Relationships of Circulating Carotenoid Concentrations with Several Markers of Inflammation, Oxidative Stress, and Endothelial Dysfunction: The Coronary Artery Risk Development in Young Adults (CARDIA)/Young Adult Longitudinal Trends in Antioxidants (YALTA) Study

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Background: Serum carotenoid concentrations relate inversely to cardiovascular disease incidence. To clarify the effect of carotenoids on atherosclerotic risk factors, we examined the association of circulating carotenoids with inflammation, oxidative stress, endothelial dysfunction, and smoking.

Methods: Black and white men and women in the Coronary Artery Risk Development in Young Adults study, ages 18 to 30 years at recruitment (1985–1986) from 4 US cities, were investigated over 15 years. We included 2048 to 4580 participants in analyses of the sum of serum α-carotene, β-carotene, zeaxanthin/lutein, and β-cryptoxanthin concentrations and of lycopene at year 0 and at year 7.

Results: The year 0 sum of 4 carotenoids was inversely associated (all $P < 0.05$) with year 0 leukocyte count (slope per sum carotenoid SD, -0.17); year 7 fibrinogen (slope, -0.10); year 7 and year 15 C-reactive protein (slope, -0.12 and -0.09); and year 15 F_2-isoprostanes (slope, -13.0), soluble P-selectin (slope, -0.48), and soluble intercellular adhesion molecule-1 (sICAM1; slope, -5.1). Leukocyte counts and sICAM1 and F_2-isoprostane concentrations had stronger associations in smokers than in nonsmokers, and sICAM1 concentrations were higher in the highest carotenoid quartile in smokers than in the lowest carotenoid quartile in nonsmokers. Superoxide dismutase was positively associated with the sum of 4 carotenoids (slope, 0.12; $P < 0.01$). Lycopene was inversely associated only with sICAM1. The year 7 carotenoid associations with these markers were mostly similar to those at year 0.

Conclusions: Circulating serum carotenoids were associated, some interactively with smoking, in apparently beneficial directions with markers of inflammation, oxidative stress, and endothelial dysfunction.
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Several epidemiologic studies have proposed an inverse relationship between serum carotenoids and cardiovascular disease (1–4). Inflammation, oxidative stress, and endothelial dysfunction are known to be associated with atherosclerosis and cardiovascular diseases (5–11). Although several cross-sectional studies reported that serum carotenoids are related inversely to these markers of the atherosclerosis pathway (12–15), we know of no

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studies that investigated whether carotenoid concentrations predict the future values of these markers.

We conducted cross-sectional and longitudinal analyses to clarify whether circulating carotenoids are associated with markers of oxidative stress, inflammation, and endothelial dysfunction. In each case we also investigated whether the relation of serum carotenoid concentrations with these variables differed according to smoking status, following our prior findings of an interaction of smoking with circulating carotenoids and several other variables (16, 17).

Materials and Methods

THE CORONARY ARTERY RISK DEVELOPMENT IN YOUNG ADULTS AND YOUNG ADULT LONGITUDINAL TRENDS IN ANTIOXIDANTS STUDIES

The Coronary Artery Risk Development in Young Adults (CARDIA)[6] study, a biethnic, prospective, multicenter epidemiologic study of the evolution of risk factors in young adults, has been described (18). Briefly, from 1985 to 1986, 5115 African American and white individuals (ages 18 to 30 years) were examined in Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. At the Birmingham, Minneapolis, and Chicago sites, participants were randomly selected from total communities or from specific census tracts. In Oakland, random selection was from members of the Kaiser Permanente Medical Care Program. Recruitment achieved nearly equal numbers at each site of race (African American, white), sex, education (high school or less, more than high school), and age (18–24 years, 25–30 years). Fifty percent of invited individuals contacted were examined (47% of African Americans and 60% of whites) and became the CARDIA cohort. Young Adult Longitudinal Trends in Antioxidants (YALTA) is a CARDIA ancillary study in which serum carotenoid concentrations were measured in most CARDIA participants at years 0 and 7, and circulating extracellular superoxide dismutase (SOD), F_2-isoprostanes, soluble P-selectin, and soluble intercellular adhesion molecule-1 (sICAM1) were measured at year 15.

We excluded individuals with missing data on smoking status (n = 20) or serum carotenoids (n = 302), confounding factors (n = 80), or who were not fasting for at least 8 h (n = 113) at year 0, leaving 4600 (89.9%) participants for analysis. After excluding the participants for whom the given measurement data were not available (not measured or participant nonfasting) or [in C-reactive protein (CRP) analyses only] who had CRP concentrations ≥10 mg/dL (possible acute inflammation), the number of participants for study of year 0 carotenoids and year 0 leukocyte counts was 4580; numbers associated with other analytes are provided in Tables 1–4.

SELF-REPORT AND ANTHROPOMETRIC MEASUREMENTS

Sex, race, date of birth, medication use, and weekly alcohol consumption were determined at each examination by structured interview or self-administered questionnaire. Participants were classified at year 0 as never, former, or current smokers (interviewer-administered questionnaire) as of year 0; the 186 "nonsmoking" participants and the 23 with missing smoking status whose year 0 cotinine concentration was ≥79.5 nmol/L (14 ng/mL) were reclassified as current smokers. A physical activity score was derived from the CARDIA Physical Activity History, a simplified version of the Minnesota Leisure Time Physical Activity Questionnaire (19). Alcohol intake (mL/day) was computed from the self-reported frequency of beer, wine, and liquor consumed per week. The interviewer-administered CARDIA diet history was obtained at years 0 and 7 and included 1609 unique food codes at either year. Body weight with light clothing was measured to the nearest 0.1 kg (0.2 pounds), and height without shoes was measured to the nearest 0.5 cm. Body mass index (BMI) was weight/height squared (kg/m^2).

COLLECTION, PROCESSING, AND WHOLE BLOOD MEASUREMENT

All participants were fasting for ≥8 h and asked to avoid smoking and heavy physical activity for at least 2 h before blood collection at each examination. Leukocyte counts at CARDIA year 0 were measured as cells ($\times 10^9$) per liter whole blood using a leukocyte counter in the local center hospital laboratory. After plasma or serum separation from whole blood, aliquots were stored at −70 °C until they were shipped on dry ice to a central laboratory.

YALTA used sera obtained at CARDIA years 0 and 7 to assay the carotenoids α- and β-carotene, lycopene, zeaxanthin/lutein, and β-cryptoxanthin (Molecular Epidemiology and Biomarker Research Laboratory, University of Minnesota), with an HPLC-based assay modified from the method of Bieri et al. (20) to optimize detection of carotenoids with calibration as described by Craft et al. (21) and sample handling as described by Gross et al. (22). Calibration was performed with pure compounds [Hoffmann-La Roche; Sigma Chemical Co. (now Sigma-Aldrich)]. Quality-control procedures included routine analysis of plasma and serum control pools containing high and low concentrations of each analyte. In addition, the laboratory routinely analyzed NIST reference sera and was a participant in the NIST Fat-Soluble Vitamin Quality Assurance Group. The CVs were <10% for all analytes and control pools. The intraclass correlation coefficients (between-person variance/between-person plus within-person variance) were 0.93 for α-carotene, 0.98 for β-carotene, 0.73 for zeaxanthin/lutein, 0.97 for β-cryptoxanthin, and 0.73 for lycopene (23).

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[6] Nonstandard abbreviations: CARDIA, Coronary Artery Risk Development in Young Adults; YALTA, Young Adult Longitudinal Trends in Antioxidants; SOD, superoxide dismutase; sICAM1, soluble intercellular adhesion molecule-1; BMI, body mass index; CRP, C-reactive protein; GGT, γ-glutamyltransferase.

Year 0 and year 7 lipid concentrations were measured by the University of Washington Northwest Lipid Research Clinic Laboratory. Triglycerides and HDL-cholesterol were measured by enzymatic procedures, with HDL-cholesterol measured after dextran sulfate–magnesium precipitation and LDL-cholesterol calculated using the Friedewald equation.

Year 5 fibrinogen was assessed by the Clauss method using reagents from the Dade Division, Baxter Healthcare Corp. *(24)*. Year 7 fibrinogen antigen was measured using the BN-II nephelometer (N Antiserum to Human Fibrinogen; Dade Behring Inc.), calibrated with standard normal plasma (SNP reagent, Dade). A high-sensitivity ELISA measured year 7 and year 15 serum CRP at the Department of Pathology, University of Vermont, using a BN-II analyzer *(25)*. Year 15 extracellular SOD activity (U/mL) was assayed according to Cayman Chemical kit procedures. The protocol was followed exactly, and the samples were diluted 1:2 with sample buffer. This analysis was automated with a Beckman Biomek. A human soluble P-selectin immunoassay was performed by ELISA with reagent sets supplied by R&D Systems, Inc, modified by pipetting by Biomek-robotic automation (Beckman) and a 1:5 dilution rather than the recommended 1:20 dilution. A human sICAM1 immunoassay was measured by ELISA with reagent sets supplied by R&D systems, Inc. Year 15 plasma free F_2-isoprostanes were measured by gas chromatography–mass spectrometry at the Molecular Epidemiology and Biomarker Research Laboratory at the University of Minnesota (Minneapolis, MN) *(8)*. Year 10 γ-glutamyltransferase (GGT) was measured with a SMAC II continuous-flow analyzer (Technicon Instruments Corp.) at American Bio-science Laboratories (now Smith-Kline Beecham). At year 10, GGT was measured colorimetrically with nitroanilide methodology on a Roche Cobas Mira Plus chemistry instrument at Linco Research Inc. *(17)*.

STATISTICAL ANALYSIS

The CARDIA and YALTA studies did not measure each analyte at each examination. Timing of measurements included in this report is provided in Table 1. In these analyses, we often related other variables to the sum of α-carotene, β-carotene, zeaxanthin/lutein, and β-cryptoxanthin; we omitted lycopene from this sum because its associations with many variables were strikingly different from those of the other 4 carotenoids. Most baseline characteristics are presented as prevalence and means of dependent variables across quartiles of year 0 serum carotenoid–independent variables with adjustment for age, sex, race, center, and education. We used multiple linear regression analyses to calculate the adjusted means of markers of inflammation, oxidative stress, and endothelial dysfunction, adjusting for center, race, sex, and year 0 values of education (years), age (years), total energy intake, alcohol consumption (mL/day), BMI (kg/m²), physical activity (continuous), LDL-cholesterol (con-

Table 1. Year of measurement of serum carotenoids and blood measures investigated in relation to them.[a]

	Year 0	Year 5	Year 7	Year 15
Carotenoids	•		•	
Leukocyte count	A			
C-reactive protein			A, B	A, B, C
Fibrinogen		A	A, B	
Superoxide dismutase				A, B
F_2-isoprostanes				A, B
P-selectin				A, B
Soluble ICAM1				A, B

[a] A, Variable investigated in relation to year 0 carotenoids. B, Variable investigated in relation to year 7 carotenoids. C, Change in the variable investigated in relation to year 7 carotenoids.

tinuous), systolic blood pressure, use of vitamin supplements (A, C, or E), and cigarette smoking (never, former, or current; further separation of current smokers by dose did not affect findings). Further adjustment for HDL-cholesterol (continuous) and triglycerides (continuous), as we have recommended *(26)*, did not basically alter the findings. For year 7 carotenoid analyses, we used year 7 covariates. We further separated the year 7 carotenoids into 2 components: the year 0 sum of 4 carotenoids and the difference (year 7 sum of 4 carotenoids − year 0 sum of 4 carotenoids). We included these 2 components in a joint model. In addition, we analyzed the relationships of the year 0 sum of 4 carotenoids with year 15 markers of inflammation, oxidative stress, and endothelial dysfunction, adjusted for each other.

We present analyses based on baseline smoking data; excluding participants who had changed smoking status at the time of measurement of each biochemical marker did not greatly alter findings. In all analyses except those presented in Table 1, we combined former and never smokers as nonsmokers after confirming that oxidative stress was lower in former smokers than in current smokers. Interaction models included a product term.

Results

YEAR 0 BASELINE CHARACTERISTICS

Among 4580 participants with measured year 0 leukocyte counts, 55.0% were women, 49.4% were white, and 60.5% had more than high school education. Mean (SD) age was 24.8 (3.6) years at year 0. At year 0, 34.3%, 11.6%, and 54.1% were current, former, and never smokers, respectively. The mean sum of 4 serum carotenoids was 80.3 (43.5) nmol/L, and that of serum lycopene was 55.6 (27.0) nmol/L.

Study participants with a higher year 0 sum of 4 serum carotenoids tended to be older, white, female, more highly educated, and less likely to be current smokers (Table 2). Year 0 BMI and total energy intake correlated inversely with the sum of 4 serum carotenoids. Vegetable or fruit intake and use of vitamin A, C, or E supplements (all of which may increase serum carotenoid concentra-

Table 2. Year 0 characteristics (mean or percentage) according to quartile of serum carotenoid concentration.[a]

Year 0 characteristic	Overall	Year 0 sum of 4 carotenoid quartile, nmol/L				Year 0 lycopene quartile, nmol/L			
		10.0–51.3	51.3–71.6	71.6–97.9	97.9–574.9	1.2–35.0	35.1–52.5	52.6–72.0	72.1–177.9
n	4580	1145	1142	1146	1147	1144	1144	1144	1148
Age (mean), year	24.8	24.3	24.6	24.8	25.7	25.1	24.8	24.6	24.8
Sex, % female	55.0	50.0	52.5	54.1	63.4	60.7	58.0	53.5	48.0
Race, % whites	49.4	47.3	43.3	48.3	58.6	50.5	49.4	49.8	47.7
Center									
Birmingham	23.4	27.7	27.8	22.4	15.7	19.3	24.0	25.3	25.0
Chicago	21.6	18.4	21.1	21.3	25.4	14.2	22.3	24.7	25.0
Minneapolis	27.8	29.4	28.7	28.7	24.3	23.0	24.7	28.4	35.0
Oakland	27.3	24.5	22.3	27.6	34.6	43.5	29.0	21.5	15.0
Education									
<12 years, %	9.9	15.7	10.9	8.7	4.2	8.9	11.1	10.8	8.7
12 years, %	29.6	37.8	32.6	27.7	20.5	28.6	31.2	28.8	29.9
≥13 years, %	60.5	46.5	56.5	63.6	75.3	62.5	57.7	60.3	61.4
Smoking status									
Current, %[b]	34.3	46	39	31	21	34	34	34	35
Former, %[b]	11.6	9	10	14	14	12	11	12	11
Never, %[b]	54.1	45	51	56	65	54	55	54	54
Total energy intake (mean), kcal/day[b]	2957	3050	3017	2947	2815	2959	2955	2960	2954
Fish intake (mean), times/week[b]	1.7	1.5	1.6	1.7	1.8	1.8	1.7	1.6	1.6
Meat intake (mean), times/week[b]	10.8	11.4	11.1	11.0	9.7	10.2	10.8	11.0	11.2
Vegetable intake (mean), times/week[b]	15.8	13.8	15.4	16.1	18.0	15.8	15.4	16.1	16.1
Fruit intake (mean), times/week[b]	7.0	5.4	6.6	7.2	8.7	7.3	7.0	7.0	6.6
Ethanol intake (mean), g/week[b]	12.1	16.5	12.7	10.8	8.6	11.1	12.1	12.2	13.1
Systolic BP (mean), mmHg[b]	110	112	111	110	109	110	111	110	111
BMI (mean), kg/m^2[b]	24.5	25.9	24.5	24.2	23.3	24.8	24.5	24.1	24.5
Physical activity (mean), exercise units[b]	419	395	411	432	437	418	422	422	414
Use of vitamin supplements (yes), %[b,c]	30	25	29	31	36	31	31	32	28
Total cholesterol (mean), mmol/L[b]	4.57	4.32	4.46	4.67	4.81	4.29	4.44	4.58	4.96
LDL cholesterol (mean), mmol/L[b]	2.82	2.61	2.73	2.91	3.01	2.59	2.70	2.81	3.16
HDL cholesterol (mean), mmol/L[b]	1.38	1.29	1.35	1.38	1.47	1.34	1.35	1.39	1.41
Triglycerides (mean), mmol/L[b]	0.82	0.92	0.81	0.82	0.72	0.78	0.83	0.82	0.84
Fasting blood glucose (mean), mg/dL[b]	4.58	4.61	4.58	4.58	4.54	4.57	4.57	4.59	4.58
α-Carotene (mean), nmol/L[b]	5.0	2.1	3.3	4.6	10.0	5.1	4.9	5.0	5.0
β-Carotene (mean), nmol/L[b]	28.2	11.8	19.4	27.7	53.8	25.4	127.2	28.9	31.3
Zeaxanthin/lutein (mean), nmol/L[b]	32.3	18.9	27.6	35.2	47.3	25.4	31.5	34.6	37.6
β-Cryptoxanthin (mean), nmol/L[b]	14.8	7.2	11.6	15.6	24.7	13.3	14.3	15.2	16.3
Sum of 4 carotenoids (mean), nmol/L[b,d]	80.3	40.1	61.9	83.1	135.8	69.2	77.9	83.6	90.2
Lycopene (mean), nmol/L[b]	55.6	42.8	55.1	61.0	63.3	24.2	44.1	62.0	91.8

[a] CARDIA 1985–2001. Restricted to 4580 participants in whom leukocyte count was assessed at year 0. n, number of participants; BP: blood pressure.

[b] Adjusted for age, sex, race, race·sex, center, and education.

[c] Vitamin supplements: vitamins A, C, and E and carotenoids.

[d] Sum of 4 serum carotenoids: α-carotene, β-carotene, zeaxanthin/lutein, and β-cryptoxanthin.

tions) were more frequent in higher serum carotenoid groups, whereas with meat or alcohol intake the carotenoid concentrations were lower. These results suggest that those with a higher sum of 4 serum carotenoids tended to have healthier lifestyles. Total and HDL cholesterol were higher in higher serum carotenoid groups, as anticipated given that the carotenoids are fat-soluble (26). This same pattern emerged individually for α- and β-carotene, zeaxanthin/lutein, and β-cryptoxanthin (data not shown). However, those with higher lycopene tended to have less healthy lifestyles. Mean intakes of meat and alcohol and concentrations of triglycerides were higher across lycopene quartiles, whereas intake of fruit and use of vitamin supplements were lower. In more specific analysis of food groups, lycopene concentration was positively related to tomatoes, ketchup, pickles, dairy desserts, low-fat cheese, red meat obtained at fast food restaurants, other red meat, and onions and inversely related to energy intake, tofu, and canned fish (data not shown). Lycopene was unrelated to cigarette smoking.

YEAR 0 SERUM CAROTENOID AND MARKERS OF
INFLAMMATION

There were inverse relationships of the sum of 4 caro-
tenoids (Table 2) and of α-carotene and β-carotene, zea-
xanthin/lutein, and β-cryptoxanthin individually (see
Table 1 in the Data Supplement that accompanies the
online version of this article at http://www.clinchem.org/
content/vol53/issue3) with year 0 leukocyte counts, a
relationship that was stronger in smokers than in non-
smokers (slope of leukocyte counts per SD of the sum of
4 carotenoids, −0.37 in smokers and −0.11 in nonsmok-
ers; P for interaction 0.01; Fig. 1A). However, year 0
leukocyte count was unrelated to circulating lycopene
(Table 3).

The year 0 serum sum of 4 carotenoids was also
inversely related to year 7 and year 15 CRP concentra-
tions; each year 0 carotenoid, except for lycopene, was
inversely related to these 2 variables. Furthermore, there
was no interaction between year 0 serum carotenoids and
year 0 smoking status in estimating CRP concentrations
(range of P for interaction: year 7, 0.04−0.97; year 15,
0.16−0.98).

The year 0 sum of 4 carotenoid concentrations was not
related to year 5 fibrinogen but was inversely related to
year 7 fibrinogen concentrations, as measured with the
more precise nephelometric method. No interactions be-
tween serum carotenoids and year 0 smoking were ob-
served in estimating fibrinogen concentration (range of
P for interaction, 0.10−0.91).

YEAR 0 CAROTENOIDS AND YEAR 15 OXIDATIVE
STRESS AND ENDOTHELIAL DYSFUNCTION

The sum of 4 carotenoids and each individual carotenoid
except lycopene were positively related to year 15 SOD
(Table 3). No interactions were observed between caro-
tenoids and smoking in predicting SOD activity (range of
P for interaction, 0.17−0.95).

Plasma F_2-isoprostane concentration was inversely re-
lated to the sum of 4 carotenoids and to each carotenoid

except lycopene. This inverse relationship of the sum of 4
carotenoids with plasma F_2-isoprostanes was stronger in
current smokers than in nonsmokers (Fig. 1B; slope of
F_2-isoprostanes per SD increase of sum of 4 carotenoids,
−26.8 in smokers and −10.5 in nonsmokers; P for inter-
action <0.01).

Soluble P-selectin concentration was significantly in-
versely related to the sum of 4 carotenoids and β-carotene,
with nonsignificant, shallow inverse relationships with
each of the other carotenoids. No interactions of caro-
tenoids and smoking in estimating P-selectin were ob-
served (range of P for interaction, 0.35−0.94).

sICAM1 was inversely related to the sum of 4 caro-
tenoids and to each individual carotenoid, including
lycopene. The inverse relationship of the sum of 4 caro-
tenoids with sICAM1 was more evident in current smok-
ers than nonsmokers (Fig. 1C; slope of sICAM1 per SD
increase of sum of 4 carotenoids in current smokers,
−10.7; in nonsmokers, −4.1; P for interaction = 0.03).

We previously reported that the inverse relationship of
serum carotenoids with GGT was stronger in current or
former smokers than in never smokers *(17)*. We recom-
puted geometric mean year 10 GGT for consistency with
this paper (comparing nonsmokers with current smok-
ers); the resulting inverse associations, stronger in smok-
ers than in nonsmokers, are shown in Fig. 1D.

YEAR 7 CAROTENOIDS AS BASELINE

Findings in relation to year 7 serum carotenoids mostly
corroborated those reported above for year 0 carotenoids
(Table 4; and Table 2 in the online Data Supplement).

We also studied year 7 carotenoid concentrations and
change in CRP concentration between years 7 and 15 (n =
2142). The slope of log-transformed year 15 CRP adjusted
for year 7 CRP per SD of sum of 4 carotenoids for all
participants was −0.02; P for slope = 0.41; with no
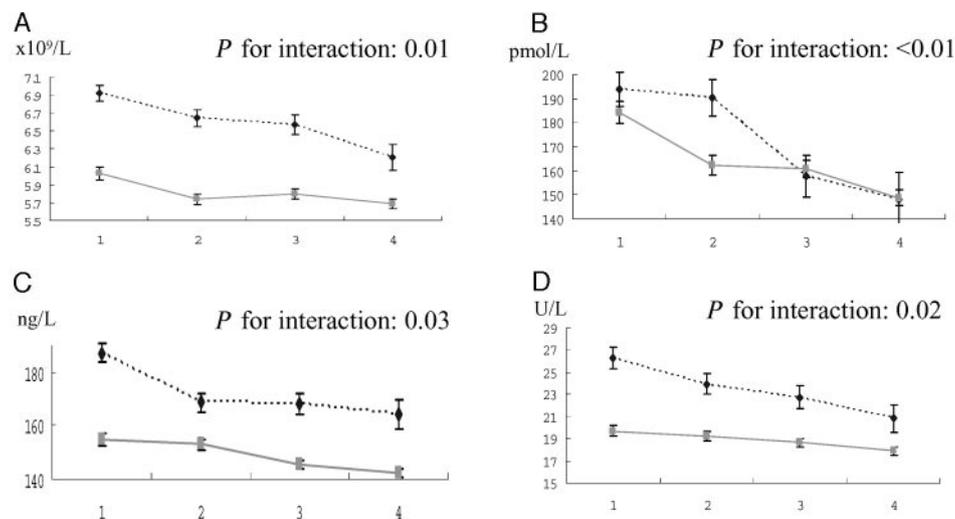difference between nonsmokers and smokers.

Fig. 1. The relationships of the year 0
sum of 4 serum carotenoid concentra-
tions with year 0 leukocyte count (*A*),
year 15 F_2-isoprostanes (*B*), year 15
sICAM1 (*C*), and year 10 geometric
means of GGT according to year 0
smoking status (current vs nonsmoker)
(*D*).

Solid line, data in nonsmokers; *broken line*,
current smokers. *Error bars* represent 1 stan-
dard error.

Table 3. Relation of year 0 serum carotenoids with markers of inflammation, oxidative stress, and endothelial dysfunction measured at year 0, 5, 7, and 15.[a]

Biochemical marker	Year 0 carotenoids	Q1	Q2	Q3	Q4	Slope[b]	P for slope
Year 0 leukocyte count, $\times 10^9$/L	Sum of 4 carotenoids[c]	6.36	6.06	6.07	5.89	−0.17	<0.01
(n = 4580, SD = 1.79)[d,e]	Lycopene	6.05	6.13	6.05	6.16	0.03	0.30
Year 7 CRP (mg/L)	Sum of 4 carotenoids	1.20	1.16	1.04	0.88	−0.12	<0.01
(n = 3039, SD (ln scale) = 1.02)[f]	Lycopene	1.04	1.11	0.99	1.11	0.00	0.92
Year 15 CRP, mg/L	Sum of 4 carotenoids	1.37	1.31	1.28	1.05	−0.09	<0.01
(n = 2581, SD (ln scale) = 1.03)[f]	Lycopene	1.23	1.28	1.28	1.19	−0.03	0.21
Year 5 Fibrinogen (mmol/L)[e]	Sum of 4 carotenoids	7.77	7.73	7.78	7.72	−0.01	0.65
(n = 3515, SD = 1.48)	Lycopene	7.74	7.72	7.73	7.81	0.02	0.41
Year 7 Fibrinogen, mmol/L[e]	Sum of 4 carotenoids	9.94	10.00	9.94	9.75	−0.10	<0.01
(n = 3250, SD = 1.94)	Lycopene	9.89	9.90	9.87	9.97	0.04	0.25
Year 15 Superoxide dismutase (U/mL)[e]	Sum of 4 carotenoids	4.55	4.59	4.57	4.86	0.12	0.02
(n = 2346, SD = 2.22)	Lycopene	4.77	4.62	4.62	4.58	−0.06	0.27
Year 15 Plasma F_2-isoprostanes, pmol/L[e]	Sum of 4 carotenoids	188	172	161	150	−13.0	<0.01
(n = 2395, SD = 84)	Lycopene	172	165	165	167	−1.9	0.34
Year 15 P-selectin (ng/L)[e]	Sum of 4 carotenoids	37.6	36.6	37.5	36.5	−0.48	0.04
(n = 2385, SD = 10.6)	Lycopene	37.0	37.4	37.0	36.7	−0.14	0.56
Year 15 Soluble ICAM1, ng/L[e]	Sum of 4 carotenoids	167	158	154	150	−5.1	<0.01
(n = 2357, SD = 41)	Lycopene	162	157	157	152	−3.6	<0.01

[a] CARDIA 1985–1986 to 2000–2001. Values adjusted for race; sex; center; age; education; hypertension; diabetes; ethanol intake; LDL cholesterol; total energy intake; BMI; physical activity; use of vitamin A, C, E, and carotenoid supplements; and smoking status. Participants with CRP concentrations >10 mg/dL were excluded from CRP analyses to avoid any effect of acute inflammation.

[b] β-Coefficient per SD of carotenoid.

[c] Sum of 4 carotenoids: sum of α-carotene, β-carotene, zeaxanthin/lutein, and β-cryptoxanthin.

[d] SD of the given variable in the adjusted model.

[e] Adjusted mean.

[f] Adjusted geometric mean (SD is given on the ln scale because it is not well-defined on the arithmetic scale for a skewed variable).

COMPARISONS AMONG SEVERAL CARDIOVASCULAR RISK FACTORS AND CAROTENOIDS

Adjustment among the year 15 markers attenuated their associations with the year 0 sum of 4 carotenoid concentrations compared with those reported in Tables 2 and 3, but all remained statistically significant except for P-selectin. The slopes of the year 15 measures regressed on the year 0 sum of 4 carotenoid concentrations and adjusted for each other (n = 2096) were SOD, $\beta = 0.11$, $P =$ 0.04; F2-isoprostanes, $\beta = -10.8$, $P < 0.01$; sICAM1, $\beta = -3.3$, $P < 0.01$; CRP, $\beta = -0.07$, $P < 0.01$; and P-selectin, $\beta = -0.14$, $P = 0.58$.

Discussion

Serum total and individual carotenoids, with the exception of lycopene, were inversely associated with markers of inflammation, oxidative stress, and endothelial dysfunction even after adjustment in multiple regression

Table 4. Relation of year 7 sum of 4 carotenoids with markers of inflammation, oxidative stress, and endothelial dysfunction measured at years 7 and 15.[a]

Biochemical marker	Slope[b]	P for slope
Year 7 CRP (n = 3044), mg/L[c]	−0.17	<0.01
Year 15 CRP (n = 2250), mg/L[c]	−0.12	<0.01
Year 7 fibrinogen (n = 3220), mmol/L[d]	−0.14	<0.01
Year 15 Superoxide dismutase (n = 2041), U/mL[d]	0.04	0.45
Year 15 Plasma F_2-isoprostanes (n = 2078), pmol/L[d]	−15.6	<0.01
Year 15 P-selectin (n = 2078), ng/L[d]	−0.75	<0.01
Year 15 Soluble ICAM1 (n = 2048), ng/L[d]	−5.09	<0.01

[a] CARDIA 1992–1993 to 2000–2001. Values adjusted for race; sex; center; age; education; hypertension; diabetes; ethanol intake; LDL cholesterol; total energy intake; BMI; physical activity; use of vitamin A, C, E, and carotenoid supplements; and smoking status. Sum of 4 carotenoids: sum of α-carotene, β-carotene, zeaxanthin/lutein, and β-cryptoxanthin. Participants with CRP concentrations >10 mg/dL were excluded from CRP analyses to avoid any effect of acute inflammation.

[b] β-Coefficient per SD of carotenoid.

[c] Adjusted geometric mean.

[d] Adjusted mean.

analyses. These associations support the inverse relation of carotenoids with cardiovascular diseases that has been reported by others *(1–4)*, in that they provide numerous pathways that might mediate the carotenoid and atherosclerotic disease relationship.

Consistent with some previous cross-sectional studies that reported an inverse association between carotenoids and markers of systemic inflammation *(12–15)*, we observed significant inverse associations between carotenoids and leukocyte counts, CRP, and fibrinogen. A recent experimental study reported that intervention with a high intake of carotenoid-rich fruit and vegetables reduced plasma CRP in healthy, nonsmoking men *(27)*.

Serum carotenoids may also beneficially affect the oxidative stress pathway to atherosclerosis, consistent with our findings for 2 biochemical variables in this pathway: positive correlations with SOD, an important antioxidant enzyme, and negative relations with F2-isoprostanes, formed in vivo from systemic oxidative damage *(28)*, with the reaction of free radicals and arachidonic acid *(29)*. Our findings are in line with some clinical trials *(30, 31)* on SOD and our previous report that most year 0 circulating antioxidants measured in YALTA, except for lycopene and γ-tocopherol, related inversely to GGT activity, which we believe indicates oxidative stress *(32)*.

One cross-sectional study reported an inverse association of sICAM1 with lutein and lycopene *(12)*. Our findings were similar: i.e., sICAM1 related inversely to all carotenoids, including lycopene. Our results were also consistent with an in vitro study of confluent human aortic endothelial cell cultures incubated for 24 h with each of the 5 most prevalent carotenoids in human plasma (α-carotene, β-carotene, β-cryptoxanthin, lutein, and lycopene); pretreatment with β-carotene, lutein, and lycopene significantly reduced the expression of sICAM-1 *(33)*.

There was evidence that smoking modified relations of circulating carotenoids with some of these biomarkers. GGT *(17)* and, as shown here, F_2-isoprostanes tend to be higher in smokers than nonsmokers. Plasma sICAM1 is also known to be higher in chronic smokers and is dose-related to daily cigarette consumption *(34)*. Furthermore, the inverse associations of year 0 leukocyte counts, year 15 F_2-isoprostanes, year 15 sICAM1, and year 10 GGT with carotenoids were stronger in current smokers than in nonsmokers (all *P* for interaction ≤0.02). Although mean F_2-isoprostanes were similar in smokers and nonsmokers in the highest carotenoid quartile, for GGT, leukocyte counts, and sICAM1, smokers in the highest quartile of the sum of 4 carotenoids actually showed higher mean concentrations than nonsmokers in the lowest quartile (Fig. 1). Because cigarette smoking leads to increased cumulative exposure to both endogenous and exogenous reactive oxygen species, cigarette smokers are exposed to higher oxidative stress than are nonsmokers *(35)*. Smoking can alter β-carotene metabolism and may alter the metabolism of other carotenoids, particularly when they are present in high concentrations, and especially with the use of supplements *(36)*. For example, ferrets living for 6 months in a smoky environment (urinary cotinine equivalent to 30 cigarettes/day in humans) had altered β-carotene metabolism compared with ferrets living in a smoke-free environment *(37, 38)*. Postmortem in vitro study of lung tissue showed evidence of altered β-carotene metabolism. Specifically, metabolites formed from excentric cleavage (e.g., β-apo-carotenals) were much more abundant in the ferrets living in the smoky environment than those in the smoke-free environment *(37, 38)*. Simultaneously, there were decreased concentrations of β-carotene, retinol, and retinoic acid in the ferrets exposed to smoke. Lower concentrations of retinol and retinoic acid are known to inhibit cell differentiation; consequent increased cell proliferation in the smoky environment may therefore promote cancer *(39, 40)*. It is possible that the alterations in β-carotene metabolism may also have an impact on atherosclerosis. Exposure of rats to smoke demonstrated the complex interaction of smoking and carotenoids *(41)*. The short-term beneficial effect of passive smoke on experimental myocardial infarction (by inuring the myocardium to small doses of oxidative stress) was negated by simultaneous administration of carotenoids, presumably by neutralizing the effects of free radicals. Our findings are consistent with the supposition that smoking changes metabolism of carotenoids, differently in different pathways. Thus, although higher serum carotenoids might partially neutralize the harmful oxidative stress due to smoking, such benefit did not completely negate the smoking effect on many endpoints demonstrated herein and did not reduce risk for incident diabetes or insulin resistance *(16)*.

We cannot discern whether the apparently beneficial effects were directly attributable to higher serum carotenoid concentrations or to other mechanisms strongly correlated with the carotenoids. For example, circulating carotenoids may reflect only a healthy diet and intake of dietary constituents that are highly correlated with intake of carotenoids. The absence of benefit of isolated β-carotene supplements in clinical trials of clinical outcomes, especially in smokers *(42, 43)*, may indicate that the circulating carotenoids are markers of other important physiologic processes, rather than acting directly on the physiology. The consistently weak associations with lycopene, a strong antioxidant, further support the notion that the circulating carotenoids mark lifestyle and other physiologic processes. If antioxidant properties were the only basis for carotenoid associations, lycopene should have associations similar to the other carotenoids. Another study has reported that lycopene was not related to any of the food groups they examined *(44)*. The present study showed that participants with higher lycopene tended to have less healthy lifestyles; mean intakes of meat and alcohol and concentrations of triglycerides were higher across lycopene quartiles, whereas intake of fruit and use of vitamin supplements were lower. The CARDIA sample includes many younger adults who frequent fast-food

restaurants. Ketchup in fast foods may be a major source of lycopene for them; fast food consumption has been shown in this cohort to result in weight gain and insulin resistance (45).

Limitations of the study included lack of measurement of several biochemical variables at year 0, and the study could not confirm whether the associations with higher concentrations of carotenoids were already present at baseline or had evolved during the study. Whether any of the observed interrelationships is causative remains unknown from studies like ours. An interpretive limitation subsumes the large number of comparisons inherent in this project, so that some apparent findings may actually have arisen by chance. However, our analyses were hypothesis-driven, and considerably more consistency was seen across biological pathways than would be expected if multiple comparisons were a large problem.

In conclusion, carotenoids were inversely associated with future oxidative stress, inflammation, and endothelial dysfunction. Although many of these relationships were not associated with smoking status, some were, consistent with the hypothesis that protective mechanisms are working harder but less effectively in smokers. Therefore, these findings offer additional insight into pathways by which higher concentrations of most serum carotenoids, or their specific beneficial components, might help to prevent cardiovascular diseases.

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