



Original Contribution

Associations of Serum Carotenoid Concentrations with the Development of Diabetes and with Insulin Concentration: Interaction with Smoking

The Coronary Artery Risk Development in Young Adults (CARDIA) Study

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Smoking is associated with low serum carotenoid concentrations. Prospective studies have found lower diabetes risk among persons with high-carotenoid diets. Whether diabetes risk is low in the rare smoker who has high serum carotenoid levels is unknown. The authors investigated the interaction of serum carotenoid concentrations and smoking with diabetes mellitus in 4,493 Black and White men and women aged 18–30 years in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. The authors assessed 15-year (1985–2001) incident diabetes (148 cases), insulin concentration, and insulin resistance (homeostasis model assessment) in smokers and non-smokers according to baseline levels of serum α -carotene, β -carotene, zeaxanthin, β -cryptoxanthin, and lycopene. Diabetes incidence was inversely associated with the sum of carotenoid concentrations in nonsmokers (per standard deviation (SD) increase, relative hazard = 0.74, 95% confidence interval: 0.55, 0.99) but not in current smokers (relative hazard = 1.13, 95% confidence interval: 0.83, 1.53) (p for interaction = 0.02). Similarly, year 15 insulin and insulin resistance values, adjusted for baseline levels, were inversely related to sum of carotenoids only in nonsmokers (per SD increase in insulin level, slope = -0.46 ($p = 0.03$); per SD increase in insulin resistance, slope = -0.14 ($p = 0.01$)). In CARDIA, higher serum carotenoid concentrations are associated with lower risk of diabetes and insulin resistance in nonsmokers but not in smokers.

carotenoids; diabetes mellitus; insulin resistance; prospective studies; smoking

Abbreviations: CARDIA, Coronary Artery Risk Development in Young Adults; HOMA, homeostasis model assessment; YALTA, Young Adult Longitudinal Trends in Antioxidants.

Oxidative stress may play a role in the pathophysiology of diabetes mellitus (1, 2). Like diabetes, cigarette smoking results in oxidative stress (3). Therefore, carotenoids—plant-derived, fat-soluble pigments that reduce oxidation by efficiently quenching the production of singlet oxygen

and free radicals (4)—should reduce risk for diabetes and adverse consequences of smoking.

Some prospective studies (5–12), but not all (13, 14), have found that a higher intake of fruit or vegetables or a vegetable-rich dietary pattern is related to lower risk of

diabetes. Similarly, one prospective study showed an inverse association between total carotenoid intake and diabetes incidence (14). These investigators either did not report on a possibly differential effect of smoking (5–9, 11) or did not find any significant interactions with smoking (10, 14). In one prospective study, Reunanen et al. (15) examined the association of circulating β -carotene concentrations with the risk of diabetes; they found a nonsignificant positive association in smokers and a nonsignificant inverse association in nonsmokers.

Thus, it is unknown whether a higher serum carotenoid concentration (or carotenoid intake) is inversely associated with the risk of diabetes and whether the relation is modified by smoking status. Therefore, we used the database of the Coronary Artery Risk Development in Young Adults (CARDIA) Study to assess the longitudinal relation of circulating carotenoids to indicators of diabetes and related metabolic abnormalities in Black and White men and women aged 18–30 years at baseline. Our main focus was on baseline serum carotenoid concentrations as the indicator of carotenoid status, recognizing that circulating carotenoids tend to be high in diets high in carotenoid-rich plant foods and in the absence of oxidative stress, as well as after intake of supplemental carotenoids. We hypothesized that serum carotenoids would be associated with reduced risk of diabetes-related conditions in nonsmokers but the apparent protection provided by high serum carotenoid levels would be attenuated or lost in smokers.

MATERIALS AND METHODS

The CARDIA and YALTA studies

The CARDIA Study, a biethnic, prospective, multicenter epidemiologic study of the evolution of cardiovascular disease risk factors in young adults, has been described in detail elsewhere (16). Briefly, in 1985 and 1986 (baseline; also called “year 0”), 5,115 Blacks and Whites aged 18–30 years were examined in four US cities: Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California. At the Birmingham, Minneapolis, and Chicago sites, participants were randomly selected from the community or from specific US Census tracts. In Oakland, participants were randomly selected from members of the Kaiser Permanente Medical Care Program. During recruitment, nearly equal numbers of participants were obtained by race (Black, White), sex, education (high school or less, more than high school), and age (18–24 years, 25–30 years) at all sites. Fifty percent of invited persons contacted were examined (47 percent of Blacks and 60 percent of Whites) and became part of the CARDIA cohort. Reexamination occurred after 2, 5, 7, 10, and 15 years. The majority of participants returned for all five follow-up examinations ($n = 3,033$ (59.3 percent)), while 812 (15.9 percent), 462 (9.0 percent), 289 (5.7 percent), and 307 (6.0 percent) returned for four, three, two, and one follow-up examinations, respectively.

The Young Adult Longitudinal Trends in Antioxidants (YALTA) Study is an ancillary study to CARDIA; it measured serum carotenoid concentrations in most CARDIA

participants at years 0 and 7. As of year 15, there were 127 verified deaths in the YALTA cohort. The top three causes of mortality were acquired immunodeficiency syndrome (28 percent), homicide (16 percent), and unintentional injury (10 percent), with only 7 percent being due to coronary heart disease (17).

For this report, we excluded participants who did not complete any follow-up surveys ($n = 212$), did not report their smoking status ($n = 17$), or were missing data on baseline serum carotenoid levels ($n = 285$). Furthermore, we excluded 32 participants with prevalent diabetes at baseline, 25 participants for whom data on baseline plasma glucose level was missing, and 51 participants with missing information on confounding factors. We excluded all observations made during pregnancy, retaining other observations made in the same woman. Thus, analyses of incident diabetes included 4,493 participants. The 622 nonrespondents were more likely to be men (50 percent in nonrespondents vs. 45 percent in the 4,493 participants) and Black (60 percent in nonrespondents vs. 50 percent in participants). The sum of serum carotenoid concentrations, available for 311 nonrespondents, was 69.9 $\mu\text{g/ml}$ as opposed to 74.7 $\mu\text{g/ml}$ in participants. No age difference or difference in smoking status was observed. Long-term follow-up was excellent in the 4,493 study participants; 86 percent were examined at either year 10 or year 15 or both (i.e., they had at least 10 years of follow-up). For analysis of serum insulin levels and homeostasis model assessment (HOMA) of insulin resistance (18), we excluded subjects for whom insulin level or insulin resistance was not measured at baseline ($n = 7$) or at year 15 ($n = 1,210$) or who were using antidiabetic medication at year 15 ($n = 68$); this left 3,208 participants for analysis.

Smoking status

Participants who reported at baseline that they had ever smoked cigarettes regularly (five or more cigarettes per week almost every week for at least 3 months) and were smokers at CARDIA baseline, or whose baseline serum cotinine level was greater than or equal to 14 ng/ml (despite a contrary self-report ($n = 186$)) were classified as current smokers. Participants who had quit smoking before baseline were designated former smokers.

Measurement of serum carotenoid levels

Stored serum samples obtained at CARDIA year 0 were used in the YALTA Study to assay levels of the carotenoids α - and β -carotene, lycopene, lutein plus zeaxanthin, and β -cryptoxanthin (Molecular Epidemiology and Biomarker Research Laboratory, University of Minnesota, Minneapolis, Minnesota). The assay of carotenoids, based on high performance liquid chromatography, was a modification of the method of Bieri et al. (19) for optimizing detection of carotenoids, with calibration as described by Craft et al. (20) and sample handling as described by Gross et al. (21). Calibration was performed with pure compounds (Hoffmann-La Roche Inc., Nutley, New Jersey; Sigma-Aldrich Chemical Company, St. Louis, Missouri). 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 National Institute of Standards and Technology reference sera and was a participant in the National Institute of Standards and Technology's Fat-Soluble Vitamin Quality Assurance Group. The coefficients of variation were less than 10 percent for all analytes and control pools. The intraclass correlation coefficients (ratio of between-person variance to between- plus within-person variance) were 0.93 for α -carotene, 0.98 for β -carotene, 0.73 for lutein plus zeaxanthin, 0.97 for β -cryptoxanthin, and 0.73 for lycopene (22).

Other baseline measurements

Sex, race, date of birth, medication use, and weekly alcohol consumption were ascertained by structured interview or self-administered questionnaire. A physical activity score was derived from the CARDIA Physical Activity History, a simplified version of the Minnesota Leisure Time Physical Activity Questionnaire (23). Alcohol intake (ml/day) was computed from self-reported frequency of beer, wine, and liquor consumption per week. Assuming that one drink of beer, wine, or liquor contains 16.7 ml, 17.0 ml, or 19.1 ml of ethanol, respectively (per CARDIA protocol), we estimated total weekly ethanol consumption in milliliters and divided it by 17.24 ml, the amount of ethanol in an average drink. In order not to obscure a nonlinear (J- or U-shaped) covariate relation, we categorized participants by their usual alcohol consumption (0, 1–6, or ≥ 7 drinks/week).

Height and weight were measured while the participant stood in light clothing without shoes; body mass index was defined as weight (kg) divided by height squared (m^2). The interviewer-administered CARDIA diet history asked open-ended questions about dietary consumption in the past month within 100 food groups; this information was obtained at years 0 and 7, referencing 1,609 separate food items. Baseline plasma lipid levels were determined at the University of Washington Northwest Lipid Research Clinic Laboratory (Seattle, Washington). Total triglyceride and cholesterol levels were measured using enzymatic procedures. High density lipoprotein cholesterol concentration was determined after dextran sulfate–magnesium precipitation.

Resting systolic and diastolic blood pressures were measured at every examination using the means of the second and third random-zero sphygmomanometer measurements. Fasting insulin concentration at baseline was determined using a modification of the immunoassay techniques of Herbert et al. (24). A radioimmunoassay was employed for year 15 insulin that used a unique antibody with less than 0.2 percent cross-reactivity to human proinsulin and its primary circulating split form, des(31,32)-proinsulin (LINCO Research, Inc., St. Louis, Missouri). Masked analysis of split serum samples resulted in a technical error of 16.6 percent of the mean ($r = 0.98$). Between-method correlation was 0.83. We measured fasting glucose level with the hexokinase method at years 0, 7, 10, and 15. Insulin resistance (HOMA) was calculated as glucose (mmol/liter) \times insulin (mU/liter)/22.5 (18).

Diabetes incidence

Diabetes was defined as a fasting glucose level of at least 126 mg/dl at year 7, 10, or 15 or the self-reported use of oral hypoglycemic medication or insulin at any examination. The numbers of cases diagnosed because of diabetes treatment were three, six, five, six, and 35 at years 2, 5, 7, 10, and 15, respectively; an additional 27, 37, and 29 cases were diagnosed because of an elevated fasting glucose level at years 7, 10, and 15, respectively (data were not available for years 2 and 5). Although no specific differentiation between type 1 and type 2 diabetes was made, most cases were apparently type 2: Of 18 participants diagnosed at or before age 30 years, only one was treated solely with insulin throughout follow-up.

Statistical analysis

We computed the sum of serum concentrations of the five carotenoids and studied this as well as individual carotenoid levels. We calculated relative hazards for diabetes incidence using Cox proportional hazards models adjusted for study center, race, sex, education (<12, 12, and >12 years), age (years), total energy intake (continuous), alcohol consumption (ml/day), body mass index (defined as above), physical activity (continuous), total cholesterol level (continuous), high density lipoprotein cholesterol level (continuous), systolic blood pressure (continuous), triglyceride level (continuous), and use of vitamin supplements (a single indicator variable for use of vitamin A, C, or E or β -carotene). Since diagnosis of diabetes could occur at only five time points, we used a discrete hazard function (the "ties=discrete" option in SAS PROC PHREG; SAS Institute, Inc., Cary, North Carolina) in these analyses. For the studied baseline carotenoids and year 15 serum insulin concentration, we used multiple linear regression with the covariates defined as above plus baseline insulin level; we performed parallel analyses for insulin resistance (HOMA). Analyses using log-transformed insulin or HOMA values yielded similar results, and thus the results are not presented. In all analyses except that shown in table 1, we combined former and never smokers as "nonsmokers," under the assumption that oxidative stress is lower in former smokers than in current smokers; in separate analyses, we verified that findings were similar for former smokers and never smokers (data not shown). Use of β -carotene supplements was reported by only six people at baseline and by only 116 people at year 7, 10, or 15; only 13 people reported such use twice. Therefore, no separate analysis was conducted for users of β -carotene supplements.

RESULTS

Baseline characteristics

The 4,493 participants included in the diabetes incidence analysis comprised 55.1 percent women, 49.6 percent Whites, and 61.1 percent persons with education beyond high school. The mean age at baseline was 24.8 years (standard deviation, 3.6). Proportions of current smokers, former

TABLE 1. Baseline characteristics of participants according to quartile of baseline sum of serum carotenoid concentrations and smoking status, Coronary Artery Risk Development in Young Adults (CARDIA) Study, 1985–2001*

Characteristic	Quartile of sum of serum carotenoid concentrations				P value	Baseline smoking status		P value
	1 (8.9–53.9 µg/dl) (n = 1,123)	2 (54.0–70.9 µg/dl) (n = 1,124)	3 (71.0–90.9 µg/dl) (n = 1,123)	4 (91.0–326.8 µg/dl) (n = 1,123)		Nonsmoker (n = 2,961)	Current smoker (n = 1,532)	
Mean age (years)	24.4	24.6	25.0	25.3	<0.01	24.8	24.9	<0.01
Sex (% female)	55.2	53.4	54.6	57.1	0.36	56.9	51.4	<0.01
Race (% White)	45.7	47.7	49.7	55.4	<0.01	52.6	43.8	<0.01
Study center (%)								
Birmingham, Alabama	25.3	26.7	24.0	18.0	<0.01	24.5	21.6	<0.01
Chicago, Illinois	15.9	20.9	23.5	25.5		21.9	20.6	
Minneapolis, Minnesota	26.4	27.2	28.1	29.9		23.8	35.9	
Oakland, California	32.3	25.2	24.4	26.6		29.9	21.9	
Education (%)								
<12 years	13.6	10.9	8.4	5.6	<0.01	5.5	17.6	<0.01
12 years	36.2	31.1	26.4	23.4		24.1	39.2	
>12 years	50.1	57.9	65.3	71.0		70.3	43.1	
Mean dietary intake								
Total energy (kcal/day)	3,044	3,002	2,918	2,815	<0.01	2,801	3,222	<0.01
Fish (no. of times/week)	1.7	1.6	1.6	1.8	0.10	1.6	1.7	0.48
Meat (no. of times/week)	11.1	11.0	10.9	10.0	<0.01	10.2	11.8	<0.01
Vegetables (no. of times/week)	14.3	15.3	16.0	17.4	<0.01	15.5	16.3	0.04
Fruit (no. of times/week)	5.9	6.7	7.3	7.8	<0.01	7.3	6.2	<0.01
Ethanol (ml/week)	13.9	13.1	12.2	9.7	<0.01	8.7	18.9	<0.01
Mean systolic blood pressure (mmHg)	111	111	110	109	<0.01	111	110	0.12
Mean body mass index†	25.6	24.4	24.2	23.7	<0.01	24.5	24.3	0.19
Mean total physical activity (exercise units‡)	399	421	423	429	0.06	421	412	0.33
Use of vitamin supplements§ (%)	26.9	30.5	31.2	34.4	<0.01	33.0	26.4	<0.01
Mean cholesterol level (mg/dl)								
Total cholesterol	164	171	180	191	<0.01	177	176	0.83
Low density lipoprotein cholesterol	99	104	112	121	<0.01	109	108	0.27
High density lipoprotein cholesterol	50	52	54	56	<0.01	54	52	<0.01
Mean triglyceride level (mg/dl)	75	75	71	68	<0.01	69	79	<0.01
Mean fasting plasma glucose level (mg/dl)	82	82	82	81	0.11	82	82	0.98
Mean insulin concentration (mU/liter)	11.9	10.8	10.6	9.9	<0.01	11.1	10.1	<0.01
Mean homeostasis model assessment level (mU/liter × mmol/liter)¶	2.4	2.2	2.2	2.0	<0.01	2.3	2.1	<0.01
Mean serum carotenoid level (µg/dl)								
α-carotene	1.3	2.0	2.6	4.9	<0.01	3.1	2.0	<0.01
β-carotene	7.4	11.3	15.0	27.2	<0.01	16.7	12.3	<0.01
Lutein plus zeaxanthin	11.6	16.0	19.8	26.3	<0.01	19.2	17.0	<0.01
β-cryptoxanthin	4.7	7.0	9.0	12.9	<0.01	9.2	7.0	<0.01
Lycopene	16.6	26.6	34.0	42.4	<0.01	29.8	30.1	0.61
Sum of above carotenoids	41.6	63.0	80.5	113.7	<0.01	78.0	68.3	<0.01

* Results for age, sex, study center, race, and education were unadjusted; results for all other variables were adjusted for age, sex, study center, race, and education.

† Weight (kg)/height (m)².

‡ Physical activity score derived from the CARDIA Physical Activity History (23).

§ Vitamin A, C, or E or β-carotene.

¶ Homoeostasis model of insulin resistance. Insulin resistance was calculated as glucose (mmol/liter) × insulin (mU/liter)/22.5 (18).

smokers, and never smokers at baseline were 34.1 percent, 11.6 percent, and 54.3 percent, respectively. The mean value for the sum of the five serum carotenoid concentrations was 74.7 $\mu\text{g}/\text{dl}$ (standard deviation, 29.9).

The groups with higher serum carotenoid levels tended to be older, White, female, and more highly educated (table 1). Baseline body mass index, total energy intake, insulin level, and HOMA insulin resistance were lower in the groups with higher serum carotenoid levels. Vegetable or fruit intake and use of vitamin A, C, or E or β -carotene supplements (all of which may increase serum carotenoid levels) were more frequent in the higher serum carotenoid groups, while intakes of meat and ethanol were lower in the higher serum carotenoid groups. Correlations between the sum of carotenoid concentrations and intakes of vegetables, fruit, meat, and fish were 0.17, 0.14, -0.11 , and 0.06 , respectively (all p 's < 0.01). These correlations were stronger in nonsmokers than in current smokers. These results suggest that the participants with higher baseline serum carotenoid levels tended to have healthier lifestyles. These same patterns emerged individually for concentrations of α - and β -carotene, lutein plus zeaxanthin, and β -cryptoxanthin. However, this was not the case with lycopene; persons with higher lycopene concentrations tended to have less healthy lifestyles (data not shown). As we reported previously, levels of total cholesterol, low density lipoprotein cholesterol, and high density lipoprotein cholesterol were higher in the higher serum carotenoid groups, which is consistent with these amphiphilic substances' equilibrating across fat pools in the body (25). Therefore, we adjusted the results of all analyses involving carotenoids for levels of total and high density lipoprotein cholesterol and triglycerides.

Total intakes of energy, alcohol, and meat were higher among current smokers than among nonsmokers, while fruit intake was lower in the current smokers (table 1). The proportion of supplement users (vitamin A, C, or E or β -carotene) was higher in nonsmokers than in current smokers. Never smokers had the highest total serum carotenoid levels; 60 percent of persons in the highest quintile were never smokers as compared with 48 percent of persons in the lowest quintile. Serum levels of α -carotene, β -carotene, β -cryptoxanthin, and lutein plus zeaxanthin were significantly lower in current smokers than in nonsmokers (table 1). Lycopene concentrations were similar in current smokers and nonsmokers.

Serum carotenoids and diabetes

The incidence rate of diabetes over the 15 years of follow-up was 2.51 per 1,000 person-years ($n = 148$ cases). Table 2 shows the relative hazard of diabetes according to quartile of baseline sum of serum carotenoids. A higher sum of serum carotenoids was inversely related to diabetes in nonsmokers but not in current smokers. Table 3 shows the relative hazard of diabetes incidence according to the sum of serum carotenoids and the level of each individual carotenoid (per standard deviation increase; continuous variables). The sum of carotenoids was significantly and inversely related to diabetes incidence in nonsmokers (relative hazard = 0.74, 95 percent confidence interval: 0.55, 0.99) but not in current

TABLE 2. Relation between baseline sum of serum carotenoid concentrations* (in quartiles) and incident diabetes mellitus, according to baseline smoking status, Coronary Artery Risk Development in Young Adults (CARDIA) Study, 1985–2001

Baseline smoking status	Quartile of sum of serum carotenoid concentrations												<i>p</i> for trend					
	1† (8.9–53.9 $\mu\text{g}/\text{dl}$) ($n = 1,123$)			2 (54.0–70.9 $\mu\text{g}/\text{dl}$) ($n = 1,124$)			3 (71.0–90.9 $\mu\text{g}/\text{dl}$) ($n = 1,123$)			4 (91.0–326.8 $\mu\text{g}/\text{dl}$) ($n = 1,123$)								
	No. of subjects	No. of cases	RH†,§	95% CI†	No. of subjects	No. of cases	RH†,§	95% CI†	No. of subjects	No. of cases	RH†,§	95% CI†	No. of subjects	No. of cases	RH†,§	95% CI†		
Overall	1,123	56	1,124	40	0.90	0.58, 1.40	1,123	21	0.55	0.32, 0.94	1,123	31	0.94	0.56, 1.57	1,123	16	0.63	0.32, 1.25
Nonsmoker	624	35	701	22	0.66	0.37, 1.19	781	16	0.51	0.27, 0.96	855	16	0.63	0.32, 1.25	855	15	2.15	0.92, 5.01
Current smoker	499	21	423	18	1.46	0.71, 3.00	342	5	0.57	0.20, 1.67	268	15	2.15	0.92, 5.01	268	15	2.15	0.92, 5.01

* Sum of serum levels of α -carotene, β -carotene, lutein plus zeaxanthin, β -cryptoxanthin, and lycopene ($\mu\text{g}/\text{dl}$).

† Reference category.

‡ RH, relative hazard; CI, confidence interval.

§ Adjusted for race, sex, study center, age, education, systolic blood pressure, ethanol intake (0, 1–6, or ≥ 7 drinks/week), plasma levels of total cholesterol, high density lipoprotein cholesterol, and triglycerides, total energy intake, body mass index, total energy expenditure, and use of vitamin supplements (vitamin A, C, or E or β -carotene).

TABLE 3. Relative hazard of incident diabetes mellitus per standard deviation increase in baseline carotenoid concentration, according to baseline smoking status (*n* = 4,681), Coronary Artery Risk Development in Young Adults (CARDIA) Study, 1985–2001

Carotenoid	Baseline smoking status				Interaction <i>p</i> value
	RH*, †	Nonsmoker		Current smoker	
		95% CI*	RH†	95% CI	
Sum of serum carotenoid concentrations‡	0.74	0.55, 0.99	1.13	0.83, 1.53	0.02
α-carotene	0.52	0.26, 1.03	0.89	0.53, 1.51	0.40
β-carotene	0.53	0.33, 0.86	1.12	0.82, 1.53	<0.01
Lutein plus zeaxanthin	0.85	0.65, 1.13	1.22	0.93, 1.61	0.03
β-cryptoxanthin	0.73	0.52, 1.02	0.90	0.63, 1.28	0.34
Lycopene	0.98	0.77, 1.25	1.05	0.78, 1.40	0.47

* RH, relative hazard; CI, confidence interval.

† Adjusted for race, sex, study center, age, education, systolic blood pressure, ethanol intake (0, 1–6, or ≥7 drinks/week), plasma levels of total cholesterol, high density lipoprotein cholesterol, and triglycerides, total energy intake, body mass index, total energy expenditure, and use of vitamin supplements (vitamin A, C, or E or β-carotene).

‡ Sum of serum levels of α-carotene, β-carotene, lutein plus zeaxanthin, β-cryptoxanthin, and lycopene (μg/dl).

smokers (*p* for interaction = 0.02). Inverse trends with diabetes incidence in nonsmokers were seen for each separate carotenoid except lycopene, though only β-carotene was significant. No significant relations were observed between individual carotenoids and diabetes incidence in current smokers. Further adjustment for intakes of fruit, vegetables, meat, and fish did not change the interaction.

Serum carotenoids, year 15 insulin, and HOMA

A total of 3,208 subjects were analyzed for the relation between baseline sum of serum carotenoids and year 15

insulin level or HOMA insulin resistance. Table 4 shows that baseline sum of serum carotenoids was significantly inversely related to year 15 insulin and HOMA insulin resistance in nonsmokers but not in smokers. Per standard deviation increase, the slope in nonsmokers was −0.46 for insulin and −0.14 for HOMA; the slope in smokers was 0.33 for insulin and 0.12 for HOMA (*p* values for interaction: 0.09 for insulin and 0.04 for HOMA) (table 5). These associations were clearest for β-carotene and lycopene (table 5). The results were unchanged when we omitted users of supplemental vitamin A, C, or E or β-carotene (insulin slope: nonsmokers, −0.42; smokers, 0.22; HOMA

TABLE 4. Relation between baseline sum of carotenoid concentrations* (in quartiles) and year 15 insulin concentration and insulin resistance (homeostasis model assessment†), Coronary Artery Risk Development in Young Adults (CARDIA) Study, 1985–2001

	No. of subjects	Quartile of sum of serum carotenoid concentrations				<i>p</i> for trend
		1 (8.9–53.9 μg/dl) (<i>n</i> = 1,123)	2 (54.0–70.9 μg/dl) (<i>n</i> = 1,124)	3 (71.0–90.9 μg/dl) (<i>n</i> = 1,123)	4 (91.0–326.8 μg/dl) (<i>n</i> = 1,123)	
Insulin concentration (mU/liter)						
Nonsmoker	2,223	14.7 (0.4)‡	14.6 (0.4)	14.1 (0.4)	13.7 (0.4)	0.06
Current smoker	985	14.2 (0.7)	14.6 (0.7)	14.0 (0.7)	15.7 (0.9)	0.35
Homeostasis model assessment						
Nonsmoker	2,223	3.28 (0.12)	3.15 (0.11)	3.02 (0.10)	2.93 (0.10)	0.02
Current smoker	985	3.11 (0.21)	3.35 (0.21)	3.16 (0.23)	3.60 (0.27)	0.27

* Sum of serum levels of α-carotene, β-carotene, lutein plus zeaxanthin, β-cryptoxanthin, and lycopene (μg/dl).

† Homeostasis model of insulin resistance. Insulin resistance was calculated as glucose (mmol/liter) × insulin (mU/liter)/22.5 (18).

‡ Adjusted mean value (with standard error in parentheses). Results were adjusted for age, sex, race, study center, education, body mass index, total energy intake, ethanol intake (0, 1–6, or ≥7 drinks/week), physical activity, systolic blood pressure, plasma levels of total cholesterol, high density lipoprotein cholesterol, and triglycerides, use of vitamin supplements (vitamin A, C, or E or β-carotene), and baseline insulin and insulin resistance (homeostasis model assessment).

TABLE 5. Difference (β) in year 15 insulin concentration and insulin resistance (homeostasis model assessment*) per standard deviation increase in baseline serum carotenoid concentrations, according to baseline smoking status, Coronary Artery Risk Development in Young Adults (CARDIA) Study, 1985–2001

Insulin outcome and carotenoid ($\mu\text{g}/\text{dl}$)	Baseline smoking status				Interaction p value [†]
	Nonsmoker		Current smoker		
	β ‡ (SE§)	p value	β ‡ (SE)	p value	
Insulin (mU/liter)					
Sum of serum carotenoid concentrations¶	−0.46 (0.21)	0.03	0.33 (0.41)	0.42	0.09
α -carotene	−0.40 (0.20)	0.04	−0.42 (0.45)	0.35	0.78
β -carotene	−0.53 (0.20)	<0.01	0.47 (0.41)	0.25	0.04
Lutein plus zeaxanthin	−0.27 (0.21)	0.19	−0.01 (0.39)	0.98	0.74
β -cryptoxanthin	−0.18 (0.20)	0.36	−0.24 (0.39)	0.53	0.96
Lycopene	−0.06 (0.21)	0.76	0.40 (0.40)	0.31	0.30
Homeostasis model assessment					
Sum of serum carotenoid concentrations	−0.14 (0.06)	0.01	0.12 (0.13)	0.34	0.04
α -carotene	−0.11 (0.05)	0.04	−0.07 (0.14)	0.60	0.74
β -carotene	−0.14 (0.05)	<0.01	0.17 (0.13)	0.18	0.05
Lutein plus zeaxanthin	−0.09 (0.06)	0.09	0.03 (0.12)	0.80	0.40
β -cryptoxanthin	−0.06 (0.05)	0.24	−0.07 (0.12)	0.55	0.95
Lycopene	−0.04 (0.06)	0.53	0.11 (0.12)	0.35	0.10

* Homeostasis model of insulin resistance. Insulin resistance was calculated as glucose (mmol/liter) \times insulin (mU/liter)/22.5 (18).

[†] p value for interaction of each carotenoid with smoking status for year 15 insulin and insulin resistance (homeostasis model assessment).

[‡] Results were adjusted for age, sex, race, study center, education, body mass index, total energy intake, ethanol intake (0, 1–6, or ≥ 7 drinks/week), physical activity, systolic blood pressure, plasma levels of total cholesterol, high density lipoprotein cholesterol, and triglycerides, use of vitamin supplements (vitamin A, C, or E or β -carotene), and baseline insulin and insulin resistance (homeostasis model assessment).

[§] SE, standard error.

[¶] Sum of serum levels of α -carotene, β -carotene, lutein plus zeaxanthin, β -cryptoxanthin, and lycopene ($\mu\text{g}/\text{dl}$).

slope: nonsmokers, −0.14; smokers, 0.09) or further adjusted for dietary factors (data not shown).

DISCUSSION

This study addressed our hypothesis that carotenoid concentrations are inversely associated with diabetes and related phenomena only in nonsmokers. We observed an inverse relation between the sum of five serum carotenoid concentrations measured at baseline and 15-year incidence of diabetes in baseline nonsmokers but not in baseline smokers. Findings for year 15 fasting insulin level and HOMA insulin resistance, both adjusted for their baseline values, were consonant with the observation about diabetes incidence.

In a nested case-control study of circulating carotenoid levels in 106 persons who started using diabetes medication and 201 age-, sex-, and municipality-matched controls, Reunanen et al. (15) reported a lower odds ratio in nonsmokers (for the highest tertile of circulating carotenoids vs. the lowest, relative risk = 0.83) than in current smokers (for highest tertile vs. lowest, relative risk = 1.64). That finding is consistent with ours, although neither relative risk was

significant and the interaction p value was not given by Reunanen et al. (15). Montonen et al. (14) studied dietary carotenoid levels in 4,304 nondiabetic men and women aged 40–69 years (~30 percent smokers) from the same Finnish sample and identified 383 persons who began using diabetes medication during 23 years of follow-up. Diabetes risk was inversely associated with the sum of intake of six carotenoids and with β -cryptoxanthin individually. The authors stated that there was no significant interaction with smoking but did not provide any details. In one recent cross-sectional study, Coyne et al. (26) also reported an inverse association between serum carotenoid levels and diabetes. However, they did not report on the interaction between smoking status and carotenoids for diabetes prevalence. We found no other reports on this topic in the published literature.

The antioxidant activity common to major carotenoids may be causally related to the stronger inverse association with diabetes observed in this study for the sum of carotenoid concentrations than for individual carotenoid concentrations. Alternatively, circulating carotenoids, rather than acting directly on risk, may reflect their strong association with intake of fruit, vegetables, and other plant foods; these foods contain

an even wider range of antioxidants (27, 28) which together may lower oxidative stress and the risk of diabetes. Although the association of circulating carotenoids with reduced diabetes risk in our study was apparently independent of diet, there may have been residual confounding, because circulating carotenoid levels are measured much more precisely than is diet.

Smoking is a potent oxidative stressor in humans, causing reductions in plasma vitamin C (29) and carotenoid (29, 30) levels. Even though we evaluated the subset of smokers who had high concentrations of serum antioxidants, tissue levels of antioxidants may still be lower in smokers than in nonsmokers (31). Smoking may neutralize the antioxidant activity of carotenoids and block their protective effect; that is, the antioxidant activity of the carotenoids may simply be overwhelmed by the prooxidant activity of smoking. While it has been suggested that smoking can produce a prooxidant form of β -carotene and perhaps other carotenoids, this does not appear to occur in humans (32, 33).

Alternatively, smoking can alter β -carotene metabolism and may change the metabolism of other carotenoids, particularly when they are present in high concentrations—especially with the use of supplements (31). Even though supplement use was low in this study, smokers with high serum carotenoid concentrations may have an altered carotenoid metabolism. Smoking-altered metabolism is associated with the formation of several retinoid products and alterations in cellular activity, especially changes in rates of apoptosis (34, 35). Smoking-derived products of carotenoids may delay or prevent apoptosis and affect cell proliferation rates. Changes in these cellular activities may influence the risk of diabetes.

There are some limitations in our study. First, it would have been desirable to study β -carotene supplementation in these data, but users of β -carotene supplements were too few for this purpose. Second, we may have missed some diabetic subjects. We did not measure glucose concentrations at either the year 2 examination or the year 5 examination, and in 1997 the fasting glucose cutpoint used to define diabetes in the United States changed from 140 mg/dl to 126 mg/dl (36); this would mean that prior to that time, participants were somewhat less likely to be diagnosed with diabetes and to receive diabetes treatment than after. However, we measured fasting glucose concentration at all other examinations and used a consistent diagnostic cutpoint throughout follow-up. We also followed the participants through 2001; therefore, people with marginal cases would have had an opportunity to be diagnosed and treated after the diabetes criteria changed. Third, since we used a single measurement of carotenoid concentrations at baseline, misclassification of circulating carotenoid levels relative to long-term average levels was expected. However, these misclassifications would probably have attenuated observed relations and diluted any interaction. Therefore, we felt that these misclassifications would not change our conclusions regarding interactions. Finally, although CARDIA is a large study, there were relatively few cases of incident diabetes, especially after subgrouping by smoking status. Thus, a small sample size may have limited our statistical power. However, these results provide insight

into factors influencing the development of diabetes at relatively young ages.

The findings of this study support the hypothesis that the composite of five serum carotenoid concentrations may act directly to combat oxidative stress and the future occurrence of diabetes-related phenomena, or that a high-plant-food diet that results in higher serum carotenoid levels is also protective. Our observations additionally suggest that smoking may nullify the protective effect, supporting the concept that antioxidant metabolism and the oxidative defense system behave differently in smokers than in nonsmokers.

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