample of men and women, lycopene, β -cryptoxanthin, lutein, and α -carotene intake values calculated from the new database were substantially better at predicting their respective plasma levels than total carotenoid vitamin A activity. In addition, lycopene and β -cryptoxanthin intake values were correlated very specifically to their respective plasma carotenoid levels.

Two previous studies, one using carotenoid precursors of vitamin A activity as a measure of dietary carotene intake (34) and one using the new USDA-NCI β -carotene values (16), had

higher correlations with plasma β -carotene than those observed here. In several other studies, the correlations between plasma β -carotene and dietary carotene (vitamin A activity) were lower than the correlations in this study (35–37). However, two of those did not adjust for factors that create extraneous variation in carotenoid plasma levels or dietary intake and did not restrict or adjust for smoking status (35, 36).

The second FFQ generally gave slightly higher correlations with plasma levels than the first FFQ, probably because the second FFQ covered the year during which the blood samples were given. Some of the correlations in the second FFQ may have been artificially raised as a result of heightened awareness of food intake due to the completion of diet records during that year.

The correlations and regression coefficients between plasma lutein, lycopene, and β -cryptoxanthin and their respective carotenoid intake were higher in men. The sex differences in correlations observed in this study are not due to differences in between-person variation in intake, which were similar and remain largely unexplained. However, hormonal changes (with menopause or menstrual cycle) may be partly responsible for plasma carotenoid variations if they affect absorption, trans-

Table 4 Correlations between total fruit, total vegetable, and both fruit and vegetable intake and plasma carotenoid level using the average of FFQ1 and FFQ2 for nonsmoking men and women and the average of two 1-week diet records for nonsmoking men^a

	Women (n = 162), FFQ	Men (n = 110), FFQ	Men (n = 110), diet records
Total fruit intake (servings/day)	2.55	2.91	2.33
vs. plasma α -carotene	0.13	0.26	0.35
vs. plasma β -carotene	0.20	0.26	0.37
vs. plasma β -cryptoxanthin	0.11	0.43	0.47
vs. plasma lycopene	-0.02	-0.02	0.18
vs. plasma lutein	0.05	0.21	0.26
Total vegetable intake (servings/day)	3.42	3.51	2.34
vs. plasma α -carotene	0.23	0.35	0.36
vs. plasma β -carotene	0.16	0.29	0.42
vs. plasma β -cryptoxanthin	-0.02	0.12	0.17
vs. plasma lycopene	0.17	0.26	0.20
vs. plasma lutein	0.16	0.30	0.33
Vegetable + fruit intake (servings/day)	5.98	6.43	4.67
vs. plasma α -carotene	0.22	0.40	0.42
vs. plasma β -carotene	0.18	0.35	0.46
vs. plasma β -cryptoxanthin	0.05	0.36	0.39
vs. plasma lycopene	0.11	0.16	0.22
vs. plasma lutein	0.13	0.33	0.35

^a Plasma carotenoid levels were adjusted for BMI, plasma lipids, and age. The second 1-week diet record, which was in close time proximity to the blood collection, did not have higher correlations than the first week of diet record over all carotenoids. Correlations of 0.20 for men and 0.16 for women were significant at the 0.05 level.

port, and/or metabolism of the carotenoids. Our data suggest that premenopausal women and postmenopausal women taking exogenous hormones have higher carotenoid plasma levels than do postmenopausal women not taking hormones, even controlling for intake. Cyclic fluctuations of plasma carotenoid concentration by menstrual cycle have been observed in subjects placed on a controlled diet (38). The heterogeneity of the women in our study, with respect to menopausal status (one-third premenopausal and two-thirds postmenopausal), may explain why the correlations for women were lower than those observed in the study by Yong *et al.* (16), in which the women were premenopausal and provided two blood samples.

Other studies have also observed sex differences in diet-plasma carotenoid relationships. In a controlled diet study, women and men who were fed high-fruit and -vegetable diets for two 15-day periods had different plasma carotenoid responses (39). In a free-living study, men and women were observed to have different diet-plasma carotenoid correlations (17).

Recent updates of the USDA-NCI database have significantly changed the values for lycopene. With these new values, the estimated mean dietary intake of lycopene in our participants was double that using the prior values, and correlations for lycopene were greater by 0.05.

Because FFQs are designed to be self-administered in 15–20 min, thousands of food items cannot possibly be represented individually, and consequently, food items must be grouped. Food items that have similar nutrient contents are often grouped together into food categories, and the nutrient composition of each category is calculated as an average of the food items present within that category. Most fruit and vegetable are represented individually on the FFQs used in this study, and although a few foods are grouped (*e.g.*, eggplant, zucchini, and summer squash), some contain two separate questions, depending on whether they are consumed cooked or raw (*e.g.*, spinach and carrots). FFQs have other limitations that result in some degree of measurement error; these include estimation of foods consumed over a 1-year period, calculation of serving sizes, and accuracy of database information (*e.g.*, nutrient contents of foods can vary over time). Yet, this study

demonstrates that the estimated carotenoid intake can still predict plasma levels, even when some measurement error is, unavoidably, present.

Low correlations between carotenoid plasma levels and dietary intake as measured by the FFQ could occur if important contributors of a carotenoid are missing from the FFQ. For example, the FFQ does not have a question on ketchup use. Lycopene concentration in ketchup (17.23 mg/100 g) is higher than that in spaghetti sauce (15.99 mg/100 g) and almost the same as that in tomato sauce (17.98 mg/100 g; Ref. 26). Although, in general, ketchup consumption may be relatively low, lycopene from ketchup may be highly bioavailable. It is also possible that recall of tomato sauce from complex dishes is poor. However, a recent study reported that the addition of more foods (including ketchup and tomato sauce in mixed dishes) to the standard Willett FFQ did not improve the estimation of intake for the main carotenoids (33). Given the high correlations for lycopene in men, the similar FFQ used and the similar mean lycopene intake for the men and the women, it is unlikely that the lower correlations in the women were due to missing food items.

Bioavailability is also an important determinant of dietary-plasma correlations because carotenoids from ingested food are not uniformly absorbed. Stahl and Sies (40) observed a large interindividual difference in lycopene absorption, with individual serum lycopene concentrations ranging from 80 to 350 nmol/liter (in five subjects) after the consumption of equal doses of lycopene. Bioavailability of specific carotenoids may also be influenced by concurrent dietary factors, particularly, vitamin E and other carotenoids. An intervention trial of α -tocopherol supplementation (800 units) had shown a moderate reduction in carotenoid levels (41).

Because many of the carotenoids have similar structures (42), supplementation of one type of carotenoid may negatively affect the absorption of other carotenoids through competition for similar absorption or transport mechanisms. A significant reduction in the concentration of plasma lycopene occurred in human subjects on β -carotene supplementation for 29 days (6 days with daily supplements of 100 mg of β -carotene, followed by 23 days of alternate-day supplementation; Ref. 43).

Table 5 Food predictors of plasma carotenoids using foods from each FFQ for nonsmoking men and women in a stepwise regression analysis^a

Plasma carotenoid	FFQ	Women (n = 162)			Men (n = 110)		
		Independent variable	Partial R	Model R ²	Independent variable	Partial R	Model R ²
α -Carotene	1	Carrots (raw)	0.32 ^b	0.10	Carrots	0.44 ^b	0.22
		Carrots (cooked)	0.13		Homemade soup	0.15	
β -Carotene	2	Carrots (cooked)	0.26 ^b	0.07	Carrots	0.53 ^c	0.28
		Yams	0.16	0.03	Carrots	0.27 ^d	0.10
β -Cryptoxanthin	2	Carrots (cooked)	0.18 ^c	0.07	Broccoli	0.16	
		Tomato sauce	0.19 ^d		Carrots	0.31 ^b	0.13
	1	Orange juice	0.28 ^b	0.14	Tomatoes	0.19 ^c	
		Peaches (canned)	0.17 ^c		Orange juice	0.29 ^d	0.17
2	Peaches (fresh)	0.18 ^c		Oranges	0.29 ^d		
	Orange juice	0.22 ^c	0.05	Orange juice	0.27 ^c	0.13	
Lycopene	1	Tomato sauce	0.20 ^d	0.04	Oranges	0.24 ^c	
		Tomato sauce	0.20 ^d	0.04	Tomato sauce	0.37 ^b	0.17
Lutein	1	Spinach (raw)	0.26 ^b	0.06	Tomatoes	0.17	
		Spinach (cooked)	0.16 ^c	0.06	Tomato sauce	0.37 ^b	0.20
	2	Zucchini	0.14		Tomatoes	0.25 ^d	
		Corn	0.13		Iceberg lettuce	0.22 ^c	0.09
				Spinach (cooked)	0.21 ^c		
				Zucchini	0.19 ^c	0.04	

^a Plasma carotenoid levels were adjusted for age, BMI, and plasma lipids. Foods stayed in the model if they met the 0.10 significant level.

^b $P < 0.001$.

^c $P < 0.05$.

^d $P < 0.005$.

Interindividual differences in plasma carotenoid levels may also be due to varying rates at which carotenoids are taken up into surrounding tissue. Although the major storage sites are the liver and adipose tissue, high concentrations of carotenoids are present in other tissues, such as the adrenal glands and the testes (44). Tissue-specific carotenoid levels vary substantially across individuals (45). The plasma contains only a small proportion of the total body carotenoid content and, thus, may not necessarily correlate well with carotenoid content in specific organs.

Calculated intakes of specific carotenoids were found to be better correlated in this study with specific plasma carotenoid levels than were intakes of total fruit and vegetables. In a cross-sectional study of free-living adults, correlations between several of the plasma carotenoids and total fruit and vegetable intake were higher than those observed in this study (lutein, 0.44; β -carotene, 0.45; and α -carotene, 0.61; Ref. 46). However, subjects were selected only if they had extremely low or high fruit and vegetable intakes, tending to inflate correlation coefficients. When using diet records in this study, correlations between fruits and vegetables and plasma carotenoid levels were only slightly higher than when using FFQs (0.10 more on average). Therefore, biological factors, such as absorption and day-to-day variation in blood levels, contribute substantially to the attenuation of diet-plasma correlations seen here and in other similar studies.

If specific carotenoids are causally related to disease, associations with disease risk should, therefore, be stronger with calculated intakes of these carotenoids than with overall fruit and vegetable intake. Furthermore, the new database values for lycopene, β -cryptoxanthin, lutein, and α -carotene intake are better predictors of their respective plasma levels than were other previously used measures of carotenoid intake. Therefore, future studies examining associations between fruit and vegetable intake and disease outcome should use the new carotenoid

database to examine whether carotenoids play a role in the etiology of the disease.

References

- Ziegler, R. G. Vegetables, fruits, and carotenoids and the risk of cancer. *Am. J. Clin. Nutr.*, 53 (Suppl.): 251s-259s, 1991.
- Steinmetz, K. A., and Potter, J. D. A review of vegetables, fruit, and cancer. I. Epidemiology. *Cancer Causes Control*, 2: 325-357, 1991.
- Khaw, K. T., and Barrett-Connor, E. Dietary fiber and reduced ischemic heart disease mortality rates in men and women: a 12-year prospective study. *Am. J. Epidemiol.*, 126: 1093-1102, 1987.
- Rimm, E. B., Ascherio, A., Giovannucci, E., Spiegelman, D., Stampfer, M. J., and Willett, W. C. Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. *J. Am. Med. Assoc.*, 275: 447-451, 1996.
- DiMascio, P., Kaiser, S., and Sie, H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.*, 274: 1-7, 1989.
- Parker, R. S. Carotenoids in human blood and tissues. *J. Nutr.*, 119: 101-104, 1989.
- Omenn, G. S., Goodman, G. E., Thornquist, M. D., Balmes, J., Cullen, M. R., Glass, A., Keogh, J. P., Meyskens, F. L., Valanis, B., Williams, J. H., Barnhart, S., and Hammar, S. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N. Engl. J. Med.*, 334: 1150-1155, 1996.
- The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and β -carotene on the incidence of lung cancer and other cancers in male smokers. *N. Engl. J. Med.*, 330: 1029-1035, 1994.
- Hennekens, C. H., Buring, J. E., Manson, J. E., Stampfer, M., Rosner, B., Cook, N. R., Belanger, C., LaMotte, F., Gaziano, J. M., Ridker, P. M., Willett, W. C., and Peto, R. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N. Engl. J. Med.*, 334: 1145-1149, 1996.
- Micozzi, M. S., Beecher, G. R., Taylor, P. R., and Khachik, F. Carotenoid analyses of selected raw and cooked foods associated with a lower risk of cancer. *J. Natl. Cancer Inst. (Bethesda)*, 82: 282-285, 1990.
- Comstock, G. W., Helzlsouer, K. J., and Bush, T. L. Prediagnostic serum levels of carotenoids and vitamin E as related to subsequent cancer in Washington County, Maryland. *Am. J. Clin. Nutr.*, 53 (Suppl.): 260s-264s, 1991.
- Giovannucci, E., Ascherio, A., Rimm, E. B., Stampfer, M. J., Colditz, A., and Willett, W. C. Intake of carotenoids and retinol in relation to risk of prostate cancer. *J. Natl. Cancer Inst. (Bethesda)*, 87: 1767-1776, 1995.

13. Chug-Ahuja, J. K., Holden, J. M., Forman, M. R., Mangels, A. R., Beecher, G. R., and Lanza, E. The development and application of a carotenoid database for fruits, vegetables, and selected multi-component foods. *J. Am. Diet. Assoc.*, *93*: 318-323, 1993.
14. Mangels, A. R., Holden, J. M., Beecher, G. R., Forman, M. R., and Lanza, E. Carotenoid content of fruits and vegetables: an evaluation of analytic data. *J. Am. Diet. Assoc.*, *93*: 284-296, 1993.
15. Forman, M. R., Lanza, E., Yong, L. C., Holden, J. M., Graubard, B. I., Beecher, G. R., Melitz, M., Brown, E. D., and Smith, J. C. The correlation between two dietary assessments of carotenoid intake and plasma carotenoid concentrations: application of a carotenoid food-composition database. *Am. J. Clin. Nutr.*, *58*: 519-524, 1993.
16. Yong, L. C., Forman, M. R., Beecher, G. R., Graubard, B. I., Campbell, W. S., Reichman, M. E., Taylor, P. R., Lanza, E., Holden, J. M., and Judd, J. T. Relationship between dietary intake and plasma concentrations of carotenoids in premenopausal women: application of the USDA-NCI carotenoid food-composition database. *Am. J. Clin. Nutr.*, *60*: 223-230, 1994.
17. Ritenbaugh, C., Peng, Y. M., Aickin, M., Graver, E., Branch, M., and Alberts, D. S. New carotenoid values for foods improve the relationship of food frequency questionnaire intake estimates to plasma values. *Cancer Epidemiol. Biomark. Prev.*, *5*: 907-912, 1996.
18. Vandenlangenberg, G. M., Brady, W. E., Nebeling, L. C., Block, G., Forman, M., Bowen, P. E., Stacewicz-Sapuntzakis, M., and Mares-Perlman, J. A. Influence of using different sources of carotenoid data in epidemiologic studies. *J. Am. Diet. Assoc.*, *96*: 1271-1275, 1996.
19. Ascherio, A., Stampfer, M. J., Colditz, G. A., Rimm, E. B., Litin, L., and Willett, W. C. Correlations of vitamin A and E intakes with the plasma concentrations of carotenoids and tocopherols among American men and women. *J. Nutr.*, *122*: 1792-1801, 1992.
20. Grobbee, D. E., Rimm, E. B., Giovannucci, E., Colditz, G. A., Stampfer, M. J., and Willett, W. C. Coffee, caffeine, and cardiovascular disease in men. *N. Engl. J. Med.*, *323*: 1026-1032, 1990.
21. Willett, W. C., Stampfer, M. J., Colditz, G. A., Rosner, B. A., Hennekens, C. H., and Speizer, F. E. Moderate alcohol consumption and the risk of breast cancer. *N. Engl. J. Med.*, *316*: 1174-1180, 1987.
22. Rimm, E. B., Giovannucci, E. L., Stampfer, M. J., Colditz, G. A., Litin, L. B., and Willett, W. C. Reproducibility and validity of an expanded self-administered semi-quantitative food frequency questionnaire among male health professionals. *Am. J. Epidemiol.*, *135*: 1114-1126, 1992.
23. Feskanich, D., Rimm, E. B., Giovannucci, E. L., Colditz, G. A., Stampfer, M. J., Litin, L. B., and Willett, W. C. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J. Am. Diet. Assoc.*, *93*: 790-796, 1993.
24. Willett, W. C., Sampson, L., Stampfer, M. J., Rosner, B., Bain, C., Witschi, J., Hennekens, C. H., and Speizer, F. E. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am. J. Epidemiol.*, *122*: 51-65, 1985.
25. Consumer and Food Economics Institute. *Composition of Foods: Handbook 8 Series, 1976-1992*. Washington, DC: United States Department of Agriculture.
26. Tonucci, L. H., Holden, J. M., Beecher, G. R., Khachik, F., Davis, C. S., and Mulokozi, G. Carotenoid content of thermally processed tomato-based food products. *J. Agric. Food Chem.*, *43*: 579-586, 1995.
27. Hess, D., Keller, H. E., Oberlin, B., Bonfanti, R., and Schuep, W. Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. *Int. J. Vitam. Nutr. Res.*, *61*: 232-238, 1991.
28. Richmond, W. Preparation and properties of a cholesterol oxidase from *Nocardia* sp. and its application to the enzymatic assay of total cholesterol in serum. *Clin. Chem.*, *19*: 1350-1356, 1973.
29. Bucolo, G. W., and David, H. Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.*, *19*: 476-482, 1973.
30. Willett, W. C., *Nutritional Epidemiology*, pp. 245-269. London: Oxford University Press, 1990.
31. Willett, W. C., and Stampfer, M. J. Total energy intake: implications for epidemiological analyses. *Am. J. Epidemiol.*, *124*: 17-24, 1986.
32. Coates, R. J., Eley, J. W., Block, G., Gunter, E. W., Sowell, A. L., Grossman, C., and Greenberg, R. S. An evaluation of a food frequency questionnaire for assessing dietary intake of specific carotenoids and vitamin E among low-income black women. *Am. J. Epidemiol.*, *134*: 658-671, 1991.
33. Enger, S. M., Longnecker, M. P., Shikany, J. M., Swenseid, M. E., Chen, M. J., Harper, J. M., and Haile, R. W. Questionnaire assessment of intake of specific carotenoids. *Cancer Epidemiol. Biomark. Prev.*, *4*: 201-205, 1995.
34. Stryker, W. S., Kaplan, L. A., Stein, E. A., Stampfer, M. J., Sober, A., and Willett, W. C. The relation of diet, cigarette smoking, and alcohol consumption to plasma β -carotene and α -tocopherol levels. *Am. J. Epidemiol.*, *127*: 283-296, 1988.
35. Rojdt, L., White, E., Goodman, G. E., Wahl, P. W., Omenn, G. S., Rollins, B., and Karkech, J. M. Association of food frequency questionnaire estimates of vitamin A intake with serum vitamin A levels. *Am. J. Epidemiol.*, *128*: 645-654, 1988.
36. Russell-Briefel, R., Bates, M. W., and Kuller, L. H. The relationship of plasma carotenoids to health and biochemical factors in middle-aged men. *Am. J. Epidemiol.*, *122*: 741-749, 1985.
37. Bolton-Smith, C., Casey, C. E., Gey, K. F., Smith, W. C. S., and Tunstall-Pedoe, H. Antioxidant vitamin intakes assessed using a food-frequency questionnaire: correlation with biochemical status in smokers and non-smokers. *Br. J. Nutr.*, *65*: 337-346, 1991.
38. Forman, M. R., Beecher, R. B., Muesing, R., Lanza, E., Olson, B., Campbell, W. S., McAdam, P., Raymond, E., Schulman, J. D., and Graubard, B. I. The fluctuation of plasma carotenoid concentrations by phase of the menstrual cycle: a controlled diet study. *Am. J. Clin. Nutr.*, *64*: 559-565, 1996.
39. Yeum, K. J., Booth, S. L., Sadowski, J. A., Liu, C., Tang, G., Krinsky, N. I., and Russell, R. M. Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. *Am. J. Clin. Nutr.*, *64*: 594-602, 1996.
40. Stahl, W., and Sies, H. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. *J. Nutr.*, *122*: 2161-2166, 1992.
41. Willett, W. C., Stampfer, M. J., Underwood, B. A., Taylor, J. O., and Hennekens, C. H. Vitamins A, E, and carotene: effects of supplementation on their plasma levels. *Am. J. Clin. Nutr.*, *38*: 559-566, 1983.
42. Olson, J. A., and Krinsky, N. I. Introduction. The colorful, fascinating world of the carotenoids: important physiologic modulators. *FASEB J.*, *9*: 1547-1550, 1995.
43. Gaziano, J. M., Johnson, E. J., Russell, R. M., Manson, J. E., Stampfer, M. J., Ridker, P. M., Frei, B., Hennekens, C. H., and Krinsky, N. I. Discrimination in absorption or transport of β -carotene isomers after oral supplementation with either all-*trans*- or 9-*cis*- β -carotene. *Am. J. Clin. Nutr.*, *61*: 1248-1252, 1995.
44. Olson, J. A. Absorption, transport, and metabolism of carotenoids in humans. *Pure Appl. Chem.*, *66*: 1011-1016, 1994.
45. Clinton, S. K., Emenhiser, C., Schwartz, S. J., Bostwick, D. G., Williams, A. W., Moore, B. J., and Erdman J. W., Jr. *cis-trans* lycopene isomers, carotenoids, and retinol in human prostate. *Cancer Epidemiol. Biomark. Prev.*, *5*: 823-833, 1996.
46. Campbell, D. R., Gross, M. D., Martini, M. C., Grandits, G. A., Slavin, J. L., and Potter, J. D. Plasma carotenoids as biomarkers of vegetable and fruit intake. *Cancer Epidemiol. Biomark. Prev.*, *3*: 493-500, 1994.