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Dietary Carotenoids Are Associated with Cardiovascular Disease Risk Biomarkers Mediated by Serum Carotenoid Concentrations[1,2]

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Abstract

Hyperlipidemia and elevated circulating C-reactive protein (CRP) and total homocysteine (tHcy) concentrations are cardiovascular disease (CVD) risk factors. Previous studies indicated that higher serum carotenoid concentrations were inversely associated with some of these biomarkers. However, whether dietary carotenoid intake is inversely associated with these CVD risk biomarkers is not well known. We assessed the associations between individual dietary carotenoid intake and CVD risk biomarkers and tested whether the serum carotenoid concentrations explain (mediate) or influence the strength of (moderate) the associations, if any association exists. Dietary data collected from 2 24-h dietary recalls and serum measurements in adult men (n = 1312) and women (n = 1544) from the NHANES 2003–2006 were used. Regression models designed for survey analysis were used to examine the associations between individual dietary carotenoids and log-transformed blood cholesterol, CRP, and tHcy. The corresponding individual serum carotenoid concentration was considered as mediator (and moderator if applicable). After adjustment for covariates, significant inverse associations with LDL cholesterol were observed for dietary β-carotene ($P < 0.05$) and lutein + zeaxanthin ($P < 0.001$), and with tHcy for dietary β-carotene ($P < 0.05$), lycopene ($P < 0.05$), and total carotenoids ($P < 0.05$). Dietary lutein + zeaxanthin intake was also positively associated with HDL cholesterol concentrations ($P < 0.01$). Most of these associations were null after additional adjustment for corresponding serum carotenoid concentrations, indicating the complete mediation effects of serum carotenoids. Serum β-carotene significantly moderated the associations between dietary β-carotene and CRP (P-interaction < 0.05), and quartile 4 of dietary β-carotene was associated with lower CRP concentrations only among participants with serum β-carotene > 0.43 μmol/L. In this population-based cross-sectional study, serum carotenoids were mediators of dietary carotenoids and CVD risk biomarker associations. Serum β-carotene was also a moderator of the dietary β-carotene and CRP association. These findings may help in the design of future intervention studies on dietary carotenoids in the prevention of CVD. J. Nutr. 144: 1067–1074, 2014.

Introduction

Increased blood cholesterol (1), C-reactive protein (CRP)[7] (2), and total homocysteine (tHcy) concentrations (3) have been associated consistently with increased cardiovascular disease (CVD) risk. Recent studies found that higher serum concentrations of carotenoids were inversely associated with several CVD risk factors, including CRP, insulin resistance, hyperuricemia, and metabolic syndrome (4–6).

Carotenoids are a widely distributed group of fat-soluble pigments that give yellow, orange, and red colors to plants, animals, and microorganisms (7). More than 700 naturally occurring carotenoids have been discovered so far, and 6 of them (α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein + zeaxanthin) represent >95% of total carotenoids in human blood (8). Preclinical studies suggested that carotenoids have cardiovascular protective effects perhaps because of their antioxidant, anti-inflammatory, and antiproliferative properties (9). Mammals do not synthesize carotenoids and need to consume them in their diet. However, serum carotenoid concentrations have only moderate correlations with intake amounts; other factors, such as smoking, and genetic factors (e.g., the β-carotene 15,15′-monooxygenase 1 gene) can also affect serum carotenoid concentrations independently of dietary

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[2] Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

[7] Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; DR, dietary recall; tHcy, total homocysteine.

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intake (10,11). Identifying the associations between dietary carotenoid intake and CVD risk biomarkers, such as serum cholesterol, CRP, and tHcy, is of public health interest for CVD prevention, but these associations were less investigated in large observational studies (12). In addition, no previous study examined both dietary carotenoids and serum carotenoids together; whether or not serum carotenoids mediate or moderate the exposure–outcome associations is essentially unknown.

The first objective of the present study was to examine the associations between dietary carotenoid intake and CVD risk biomarkers. The second objective was to test whether corresponding serum carotenoid concentrations mediate or moderate the diet–outcome associations, if any association exists. We conducted mediation analyses using national representative data from the NHANES.

Materials and Methods

Data source and study population. The NHANES, conducted by the National Center for Health Statistics, is designed to obtain nationally representative information on the health and nutritional status of adults and children in the United States since the early 1960s. It was changed in 1999 from a periodic annual survey to a continuous annual survey, and the continuous NHANES data have been released in 2-y increments for public use (13). Selected individuals were interviewed in their homes and then invited to the mobile examination center, where physical measurements and other health interviews were conducted by trained medical personnel. Written informed consent was obtained from all participants or proxies, and the survey protocol was approved by the Research Ethics Review Board of the National Center for Health Statistics (14).

A total of 10,577 individuals, aged ≥20 y, were selected from the NHANES 2003–2006. Individuals with a history of coronary heart disease or cancer were excluded from the analysis (n = 1867). We further excluded participants with only 1 24-h dietary recall (DR) or unreliable DR (no data on total nutrient intakes or the total number of foods were provided) or missing dietary carotenoid intake data (n = 1600), those with missing serum carotenoid concentrations (n = 651), and those with missing blood CVD risk biomarkers (lipids, CRP, and tHcy concentrations; n = 3483). Individuals with extreme dietary energy intake (males: >4200 or <800 kcal/d; females: >3500 or <600 kcal/d) were also excluded from the study population (n = 98). Last, the top 0.2% of individual dietary carotenoid intake was excluded as outliers (n = 22). In total, 1312 men and 1544 women were included in the analysis.

Dietary assessment. Dietary data were collected by 2 24-h DRs. The first 24-h DR was administered in person during the examination in the NHANES mobile examination center. Multiple measurement guides, such as glasses, bowls, mugs, household spoons, measuring cups and spoons, and circles, were available for participants to estimate the portion size. The second dietary interview was a phone interview that occurred 3–10 d after the in-person interview. The USDA Automated Multiple Pass Method (AMPM) program, a 5-step computerized DR instrument, was used for both dietary interviews (15). The purpose of releasing 2-d data was to permit the estimate of usual dietary intake in the United States, because repeated measures can reduce measurement error. Previously, 2 Automated Multiple Pass Method DRs were validated for total energy and several energy-adjusted micronutrients, including β-carotene and β-cryptoxanthin intake (16).

Measurement of serum carotenoids and CVD risk biomarkers. HPLC with photodiode array detection was used to measure 6 serum carotenoid concentrations (α-carotene, *trans*-β-carotene, *cis*-β-carotene, β-cryptoxanthin, lutein + zeaxanthin, and lycopene) (17,18).

TG, total cholesterol, and HDL cholesterol were measured directly from serum (19). LDL cholesterol was calculated according to the Friedewald calculation: LDL cholesterol = total cholesterol − HDL cholesterol − TG/5 (20). CRP was measured by latex-enhanced nephelometry as described in detail previously (21,22). Plasma tHcy was measured by the Abbott homocysteine assay on the Abbott AxSym analyzer, a fully automated fluorescence polarization immunoassay from Abbott Diagnostics (23,24).

Statistical analysis. All statistical analyses were performed using SAS software, release 9.3 (SAS Institute). Sample weights were applied to all analyses to account for the unequal probability of selection, noncoverage, and nonresponse bias resulting from oversampling of low-income individuals, adolescents, the elderly, African Americans, and Mexican Americans.

Because of skewed distributions of serum carotenoid and CVD risk biomarkers, ln-transformed values were used to improve normality when they were dependent variables. Energy-adjusted values of dietary carotenoids were calculated using the residual method (25). PROC SURVEYREG was used to obtain least-square means and 95% CIs for dietary and serum carotenoids by covariates and to obtain β coefficients and P values for mediation analyses. Geometric means were the exponentials of least-square means.

Multivariable models were adjusted for significant confounders that were related to either dietary or serum carotenoids, including the following: *1*) age; *2*) gender; *3*) ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, others); *4*) poverty income ratio (ratio of the median family income over the poverty index: <1.30, 1.30 to <1.85, ≥1.85, unknown); *5*) dietary supplement use (yes or no to "Have you taken any dietary supplements in the past month?"); *6*) alcohol consumption (yes or no to "Do you have at least 12 drinks per year?", unknown); *7*) smoking (yes or no to "Have you smoked at least 100 cigarettes during your life?"); *8*) physical activity (metabolic equivalent score in quartile); *9*) BMI (<18.5, 18.5–24.9, 25–29.9, ≥30 kg/m^2); *10*) self-reported diabetes (yes or no to "Have you ever been told by a doctor or health professional that you have diabetes or sugar diabetes?"); *11*) self-reported high blood cholesterol (yes or no to "Have you ever been told by a doctor or health professional that your blood cholesterol was high?", unknown); *12*) prescription medication use in the past month (yes, no, unknown); *13*) total energy intake (kilocalories per day); *14*) plasma TGs; and *15*) total cholesterol. We further adjusted serum vitamin B-12 and folate concentrations for plasma tHcy because they are essential vitamins in homocysteine metabolism and are associated with tHcy concentrations (26).

Mediation analysis hypothesizes that the association between exposure and outcome is mediated by a mechanism factor: the mediator as shown in **Figure 1**. To be considered a mediator, the following criteria

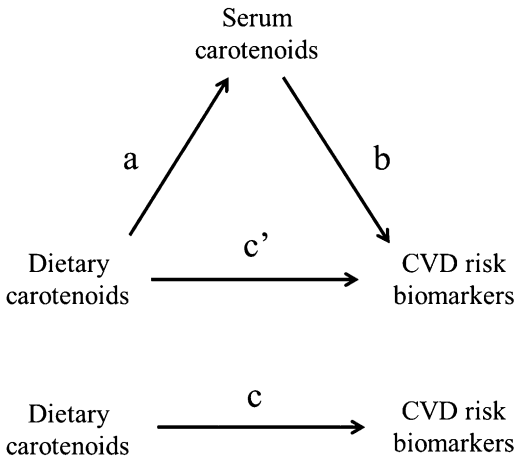

FIGURE 1 Mediation models among 2856 U.S. adults aged ≥20 y in the NHANES 2003–2006. a indicates the path from dietary carotenoids (exposure) to serum carotenoids (mediator). b indicates the path from serum carotenoids (mediator) to CVD risk biomarkers (outcome). c indicates the path from dietary carotenoids (exposure) to CVD risk biomarkers (outcome). c′ indicates the path from dietary carotenoids (exposure) to CVD risk biomarkers (outcome) when controlled for serum carotenoids (mediator). CVD, cardiovascular disease.

TABLE 1 Carotenoid intake by selected characteristics of 2856 U.S. adults aged ≥20 y in the NHANES 2003–2006[1]

		Dietary carotenoids											
		α-Carotene		β-Carotene		β-Cryptoxanthin		Lycopene		Lutein + zeaxanthin		Total	
Sociodemographic factors	*n*	Value	*P*	Value	*P*	Value	*P*	Value	*P*	Value	*P*	Value	*P*
		mg/d		*mg/d*		*mg/d*		*mg/d*		*mg/d*		*mg/d*	
Gender			<0.0001		<0.0001		<0.0001		<0.001		<0.0001		<0.0001
Men (reference)	1312	0.06 (0.05, 0.07)		0.75 (0.69, 0.82)		0.03 (0.03, 0.04)		0.48 (0.33, 0.7)		0.58 (0.54, 0.63)		3.9 (3.5, 4.2)	
Women	1544	0.10 (0.08, 0.11)		1.2 (1.1, 1.4)		0.05 (0.04, 0.05)		1.17 (0.88, 1.5)		0.92 (0.85, 0.99)		7.1 (6.5, 7.8)	
Age (y)			<0.0001		<0.0001		<0.0001		0.10		<0.0001		<0.0001
20–34 (reference)	901	0.05 (0.04, 0.06)		0.72 (0.63, 0.83)		0.03 (0.03, 0.04)		1.0 (0.75, 1.4)		0.58 (0.52, 0.64)		4.5 (4.0, 5.1)	
35–49	749	0.07 (0.06, 0.09)		0.93* (0.82, 1.0)		0.03 (0.03, 0.04)		0.82 (0.57, 1.2)		0.71* (0.64, 0.78)		5.1 (4.6, 5.7)	
50–64	618	0.11* (0.09, 0.15)		1.3* (1.1, 1.5)		0.05* (0.04, 0.06)		0.60 (0.34, 1.0)		0.93* (0.85, 1.0)		6.2* (5.5, 7.1)	
≥65	588	0.12* (0.10, 0.16)		1.4* (1.3, 1.7)		0.07* (0.06, 0.08)		0.50 (0.30, 0.85)		1.0* (0.93, 1.1)		6.9* (6.0, 8.0)	
Ethnicity			<0.0001		<0.001		<0.01		<0.0001		<0.01		<0.001
Non-Hispanic whites (reference)	1414	0.08 (0.07, 0.09)		1.0 (0.94, 1.1)		0.04 (0.03, 0.04)		0.81 (0.61, 1.08)		0.76 (0.69, 0.82)		5.5 (5.0, 6.0)	
Non-Hispanic blacks	581	0.05* (0.04, 0.06)		0.78* (0.69, 0.88)		0.05* (0.04, 0.06)		0.31* (0.20, 0.49)		0.76 (0.68, 0.85)		4.3* (3.9, 4.8)	
Mexican Americans	637	0.09 (0.06, 0.12)		0.81* (0.71, 0.92)		0.06* (0.05, 0.07)		1.6* (1.1, 2.2)		0.60* (0.56, 0.65)		5.6 (5.1, 6.3)	
Others	224	0.09 (0.06, 0.13)		0.93 (0.77, 1.14)		0.04 (0.03, 0.06)		0.77 (0.43, 1.4)		0.74 (0.63, 0.87)		5.3 (4.5, 6.1)	
Poverty income ratio[2]			<0.0001		<0.0001		0.06		0.11		<0.001		<0.05
<1.3 (reference)	700	0.06 (0.04, 0.07)		0.72 (0.63, 0.83)		0.03 (0.03, 0.04)		0.48 (0.31, 0.74)		0.59 (0.52, 0.67)		4.6 (3.9, 5.4)	
1.3–1.84	353	0.04 (0.03, 0.07)		0.76 (0.62, 0.92)		0.03 (0.02, 0.04)		0.64 (0.35, 1.15)		0.60 (0.51, 0.70)		4.6 (3.8, 5.6)	
≥1.85	1679	0.09* (0.07, 0.10)		1.1* (1.0, 1.2)		0.04 (0.04, 0.05)		0.89 (0.65, 1.21)		0.81* (0.75, 0.87)		5.7* (5.3, 6.1)	
Dietary supplement use[3]			<0.0001		<0.0001		<0.0001		<0.01		<0.0001		<0.0001
No (reference)	1332	0.05 (0.04, 0.06)		0.74 (0.68, 0.82)		0.03 (0.03, 0.04)		0.61 (0.45, 0.82)		0.59 (0.54, 0.65)		4.5 (4.1, 4.9)	
Yes	1524	0.10 (0.09, 0.12)		1.2 (1.1, 1.3)		0.05 (0.04, 0.05)		0.93 (0.70, 1.2)		0.89 (0.83, 0.96)		6.2 (5.7, 6.7)	
Alcohol consumption[4]			0.14		0.58		0.39		0.28		0.22		0.27
No (reference)	836	0.09 (0.07, 0.11)		1.04 (0.93, 1.2)		0.04 (0.03, 0.05)		0.57 (0.36, 0.92)		0.80 (0.74, 0.87)		5.8 (5.2, 6.5)	
Yes	1901	0.07 (0.06, 0.08)		0.96 (0.87, 1.1)		0.04 (0.03, 0.04)		0.84 (0.62, 1.12)		0.73 (0.66, 0.79)		5.2 (4.7, 5.7)	
Cigarette smoking[5]			<0.001		<0.0001		<0.0001		0.98		<0.0001		<0.01
No (reference)	1520	0.10 (0.08, 0.11)		1.1 (1.0, 1.2)		0.05 (0.05, 0.06)		0.77 (0.57, 1.04)		0.82 (0.77, 0.87)		5.8 (5.4, 6.3)	
Yes	1336	0.06 (0.05, 0.07)		0.85 (0.76, 0.95)		0.03 (0.02, 0.03)		0.77 (0.56, 1.05)		0.67 (0.61, 0.73)		4.9 (4.4, 5.4)	
Physical activity amount (MET-h/wk)			<0.001		<0.001		<0.01		<0.001		<0.001		<0.01
<2.5 (reference)	1103	0.05 (0.04, 0.06)		0.78 (0.70, 0.88)		0.03 (0.03, 0.04)		0.45 (0.31, 0.64)		0.65 (0.59, 0.71)		4.6 (4.1, 5.3)	
2.5 to <4	423	0.09* (0.07, 0.12)		1.18* (1.03, 1.35)		0.04 (0.03, 0.06)		0.94* (0.60, 1.5)		0.84* (0.75, 0.94)		6.2* (5.4, 7.1)	
4 to <11.5	595	0.10* (0.08, 0.12)		1.10* (0.97, 1.24)		0.04 (0.03, 0.05)		0.98* (0.72, 1.3)		0.82* (0.73, 0.92)		5.8* (5.2, 6.4)	
≥11.5	735	0.09* (0.07, 0.10)		1.04* (0.93, 1.16)		0.05* (0.04, 0.05)		1.0* (0.76, 1.4)		0.75 (0.68, 0.83)		5.5* (4.9, 6.1)	
BMI (kg/m^2)			0.38		0.69		0.07		0.31		0.45		0.83
<18.5	41	0.07 (0.04, 0.14)		1.1 (0.68, 1.7)		0.04 (0.02, 0.12)		1.0 (0.36, 2.7)		0.88 (0.52, 1.48)		5.5 (3.5, 8.8)	
18.5–24.9 (reference)	803	0.08 (0.06, 0.10)		1.0 (0.90, 1.2)		0.04 (0.04, 0.05)		1.0 (0.69, 1.4)		0.78 (0.70, 0.87)		5.6 (4.9, 6.4)	
25–29.9	1017	0.08 (0.07, 0.10)		0.97 (0.88, 1.1)		0.04 (0.03, 0.05)		0.71 (0.48, 1.0)		0.72 (0.66, 0.78)		5.2 (4.7, 5.8)	
≥30	995	0.07 (0.06, 0.08)		0.93 (0.82, 1.1)		0.03 (0.03, 0.04)		0.65 (0.42, 1.0)		0.73 (0.66, 0.81)		5.2 (4.6, 5.9)	

(Continued)

TABLE 1 *Continued*

		Dietary carotenoids									
		α-Carotene		β-Carotene		β-Cryptoxanthin		Lycopene		Lutein + zeaxanthin		Total	
Sociodemographic factors	*n*	Value	*P*	Value	*P*	Value	*P*	Value	*P*	Value	*P*	Value	*P*
History of diabetes[6]			<0.01		<0.05		<0.01		0.07		<0.001		0.43
No (reference)	2562	0.07 (0.06, 0.09)		0.96 (0.88, 1.05)		0.04 (0.03, 0.04)		0.80 (0.61, 1.05)		0.72 (0.67, 0.78)		5.3 (4.9, 5.8)	
Yes	294	0.11 (0.09, 0.15)		1.2 (1.0, 1.37)		0.06 (0.05, 0.07)		0.44 (0.24, 0.80)		1.0 (0.88, 1.14)		5.7 (4.9, 6.6)	
History of hypercholesterolemia[7]			0.61		0.71		0.84		<0.05		0.09		0.46
No (reference)	1151	0.10 (0.08, 0.12)		1.2 (1.1, 1.3)		0.04 (0.04, 0.05)		1.1 (0.84, 1.4)		0.86 (0.79, 0.93)		6.1 (5.6, 6.6)	
Yes	786	0.09 (0.07, 0.11)		1.1 (1.0, 1.2)		0.04 (0.04, 0.05)		0.64 (0.41, 1.0)		0.80 (0.74, 0.86)		5.9 (5.3, 6.4)	
Prescription medication[8]			<0.001		<0.0001		<0.001		0.69		<0.0001		<0.001
No (reference)	1342	0.06 (0.05, 0.08)		0.83 (0.73, 0.94)		0.03 (0.03, 0.04)		0.74 (0.51, 1.08)		0.65 (0.60, 0.71)		4.7 (4.2, 5.2)	
Yes	1511	0.09 (0.08, 0.11)		1.1 (1.0, 1.2)		0.04 (0.04, 0.05)		0.79 (0.64, 0.97)		0.83 (0.77, 0.90)		6.0 (5.5, 6.6)	

[1] Values are geometric means (95% CIs). *Significantly different from reference, $P < 0.017$. MET, metabolic equivalent score.
[2] Ratio of the median family income over the poverty index. A poverty income ratio of ≤1.30 is required to be eligible for food assistance programs.
[3] Yes indicates taking any dietary supplements at the time of the interview. Dietary supplements include any vitamins, minerals, or other dietary supplements.
[4] Yes indicates having at least 12 alcoholic drinks per year.
[5] Yes indicates having smoked at least 100 cigarettes in the lifetime.
[6] Yes indicates ever being told by a doctor or health professional that individual has diabetes or sugar diabetes.
[7] Yes indicates ever being told by a doctor or health professional that individual's blood cholesterol is high.
[8] Yes indicates using prescription medication in the past month.

need to be satisfied: *1*) a change in amounts of the exposure significantly affects the changes in the mediator (path a is significant); *2*) there is a significant relation between the mediator and the outcome (path b is significant); and *3*) the effect of the exposure on the outcome is reduced when the mediator is present (mediation effect is significant) (27). Mediation analyses were performed per the method proposed by Valeri and VanderWeele (27) that allows for exposure–mediator interactions. We tested interaction (moderation) effects of serum carotenoids on the associations between dietary carotenoids and CVD risk biomarkers using the likelihood ratio test. If significant, an interaction term between the exposure and the mediator was added into the mediational model (path c′). The mediation effect was calculated and tested for significance using the partial posterior method (28). All *P* values were 2 sided and considered statistically significant at <0.05. Bonferroni's correction was applied for multiple comparisons, and $P < 0.017$ was considered statistically significant for the 3-pair comparison in **Table 1**.

Results

Dietary, serum carotenoids, and selected characteristics. The mean intakes of dietary carotenoids were calculated from 2 24-h DRs. Energy-adjusted dietary carotenoids were calculated by the residual method. Geometric means of energy-adjusted dietary carotenoids by sociodemographic, clinical, and lifestyle factors are shown in Table 1. Participants with higher dietary total carotenoid intakes were more likely to be women, older individuals (≥50 y), individuals with higher family income amounts, supplement users, and individuals with higher physical activity amounts. They were less likely to be non-Hispanic blacks, smoke, or use prescription medication. Similarly, individuals with higher serum carotenoid concentrations were more likely to be women, individuals with higher family income amounts, supplement users, nonsmokers or less frequent smokers, and individuals with higher physical activity amounts. Moreover, they were less likely to be overweight or obese, have a history of diabetes, or use prescription medication (data not shown). **Supplemental Table 1** presents the top 10 food sources of the individual carotenoid subclass.

Associations between dietary carotenoids and serum carotenoids (path a). After adjustment for the covariates shown in Table 1, dietary carotenoids had low-to-moderate associations with their serum concentrations, with β coefficients that varied from 0.09 for lutein + zeaxanthin to 0.40 for β-cryptoxanthin (**Table 2**).

Serum carotenoids and CVD risk biomarkers (path b). After adjustment for covariates and exposure, serum total carotenoids and all individual subclasses (mediator) had significant inverse associations with blood CRP and tHcy concentrations (**Table 3**). Several serum carotenoids had significant positive associations with HDL cholesterol concentrations, among which only lutein + zeaxanthin had an inverse association with LDL cholesterol concentrations. According to the criteria, to be considered mediators, carotenoids must have significant associations with both exposure and outcome.

Dietary carotenoids and CVD risk biomarkers (paths c and c′) and mediation analyses. After adjustment for covariates but not mediators (path c), dietary β-carotene and lutein + zeaxanthin were inversely associated with LDL cholesterol concentrations (Table 3). In the mediational model (path c′) (i.e., additional adjustment for corresponding serum carotenoid concentrations), the association between β-carotene and LDL cholesterol was not attenuated (i.e., the association was not

TABLE 2 Associations between dietary carotenoids and serum carotenoid concentrations among 2856 U.S. adults aged ≥20 y in the NHANES 2003–2006[1]

	Dietary carotenoids (mg/d)											
	α-Carotene		β-Carotene		β-Cryptoxanthin		Lycopene		Lutein + zeaxanthin		Total	
Serum carotenoids (μmol/L)	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
α-Carotene	0.33	<0.0001										
β-Carotene			0.29	<0.0001								
β-Cryptoxanthin					0.40	<0.0001						
Lycopene							0.12	<0.0001				
Lutein + zeaxanthin									0.09	<0.0001		
Total											0.18	<0.0001

[1] Adjusted for age, gender, ethnicity, BMI, supplement use, alcohol consumption, poverty income ratio, physical activity, history of diabetes, history of high blood cholesterol, prescription medication use in the past month, total energy intake, plasma TGs, and plasma total cholesterol.

mediated by serum β-carotene concentrations). The association between dietary lutein + zeaxanthin and LDL cholesterol was partially mediated by serum lutein + zeaxanthin concentrations in that the inverse association was attenuated (β coefficient changed from −0.0083 to −0.0052) but still significant after adjustment for serum concentration. Lutein + zeaxanthin intake

TABLE 3 Individual dietary carotenoid and CVD risk biomarkers mediated by corresponding serum carotenoid among 2856 U.S. adults aged ≥20 y in the NHANES 2003–2006[1]

	CVD risk biomarkers							
	Log LDL cholesterol		Log HDL cholesterol		Log CRP		Log tHcy	
Dietary carotenoids (per interquartile increment)[2]	β	*P*	β	*P*	β[3]	*P*	β	*P*
α-Carotene								
Path c[4]	0.0007	0.85	0.0017	0.76	−0.0135	0.62	−0.0093	0.09
Path c′[5]	0.0020	0.53	−0.0028	0.62	0.0419	0.17	−0.0120	0.07
Path b[6]	−0.0314	0.32	0.1068	0.01	−1.2940	<0.0001	−0.2649	<0.01
Mediated effect[7]	−0.0103	0.31	0.0352	<0.01	−0.4260	<0.0001	−0.0644	<0.01
β-Carotene								
Path c[4]	−0.0098	0.04	0.0088	0.24	−0.0412	0.40	−0.0169	0.03
Path c′[5]	−0.0093	0.04	0.0061	0.39	0.1416	0.05	−0.0046	0.56
Path b[6]	−0.0038	0.67	0.0204	0.14	−0.2893	0.02	−0.0701	<0.001
Mediated effect[7]	−0.0011	0.67	0.0058	0.13	0.1455[7]	—	−0.0201	<0.0001
β-Cryptoxanthin								
Path c[4]	0.0012	0.81	0.0056	0.43	−0.0342	0.48	−0.0046	0.63
Path c′[5]	0.0040	0.37	−0.0077	0.21	0.0572	0.28	0.0268	0.01
Path b[6]	−0.0378	0.28	0.1785	<0.01	−1.2289	<0.0001	−0.3311	<0.0001
Mediated effect[7]	−0.0151	0.28	0.0710	<0.0001	−0.4890	<0.0001	−0.1318	<0.0001
Lycopene								
Path c[4]	0.0009	0.82	0.0002	0.98	−0.0550	0.13	−0.0222	0.03
Path c′[5]	−0.00001	1.00	−0.0014	0.80	−0.0366	0.30	−0.0102	0.23
Path b[6]	0.0199	0.37	0.0356	0.33	−0.4215	<0.01	−0.2077	<0.0001
Mediated effect[7]	0.0023	0.34	0.0042	0.31	−0.0500	<0.01	−0.0244	<0.0001
Lutein + zeaxanthin								
Path c[4]	−0.0083	<0.001	0.0095	<0.01	−0.0160	0.55	−0.0059	0.21
Path c′[5]	−0.0052	0.04	0.0016	0.63	0.0154	0.58	0.0023	0.62
Path b[6]	−0.1106	<0.01	0.2831	<0.0001	−1.1324	<0.0001	−0.2230	<0.0001
Mediated effect[7]	−0.0105	<0.01	0.0269	<0.0001	−1.0760	<0.0001	−0.0212	<0.0001
Total								
Path c[4]	−0.0039	0.35	0.0054	0.40	−0.0759	0.06	−0.0307	0.01
Path c′[5]	−0.0021	0.66	−0.0025	0.72	0.0055	0.89	−0.0096	0.33
Path b[6]	−0.0070	0.34	0.0308	<0.01	−0.3147	<0.0001	−0.0645	<0.0001
Mediated effect[7]	−0.0013	0.33	0.0055	<0.01	−0.0560	<0.0001	−0.0116	<0.0001

[1] CRP, C-reactive protein; CVD, cardiovascular disease; tHcy, total homocysteine.
[2] Differences in medians of quartiles 1 and 4: α-carotene, 0.90 mg/d; β-carotene, 3.9 mg/d; β-cryptoxanthin, 0.33 mg/d; lycopene, 12.3 mg/d; lutein + zeaxanthin, 2.1 mg/d.
[3] Calculated as the sum of the cross-product of coefficients of path a and path b and the cross-product of coefficients of path a and the interaction term (27).
[4] Adjusted for covariates (age, gender, ethnicity, BMI, supplement use, alcohol consumption, poverty income ratio, physical activity, history of diabetes, history of high blood cholesterol, prescription medication use in the past month, total energy intake, plasma TGs, and plasma total cholesterol); further adjusted for serum folate and vitamin B-12 for tHcy.
[5] Adjusted for covariates + mediator (the serum carotenoid corresponding to the dietary carotenoid).
[6] The regression coefficient of the mediator after adjustment for exposure and covariates.
[7] Cross-product of coefficients of path a (Table 2) and path b.

was positively associated with HDL cholesterol concentrations in path c. Because the positive association was no longer significant after additional adjustment for serum lutein + zeaxanthin concentrations, serum lutein + zeaxanthin concentrations had a complete mediation effect on this association. Similarly, the inverse associations between dietary intakes of β-carotene, lycopene, total carotenoids, and plasma tHcy (path c) were completely mediated by the corresponding serum carotenoid concentrations. Serum β-carotene, lycopene, and total carotenoids accounted for 81% (ab/ab + c′), 71%, and 55% of the associations between their intake amounts and tHcy concentrations, respectively. Although dietary carotenoids were not significantly associated with CRP, their serum concentrations were significant mediators in these associations.

We observed significant interactions between dietary β-carotene and serum β-carotene concentrations (P-interaction < 0.05) in their effects on CRP concentrations, and thus interaction terms of the exposure and the mediator were added to the mediational regression models (27). Stratification analysis showed that dietary β-carotene was only associated with CRP with higher serum β-carotene concentrations (**Fig. 2**). For example, individuals in quartile 4 of both dietary β-carotene and serum β-carotene had 47% lower CRP concentrations compared with those in quartile 1 of both dietary β-carotene and serum β-carotene.

Discussion

This study examined the associations between individual dietary carotenoids and serum cholesterols, CRP, and plasma tHcy concentrations in a representative sample of the U.S. population. We also tested mediation/moderation effects of corresponding serum carotenoids on these associations. We observed several significant associations between dietary carotenoids and LDL cholesterol, HDL cholesterol, and tHcy. Most of the associations were completely mediated by corresponding serum carotenoid concentrations. Although dietary carotenoids were not significantly associated with serum CRP concentrations, a significant interaction between dietary β-carotene and serum β-carotene in CRP concentrations was observed, with significant associations between dietary β-carotene intake and CRP observed among individuals with the highest serum β-carotene concentrations.

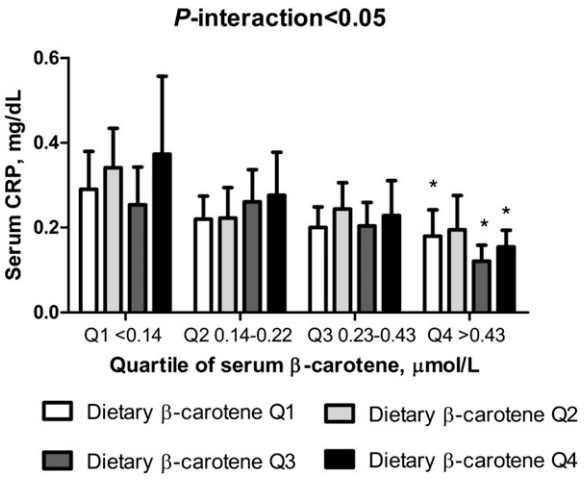

FIGURE 2 Geometric means and 95% CIs of CRP across quartiles of dietary β-carotene and quartiles of serum β-carotene concentrations among 2856 U.S. adults aged ≥20 y in the NHANES 2003–2006. Adjusted for age, gender, ethnicity, BMI, supplement use, alcohol consumption, poverty income ratio, physical activity, history of diabetes, history of high blood cholesterol, prescription medication use in the past month, total energy intake, plasma TGs, and plasma total cholesterol. Range for dietary β-carotene quartiles: <0.38, 0.39–0.93, 0.94–2.42, and ≥2.43 mg/d. *Different from reference, $P < 0.0033$. CRP, C-reactive protein; Q, quartile.

Epidemiologic studies suggest that a high intake of fruits and vegetables is inversely associated with plasma CRP and homocysteine concentrations (29,30). Antioxidants present in fruits and vegetables, such as vitamin C, vitamin E, carotenoids, and flavonoids, may contribute to this anti-inflammatory effect. Associations between dietary carotenoids and CVD risk biomarkers were seldom reported in previous studies. Sluijs et al. (31) documented that intake of lycopene estimated from a validated FFQ consisting of 178 food items was inversely associated with serum TG concentrations among 374 men aged 40–80 y. One cross-sectional study of the NHANES 1999–2002 found that carotene intake was associated with a lower risk of elevated blood CRP and tHcy concentrations (12). In the present study, we observed significant inverse associations between several dietary carotenoids and plasma tHcy concentrations. Although the associations might be confounded by other dietary factors, mediation analysis revealed that serum carotenoids completely mediated these associations.

Although dietary carotenoids were not significantly associated with serum CRP generally, serum carotenoids had significantly inverse associations with serum CRP concentrations in this study, which is similar to the findings reported previously. In an analysis of data from 4557 nonsmokers from the NHANES III, Kritchevsky et al. (5) reported that serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein + zeaxanthin (measured together) were lower in participants with elevated CRP concentrations. A subsequent analysis of the NHANES III by Ford et al. (32) extended the inverse associations between serum carotenoids and CRP concentrations to a larger and broader population consisting of 14,519 men and women, regardless of smoking status. A cross-sectional analysis of 2895 women free of CVD and cancer from the Women's Health Study also found inverse associations between serum α-carotene, β-carotene, and CRP concentrations (6).

One of the strengths of the present study is that both dietary carotenoids and serum carotenoids were examined at the same time. In this study, interestingly, we observed significant interactions between dietary β-carotene and serum β-carotene on serum CRP concentrations, with lower serum CRP concentrations found among participants with both higher dietary and serum β-carotene concentrations. Because previous intervention studies of dietary β-carotene supplements in prevention of CVD failed to evaluate baseline serum carotenoid concentrations or consider such interactions, it is plausible that this failure is 1 of the reasons that most of them reported null findings (33–36) or even adverse effect (37) on CVD outcomes.

In this cross-sectional study, serum lutein + zeaxanthin concentrations were inversely associated with LDL cholesterol and positively associated with HDL cholesterol, maybe because lutein + zeaxanthin are primarily carried by HDL compared with other carotenoid classes (38,39). Although α-carotene is primarily carried by LDL and β-cryptoxanthin is carried equally by LDL and HDL in blood (38,39), we observed positive associations between α-carotene, β-cryptoxanthin, and HDL cholesterol and no association with LDL cholesterol.

The present study has limitations. The cross-sectional study design prevented us from drawing any conclusions as to cause and effect. In addition, although multiple covariates were

carefully adjusted for in our analyses, residual confounding caused by incomplete categorization, misclassification, or unmeasured factors may exist. Because other dietary components might be correlated with both carotenoids and CVD risk biomarkers, we cannot rule out the possibility that other dietary components might confound the associations we observed.

We found that dietary carotenoids were associated with several CVD risk factors in this population-based cross-sectional study. Corresponding serum carotenoid concentrations partially, but in most cases completely, mediated these associations. Meanwhile, serum β-carotene significantly modified the association between dietary β-carotene and serum CRP concentrations. Our findings suggested that serum carotenoids, although moderately correlated with dietary amounts, are significant mediators and/or moderators in the relation between dietary carotenoids and CVD risk biomarkers. Future clinical trials that study dietary carotenoids or carotenoid supplement in CVD prevention should monitor serum carotenoid concentrations during follow-up for better interpretation of the findings.

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Y.W. and O.K.C. designed the study. S.-J.C. helped with dataset preparation. Y.W. conducted the statistical analysis and drafted the manuscript. M.L.M., W.O.S., M.L.F., and S.I.K. provided professional advice. O.K.C. had primary responsibility for the final content. All authors were involved in the data interpretation and manuscript preparation. All authors read and approved the final manuscript.

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