

Serum carotenoid concentrations predict lung function evolution in young adults: the Coronary Artery Risk Development in Young Adults (CARDIA) Study^{1–3}

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ABSTRACT

Background: A higher dietary intake of carotenoid-rich foods and higher circulating concentrations of carotenoids have been associated with better lung function in cross-sectional studies; however, the longitudinal association between carotenoids and lung function has shown conflicting results.

Objective: We examined the longitudinal association between serum carotenoids (β -cryptoxanthin, α -carotene, β -carotene, lutein/zeaxanthin, and lycopene) and the evolution of lung function.

Design: We evaluated our hypothesis in the Coronary Artery Risk Development in Young Adults (CARDIA) prospective cohort study. Spirometry testing was conducted at year 0 (1985–1986) and at follow-up in years 2, 5, 10, and 20; serum carotenoids were assayed at years 0 and 15, and diet was assessed at years 0 and 20.

Results: Year 0 sum of provitamin A carotenoids and β -cryptoxanthin concentrations were associated with maximum forced vital capacity (FVC) ($P \leq 0.01$) and forced expiratory volume in 1 s (FEV_1) ($P \leq 0.05$) (maximum across years 0–10) in linear regression models adjusted for age, race, height, study center, amount of physical activity, smoking status, and BMI. Year 0 lutein/zeaxanthin and lycopene were not associated with maximum lung function. Baseline concentrations of lutein/zeaxanthin, lycopene, sum of the 3 provitamin A carotenoids, β -carotene, and β -cryptoxanthin were each inversely associated with a decline from maximum FVC and FEV_1 ($P \leq 0.04$). The sum of provitamin A carotenoids and lycopene remained significant after adjustment for dietary intake related to serum carotenoids ($P \leq 0.03$). The 15-y change in provitamin A carotenoid and lutein/zeaxanthin concentrations was associated with a slower decline from maximum FVC and FEV_1 ($P \leq 0.04$).

Conclusion: These findings support an association between serum carotenoid concentrations and a decline in lung function. *Am J Clin Nutr* 2011;94:1211–8.

INTRODUCTION

Reduced lung function, even in asymptomatic and otherwise healthy adults, is associated with cardiovascular disease and overall mortality in the general population (1, 2). Increased oxidative stress (3) and inflammation (4, 5) have been negatively associated with lung function. Carotenoids are phytochemicals with antioxidant properties. Many cross-sectional epidemiologic studies have shown positive associations between higher lung function and higher intakes of carotenoid (6–10) and higher serum concentrations of carotenoids (11, 12). In contrast, only 2

studies have evaluated the longitudinal association between diets rich in fruit and vegetables and changes in lung function, with one study showing no association between increasing fresh fruit intake and lung function decline and the other study showing a significant association between increasing fresh fruit intake and reduced lung function decline (13, 14). One longitudinal study found that a higher serum β -carotene concentration was associated with smaller declines in lung function (15). A placebo-controlled clinical trial of carotenoid supplementation showed that increases in β -carotene and retinol concentrations were associated with improvements in lung function in current and former smokers (16). Thus, the role of carotenoids in the longitudinal decline in lung function needs to be further clarified.

In this study we evaluated the longitudinal association between baseline serum carotenoid concentrations and the evolution of lung function (both attainment of maximum lung function and subsequent decline from the maximum through year 20 of follow-up) in a large population-based study of young adults. We also evaluated the association between the 15-y change in serum carotenoid concentrations (year 15 – year 0) and the decline from maximum lung function to year 20. We hypothesized that higher serum carotenoid concentrations would be associated with higher maximum lung function and a slower rate of lung function decline.

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SUBJECTS AND METHODS

Participants and measurements

Data are from the US CARDIA⁴ Study, a multicenter cohort study. The CARDIA Study is reviewed annually by the institutional review boards at each participating institution, and participants sign a new informed consent form at every examination. Details of the methods and instruments used and of quality-control procedures were described previously (17). Briefly, in 1985–1986 (year 0), 5115 black and white men and women between the ages of 18 and 30 y were screened in the baseline examination. Fifty percent of the invited individuals contacted were examined (47% of blacks and 60% of whites) at baseline. The examination in 2005–2006 (year 20) included 3549 of the 4907 (73%) participants still alive at that time. We excluded from the analyses participants with prevalent or incident asthma during the study ($n = 718$), those who did not participate in or had missing lung function at year 20 ($n = 1498$), those with missing baseline carotenoid concentrations ($n = 176$), and those who were missing covariates at baseline ($n = 22$). After these exclusions, data for 2701 participants were available for the analyses. Participants included in this study were more likely to be white, to have higher education status, to be never smokers at baseline and at the year 20 exam, and to have higher maximum FVC and FEV₁. A detailed description of the differences in characteristics of the participants included in study and those excluded from the study is shown elsewhere (see Supplement Table 1 under “Supplemental data” in the online issue).

Physical activity was estimated by using an interviewer-administered questionnaire (18) concerning the frequency of participation in 13 different activities during the past 12 mo. Smoking status was categorized into 3 groups: never smokers, former smokers, and current smokers. Pack-years of smoking were calculated based on the duration and frequency of cigarettes smoked. Change in smoking status over the 20-y follow-up was also evaluated. Diet was assessed at years 0, 7, and 20 by using the interviewer-administered CARDIA Diet History questionnaire. Interviewers asked open-ended questions about dietary consumption in the past month within 100 food categories, referencing 1609 separate food items during years 0 and 7 and many more at year 20. The diet assessment methods were standardized across examinations, despite the increase in the number of food items reported, which was a reflection of the changing food supply. In addition, fast food restaurant use (number of visits/wk) was queried at each examination, and eating breakfast (d/wk) was queried at years 7 and 20. Foods were classified into 166 food groups according to the Nutrient Data System (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN) and summed on the basis of serving sizes (19). Food group intake was in servings per day of constituent foods. We excluded 6 infant product food groups (not relevant), unsweetened water (collected at year 20 only), and nongrain flour (rarely consumed). We further collapsed these 158 food groups into 46, which in turn were based on considerations of similar

nutrient characteristics, hypothesized biologic effects, and comparability with food groups defined in previous studies (20).

Lung function was measured by using a Collins Survey 8-L water-sealed spirometer and an Eagle II Microprocessor (Warren E Collins Inc) at the year 0, 2, 5, and 10 exams. At year 20, a dry rolling-seal OMI spirometer was used (Viasys Corp). Standard quality-control and testing procedures (21–23) were followed at all examinations. A detailed description of the testing procedures used during different times was described previously (24).

Overnight fasting blood samples were collected, processed within 90 min of blood collection, and stored at -70°C . Sera obtained at years 0 and 15 were assayed by the Young Adult Longitudinal Trends in Antioxidants ancillary study for the carotenoids α - and β -carotene, lycopene, lutein/zeaxanthin, and β -cryptoxanthin (Molecular Epidemiology and Biomarker Research Laboratory, University of Minnesota). The HPLC-based assay of carotenoids was described previously (25, 26). The CVs were $<10\%$ for all analytes and control pools.

A flow chart detailing the measurement of all demographic and lifestyle variables, lung function, diet history, and serum carotenoids at various time points in the CARDIA Study is shown elsewhere (see Supplement Figure 1 under “Supplemental data” in the online issue).

Statistical analysis

We used linear regression to evaluate the associations between year 0 individual concentrations for the 5 carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lutein/zeaxanthin, and lycopene), the sum of year 0 provitamin A carotenoids (α -carotene, β -carotene, and β -cryptoxanthin), and the maximum FVC, FEV₁, or FEV₁/FVC observed over the first 10 y of follow-up (maximum observed lung function). Because age and height have sex-specific effects on lung function and because the concentrations of individual carotenoids were different between men and women, we initially evaluated the association between serum carotenoids and lung function stratified by sex. Because no sex-specific interactions were observed for the individual carotenoids, we performed meta-analytic pooling of the sex-stratified models and presented the overall association between serum carotenoids and lung function. We specifically examined the relation between year 0 serum carotenoid concentrations and maximum observed lung function, and the decline from maximum observed lung function, calculated as the maximum minus the year 20 lung function. A negative regression coefficient for the decline from maximum observed lung function indicates less of a decline per unit increase in the year 0 serum carotenoid concentration. In addition to year 0 serum carotenoid concentrations, we also examined the relation between the 15-y change (year 15 – year 0) in serum carotenoids and the decline from maximum observed lung function. Regression models were adjusted for potential confounders, including age, age², race, study center, height, height², BMI, amount of self-reported physical activity, and smoking status (never, former, or current), all at year 0. We included age² and height² because age and height were nonlinearly related to lung function. Further adjustment for educational status, the 15-y change in BMI, or physical activity did not significantly change the results; therefore, these terms were not included in the final model. Finally, we examined whether the association between serum carotenoids and lung

⁴ Abbreviations used: CARDIA, Coronary Artery Risk Development in Young Adults; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

function was affected by adjustment for a dietary score, which was described previously (27). For the provitamin A carotenoids and lutein/zeaxanthin, we included carotenoid-rich fruit and vegetables (CARDIA food groups: fruit, fruit juices, green vegetables, and yellow vegetables), red and processed meat, beer, and sugar-sweetened soda groups based on empirical regression findings of foods that plausibly affect oxidative stress. For lycopene, we included tomato-based sauces, salsas, and pastes (excluding catsup, which was not separately available for analysis) and red and processed meat. We examined maximum lung function across 4 examinations because lung function decline from a single time point (eg, year 0 – year 20) would be confounded by ongoing lung maturation during the study. Nevertheless, the analyses of maximum lung function are complicated because of maximum lung function being achieved at different time points. We therefore also presented the results of sensitivity analyses that evaluated the cross-sectional asso-

ciation between year 0 carotenoids and year 0 lung function and the 20-y decline in lung function (year 0 – year 20) (see Supplement 1 under “Supplemental data” in the online issue). All analyses were conducted in SAS, version 9.1 (SAS Institute).

RESULTS

Participant characteristics

At year 0, participants in the highest quartile of the sum of the 5 serum carotenoids were more likely to be white, to have higher educational attainment, and to have lower BMI and greater physical activity ($P = 0.09$) and less likely to be current smokers (Table 1). The smoking status over 20 y was constant for 86% of the participants. Although the sum of all 5 serum carotenoids was not significantly different between men and women, all the provitamin A carotenoids (β -cryptoxanthin, α -carotene, and

TABLE 1

Clinical characteristics according to quartiles of year 0 sum of 5 serum carotenoid concentrations in the CARDIA Study ($n = 2701$)¹

Characteristic	Year 0 serum carotenoid concentration ($\mu\text{mol/L}$) ²				<i>P</i>
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Total no. of subjects	675	676	675	675	
Race (% white)	50	55	55	60	0.005
Sex (% female)	55	53	56	59	0.24
Year 0 education (% $\leq$ high school education)	45	33	31	25	<0.0001
Age, year 0 (y)	24.6 $\pm$ 3.7 ³	25.0 $\pm$ 3.6	25.2 $\pm$ 3.6	25.5 $\pm$ 3.4	0.0002
Age at maximum FVC (y)	28.7 $\pm$ 4.7	29.3 $\pm$ 4.8	29.4 $\pm$ 4.6	29.9 $\pm$ 4.6	<0.0001
Age at maximum FEV ₁ (y)	27.3 $\pm$ 4.3	27.8 $\pm$ 4.5	27.6 $\pm$ 4.2	28.3 $\pm$ 4.3	0.001
BMI, year 0 (kg/m^2) ³	25.1 $\pm$ 5.4	24.2 $\pm$ 4.5	24.2 $\pm$ 4.5	23.5 $\pm$ 4.1	<0.0001
Maximum FVC	4323 $\pm$ 1038	4417 $\pm$ 1020	4349 $\pm$ 1024	4402 $\pm$ 1015	0.61
Maximum FEV ₁	3573 $\pm$ 793	3663 $\pm$ 775	3610 $\pm$ 881	3649 $\pm$ 778	0.58
Physical activity, year 0 (exercise units)	405 $\pm$ 295	412 $\pm$ 290	423 $\pm$ 309	443 $\pm$ 296	0.09
Diet score, year 0 (sum of servings/d of available food groups)	0.34 $\pm$ 5.18	1.32 $\pm$ 5.27	2.12 $\pm$ 5.42	4.55 $\pm$ 6.05	<0.0001
Sum of all 5 carotenoids, year 15 ($\mu\text{mol/L}$)	82.64 $\pm$ 33.16	95.07 $\pm$ 32.78	109.25 $\pm$ 37.87	134.70 $\pm$ 48.51	<0.0001
Smoking status, year 0 (%)					<0.0001
Never smokers	54	61	64	66	
Former smokers	11	12	13	18	
Current smokers	35	27	23	16	
Smoking status, year 20 (%)					<0.0001
Never smokers	55	63	64	68	
Former smokers	17	20	18	19	
Current smokers	28	17	16	13	
Smoking change, year 20 – year 0 (%)					0.14
Unchanged	87	86	88	89	
Quit smoking	9	11	8	6	
Started smoking	4	3	4	5	
Visit at which maximum FVC recorded (%)					0.48
Year 0	30	32	29	27	
Year 2	18	15	19	17	
Year 5	29	27	28	29	
Year 10	23	26	24	27	
Visit at which maximum FEV ₁ recorded (%)					0.04
Year 0	42	42	45	40	
Year 2	24	22	22	21	
Year 5	25	26	27	30	
Year 10	9	10	6	9	

¹ CARDIA, Coronary Artery Risk Development in Young Adults; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

² Serum carotenoids include the 3 provitamin A carotenoids (α -carotene, β -carotene, and β -cryptoxanthin), lutein/zeaxanthin, and lycopene. Quartile cutoffs: 1, 9.96–55.71 $\mu\text{mol/L}$; 2, 55.72–72.93 $\mu\text{mol/L}$; 3, 72.94–93.78 $\mu\text{mol/L}$; and 4, 93.79–326.81 $\mu\text{mol/L}$.

³ Mean $\pm$ SD (all such values).

β -carotene) were significantly lower in men than in women [8.38 compared with 8.96 ($P = 0.008$), 2.44 compared with 3.35 ($P < 0.0001$), and 13.86 compared with 17.76 ($P < 0.0001$), respectively]. Serum lutein/zeaxanthin concentrations were similar between men and women ($P = 0.19$), and serum lycopene concentrations were significantly higher in men than in women (32.02 compared with 28.90; $P < 0.0001$). The diet score for the 3 provitamin A carotenoids and lutein/zeaxanthin at year 0 showed significant correlations with the year 0 sum of provitamin A carotenoids ($r = 0.48$), α -carotene ($r = 0.48$), β -carotene ($r = 0.41$), lutein/zeaxanthin ($r = 0.26$), β -cryptoxanthin ($r = 0.39$), and the sum of all 5 carotenoids ($r = 0.32$) (all P values < 0.0001). The diet score for lycopene was positively correlated with serum lycopene ($r = 0.17$, $P < 0.0001$). The correlation between individual year 0 and year 15 carotenoid concentrations ranged from 0.32 to 0.52 ($P < 0.0001$). CARDIA participants achieved maximum lung function at different study visits during the first 10 y (Table 1); in $\sim 25\%$ of the sample, maximum FVC was not achieved until year 10; however, the year 10 value was the highest value achieved during CARDIA, because only a small number of participants were observed to have an even higher value at year 20, and then by < 100 mL

in almost all cases. The mean ($\pm$ SD) age at peak FVC was $29.3 \text{ y} \pm 4.7 \text{ y}$, and the average age at peak FEV₁ was $27.3 \pm 4.3 \text{ y}$.

Year 0 serum carotenoid concentrations were positively associated with maximum lung function

No significant interactions were found between sex and year 0 serum carotenoids in determining maximum observed FVC, FEV₁, or FEV₁/FVC ($P \geq 0.26$). The sex-stratified analyses are presented elsewhere (see Supplement Table 2 under "Supplemental data" in the online issue). Year 0 sum of all provitamin A carotenoids, β -cryptoxanthin and α -carotene concentrations were associated with maximum observed FVC without adjustment for the year 0 diet score ($P = 0.01$, $P = 0.005$, and $P = 0.03$, respectively) (Table 2, model 1). However, none of these associations remained significant after adjustment for the year 0 diet score (Table 2, model 2). Similarly, the year 0 sum of all provitamin A carotenoids and β -cryptoxanthin were associated with the maximum observed FEV₁ before adjustment for the year 0 diet score ($P = 0.04$ and $P = 0.05$, respectively) (Table 2, model 1) but not after adjustment for the year 0 diet score (Table

TABLE 2

Slope (95% CI) of maximum observed lung function and decline from maximum observed lung function (peak lung function – year 20) on year 0 serum carotenoid concentrations for 2 models¹

		FVC				FEV ₁			
		Model 1 ³ (<i>n</i> = 2701)		Model 2 ⁴ (<i>n</i> = 2647)		Model 1 ³ (<i>n</i> = 2701)		Model 2 ⁴ (<i>n</i> = 2647)	
	Year 0 carotenoid concentration ²	Slope (95% CI)	<i>P</i>	Slope (95% CI)	<i>P</i>	Slope (95% CI)	<i>P</i>	Slope (95% CI)	<i>P</i>
<i>μmol/L</i>									
Maximum observed lung function									
Sum of provitamin A carotenoids ⁵	27.67 ± 20.39	24 (4, 43)	0.01	15 (−6, 36)	0.16	17 (1, 32)	0.04	13 (−4, 30)	0.14
β-Cryptoxanthin	8.70 ± 5.60	27 (8, 46)	0.005	19 (−2, 39)	0.07	16 (0, 31)	0.05	12(−5, 29)	0.16
α-Carotene	2.94 ± 3.78	21 (1, 41)	0.03	12 (−9, 34)	0.25	11 (−5, 28)	0.16	7 (−10, 25)	0.41
β-Carotene	16.02 ± 14.24	17 (−2, 36)	0.07	9 (−11, 29)	0.37	14 (−2, 29)	0.08	10 (−6, 27)	0.21
Lutein/zeaxanthin	18.91 ± 8.83	9 (−10, 29)	0.32	5 (−15, 24)	0.63	6 (−9, 22)	0.41	5 (−11, 21)	0.57
Lycopene	30.29 ± 14.58	4 (−15, 23)	0.66	5 (−14, 24)	0.62	4 (−12, 19)	0.63	3 (−13, 19)	0.71
Decline from maximum observed lung function to year 20									
Sum of provitamin A carotenoids ⁵	27.67 ± 20.39	−19 (−33, −5)	0.01	−17 (−31, −2)	0.02	−15 (−27, −4)	0.01	−13 (−26, −1)	0.03
β-Cryptoxanthin	8.70 ± 5.60	−16 (−30, −2)	0.02	−14 (−28, 0)	0.05	−12 (−23, 0)	0.04	−9 (−21, 3)	0.15
α-Carotene	2.94 ± 3.78	−12 (−26, 3)	0.11	−7 (−22, 8)	0.34	−13 (−24, −1)	0.03	−10 (−23, 3)	0.13
β-Carotene	16.02 ± 14.24	−17 (−31, −4)	0.01	−14 (−28, 0)	0.05	−14 (−25, −2)	0.02	−11 (−23, 1)	0.06
Lutein/zeaxanthin	18.91 ± 8.83	−17 (−31, −3)	0.01	−13 (−27, 1)	0.07	−13 (−25, −2)	0.02	−12 (−24, 0)	0.05
Lycopene	30.29 ± 14.58	−19 (−33, −6)	0.004	−20 (−33, −7)	0.003	−18 (−29, −7)	0.001	−21 (−33, −10)	0.0002

¹ Unit of slope was defined as mL/SD of the indicated predictor. Maximum lung function was defined as the maximum observed value across years 0, 2, 5, and 10. A negative regression coefficient indicates less deterioration per unit of the carotenoid predictor. FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

² Values are means $\pm$ SDs.

³ Represents a meta-analytic pooling of sex-stratified linear regression models for the carotenoid predictors, which were adjusted for year 0 age, race, study center, height, height², age, age², BMI, amount of self-reported physical activity, and smoking status (never, former, or current).

⁴ Represents a meta-analytic pooling of sex-stratified linear regression models for the carotenoid predictors, which were adjusted as for model 1 plus the respective dietary intake score.

⁵ Defined as β -cryptoxanthin + α -carotene + β -carotene.

2, model 2). Serum carotenoids were not associated with maximum observed FEV₁/FVC (data not shown).

Year 0 carotenoid concentrations were inversely associated with the decline from maximum lung function

No significant interactions were found between sex and year 0 serum carotenoids in determining the decline from maximum observed FVC, FEV₁, or FEV₁/FVC ($P \geq 0.08$). The sex-stratified analyses are presented elsewhere (see Supplement Table 3 under "Supplemental data" in the online issue). All individual year 0 serum carotenoids (β -carotene, β -cryptoxanthin, lutein/zeaxanthin, and lycopene) except α -carotene were inversely and significantly associated with the 20-y decline in maximum observed FVC without adjustment for the year 0 diet score (Table 2, model 1). The sum of year 0 provitamin carotenoid concentrations was significantly associated with a change of -19 mL (19 mL less deterioration; 95% CI: -33 , -5 mL) from the maximum observed FVC per SD (20.39 μ mol/L) of the sum of year 0 provitamin A carotenoids ($P = 0.01$). Except for year 0 lutein/zeaxanthin concentrations ($P = 0.07$), all other associations remained significant after adjustment for the year 0 diet score (Table 2, model 2). All individual year 0 carotenoid concentrations, including α -carotene, were inversely and significantly associated with a decline from maximum observed FEV₁ without adjustment for year 0 diet score ($P \leq 0.04$) (Table 2, model 1). All associations except for the 3 provitamin A carotenoids (β -cryptoxanthin, β -carotene, and α -carotene) remained significant after adjustment for the year 0 diet score (Table 2, model 2). Year 0 lycopene concentrations showed no association with change from the maximum observed FEV₁/FVC to year 20 FEV₁/FVC ($P = 0.17$; 0.88%; 95% CI: -0.05% ,

0.26%) without adjustment for the diet score, but this association became significant after adjustment for the diet score ($P = 0.04$; 0.09%; 95% CI: 0.01%, 0.35%). None of the other carotenoids showed significant associations with change in FEV₁/FVC (data not shown).

Fifteen-year increase in carotenoid concentrations was inversely associated with decline from maximum lung function

No significant interactions were found between sex and the 15-y increase in serum carotenoids in determining decline from maximum observed FVC, FEV₁, or FEV₁/FVC ($P \geq 0.11$). The sex-stratified analyses are presented elsewhere (see Supplement Table 4 under "Supplemental data" in the online issue). After adjustment for all covariates except the 20-y change in diet score, the 15-y increase in all provitamin A carotenoid concentrations (β -cryptoxanthin, α -carotene, and β -carotene) and sum of year 0 provitamin A carotenoids were associated with a decline from the maximum observed FVC (peak FVC – year 20 FVC) ($P = 0.01$, $P = 0.02$, $P = 0.03$, and $P = 0.002$, respectively) (Table 3, model 1). Except for α -carotene ($P = 0.11$), these associations remained unchanged after adjustment for the 20-y change in the diet score (Table 3, model 2). Before adjustment for the 20-y change in diet score, lutein/zeaxanthin concentrations were not significantly associated with a decline from the maximum observed FVC ($P = 0.08$) (Table 3, model 1), but this association became significant after this adjustment ($P = 0.03$) (Table 3, model 2). The 15-y increase in lycopene concentrations was not associated with a decline from the maximum observed FVC. A similar pattern of associations was seen between circulating carotenoids and FEV₁ (Table 3), but the 15-y

TABLE 3

Slope (95% CI) of decline from maximum observed lung function (peak lung function – year 20) on change (Δ) in serum carotenoid concentrations for 2 models¹

		Decline from maximum observed lung function							
		FVC				FEV ₁			
		Model 1 ³ (<i>n</i> = 1981)		Model 2 ⁴ (<i>n</i> = 1726)		Model 1 ³ (<i>n</i> = 1981)		Model 2 ⁴ (<i>n</i> = 1726)	
15-y Change in carotenoid concentrations ²		Slope (95% CI)	<i>P</i>	Slope (95% CI)	<i>P</i>	Slope (95% CI)	<i>P</i>	Slope (95% CI)	<i>P</i>
	μ mol/L								
Sum of provitamin A carotenoids ⁵	12.87 $\pm$ 27.21	-22 (-37, -7)	0.002	-20 (-35, -5)	0.01	-19 (-33, -6)	0.003	-18 (-31, -4)	0.01
$\Delta\beta$ -Cryptoxanthin	3.39 $\pm$ 8.52	-19 (-33, -4)	0.01	-21 (-37, -4)	0.01	-18 (-31, -6)	0.004	-21 (-35, -7)	0.002
$\Delta\alpha$ -Carotene	2.99 $\pm$ 6.35	-16 (-31, -2)	0.02	-12 (-27, 3)	0.11	-15 (-27, -2)	0.02	-12 (-25, 2)	0.08
$\Delta\beta$ -Carotene	6.49 $\pm$ 19.36	-15 (-29, -1)	0.03	-15 (-30, 0)	0.04	-14 (-27, -1)	0.03	-12 (-25, 1)	0.06
Δ Lutein/zeaxanthin	6.75 $\pm$ 10.28	-13 (-27, 2)	0.08	-17 (-32, -1)	0.03	-13 (-27, 1)	0.06	-17 (-30, -3)	0.01
Δ Lycopene	8.98 $\pm$ 18.59	5 (-10, 19)	0.52	6 (-9, 22)	0.41	6 (-7, 19)	0.33	11 (-3, 4)	0.11

¹ Unit of slope was defined as mL/SD of the indicated predictor. Maximum lung function was defined as the maximum observed value across years 0, 2, 5, and 10. A negative regression coefficient indicates less deterioration per unit of the carotenoid predictor. FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

² Values are means $\pm$ SDs. Calculated as year 15 – year 0.

³ Represents a meta-analytic pooling of sex-stratified linear regression models for the carotenoid predictors, which were adjusted for year 0 age, race, study center, height, height², age, age², BMI, amount of self-reported physical activity, and smoking status (never, former, or current).

⁴ Represents a meta-analytic pooling of sex-stratified linear regression models for the carotenoid predictors, which were adjusted as for model 1 plus the respective dietary intake score.

⁵ Defined as β -cryptoxanthin + α -carotene + β -carotene.

change in carotenoid concentrations was not significantly associated with the change in FEV₁/FVC (data not shown).

No significant interaction was found between smoking status and carotenoid concentrations in predicting maximum lung function or a decline from maximum lung function. Also, no interaction was found between smoking status and the 15-y change in carotenoid concentrations in predicting the decline from maximum lung function. Adjustment for pack-years of smoking instead of smoking status or the 20-y change in smoking status did not significantly change the observed associations. Among non-smokers, passive smoking, which was uncommon after the first few years of CARDIA, did not significantly change the observed association between serum carotenoids and lung function (data not shown).

Sensitivity analyses

Replacing maximum observed lung function with year 0 lung function yielded associations between serum carotenoid concentrations and lung function that were similar in magnitude and direction to those observed with maximum observed lung function (*see* Supplement Tables 5–9 under “Supplemental data” in the online issue). The notable exceptions were the lack of association between the year 0 lycopene concentration and the 20-y decline in FVC with ($P = 0.20$) and without ($P = 0.39$) adjustment for the year 0 diet score (*see* Supplement Table 5 under “Supplemental data” in the online issue), a statistically significant interaction of sex and year 0 lutein/zeaxanthin interaction with the 20-y decline in FVC after adjustment for the year 0 diet score ($P = 0.04$), and a significant interaction of sex and year 0 β -cryptoxanthin with the 20-y decline in FEV₁ with ($P = 0.05$) and without ($P = 0.02$) adjustment for the year 0 diet score (*see* Supplement Table 8 under “Supplemental data” in the online issue).

DISCUSSION

Baseline carotenoid concentrations and the 15-y increase in provitamin A carotenoid concentrations were inversely associated with a decline from maximum observed lung function. The associations between serum carotenoids and a decline in lung function were, for the most part, independent of the dietary intake of carotenoids. This findings of this study are consistent with those of cross-sectional studies that have shown positive associations between an increased dietary intake of carotenoids (6–10), serum carotenoid concentrations (11, 12), and lung function. The findings of the current study are also consistent with the prospective findings from 1194 French participants within the European Community Respiratory Health Study, which showed that lower β -carotene concentrations were associated with steeper declines in FEV₁ over an 8-y period (15) and with a clinical trial that showed improvements in lung function with increases in β -carotene and retinol concentrations (16). Our findings are also consistent with those of one study that found a significant association between increasing fresh fruit consumption and reduced lung function decline (14). However, another study found no association between fresh fruit intake or longitudinal increase in fresh fruit intake and lung function over a 5-y period (13). This was the first study to show an association between provitamin A carotenoid concentrations and maximum

lung function. However, because this association was no longer significant after adjustment for relevant dietary intake and detailed information regarding lung development was not available, the role of provitamin A carotenoids on lung development needs to be investigated in more detail in future studies.

Circulating carotenoid concentrations are determined by both dietary consumption and metabolism after intake (28–30). Although dietary and supplement intakes are the only source for circulating carotenoids, individual carotenoid concentrations are imperfectly associated with dietary estimates, ranging from $r = 0.31$ to 0.51 , with dietary intake estimated by food frequency questionnaires (29, 31). In the current study, serum carotenoid concentrations remained associated with lung function even after adjustment for relevant dietary intake, which suggests that diet alone is insufficient to explain the association between circulating carotenoid concentrations and lung function. This suggests, among other possibilities, that interindividual variation in the bioavailability of various dietary carotenoids (28, 30) or systemic oxidative stress (32) may play a role in determining serum carotenoid concentrations. Because greater serum carotenoid concentrations are associated with lower BMI, increased physical activity, and a lower prevalence of current smoking in addition to a diet rich in fruit and vegetables, higher serum carotenoid concentrations may be a marker for a healthy lifestyle. Despite adjustment for these covariates in the analyses, we cannot exclude the possibility of residual confounding from an unmeasured covariate or measurement error in assessment of covariates. Furthermore, serum measurement of carotenoids has been shown to correlate ($r = 0.61$, $P = 0.004$) with measurements of carotenoids in lung tissue (33), which suggests that serum measurements of carotenoids may reflect the amount available in the airways to protect against damage to airways caused by oxidative stress.

This study did not show a significant interaction between smoking—a well-recognized source of oxidants—and carotenoid concentrations in determining lung function. Significant interactions between smoking status and carotenoid concentrations have been reported in determining diabetes incidence (34), BMI (32), and levels of oxidative stress markers (35) in CARDIA. The CARDIA cohort is a young cohort with a relatively short lifetime exposure to cigarette smoke. Thus, despite significant interactions between carotenoid concentrations and smoking status in determining levels of oxidative stress, BMI, and diabetes, it is possible that the interactions between cigarette smoke and carotenoid concentrations may not play a significant role in determining lung function until later in life.

The current study has several strengths, including the large number of young adult participants, the relatively narrow age range at entry, the inclusion of blacks and whites and men and women, the long duration of follow-up (including the period in which maximum lung function is achieved), and the presence of contemporaneously measured dietary and circulating measures of carotenoids. The simultaneous availability of serum biomarkers and dietary intakes of carotenoids also allowed us to evaluate the relative contribution of diet and metabolic variability in the metabolism of carotenoids in determining lung function. In addition, dietary data were obtained prospectively in the young and relatively disease-free cohort, thereby minimizing any recall bias in the collection of dietary information. It also assured a high quality of data collection through strict quality control across

examinations. Because the sample studied by CARDIA included young healthy people, few individuals were lost because of disease, avoiding survivorship bias (36). Limitations include biases common to observational and longitudinal studies, such as bias introduced because of the loss of follow-up. This bias is minimized in CARDIA because of the excellent retention of the original cohort and because of the lack of differences in baseline lung function measures between those who were lost to follow-up and those who continued to participate in the study. Another limitation of this study was that serum carotenoids were not measured at the same time points that lung function was measured in the CARDIA cohort. The large number of statistical tests performed could limit the statistical significance of the observed findings. However, the consistency of results across multiple analyses and the similarity of findings across different related carotenoids (eg, the 3 provitamin A carotenoids) is a strength, supporting the hypothesis that serum carotenoid concentrations are associated with lung function.

In summary, several criteria for determining a causal protective role for serum carotenoid concentrations in lung function decline were fulfilled in these data, including that year 0 carotenoid concentrations predicted the future decline in lung function and that changes in carotenoid concentrations corresponded to a decline in lung function. These findings suggest that both a healthy lifestyle, which leads to higher serum carotenoid concentrations, and factors that influence serum carotenoid concentrations (eg, interindividual variations in carotenoid metabolism) may play a role in the age-related decline in lung function.

The authors' responsibilities were as follows—DRJ, WSB, and ODW; designed the research question; BT: conducted the research and analyzed the data; DRJ: directed the data analysis; MDG: performed all the carotenoid measurements; and BT, LJS, KM, WSB, ODW, DRJ, and MDG: wrote the manuscript. The manuscript was reviewed and approved by the CARDIA Steering Committee. All authors have read and approved the final manuscript. None of the authors had any conflicts of interest to disclose.

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