

## Longitudinal associations between body mass index and serum carotenoids: the CARDIA study

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Cross-sectional studies report an inverse association between BMI and serum carotenoid concentration. The present study examined the prospective association between BMI and the serum concentration of five carotenoids in the Coronary Artery Risk Development in Young Adults (CARDIA) study. Serum carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, zeaxanthin/lutein, lycopene), BMI, dietary intake, physical activity and dietary supplement use were measured at years 0 and 7 in 3071 black and white male and female participants, who were either persistent smokers or non-smokers. Among non-smokers, year 0 BMI predicted year 7 serum carotenoid levels: obese subjects (BMI  $\geq 30$  kg/m<sup>2</sup>) had an average concentration of the sum of four carotenoids ( $\alpha$ -carotene +  $\beta$ -carotene + zeaxanthin/lutein +  $\beta$ -cryptoxanthin) that was 22% lower than the concentration among subjects with a BMI of less than 22 kg/m<sup>2</sup>. In contrast, the sum of carotenoids among smokers was only 6% lower. Relationships between BMI and serum lycopene were weak. The change from year 0 to year 7 in serum carotenoids, except for lycopene, was inversely associated with the change in BMI among non-smokers but not among smokers. Parallel findings were observed for BMI and serum  $\gamma$ -glutamyl transferase level. In summary, the observation that BMI predicted the evolution of serum carotenoids during a 7-year follow-up among young non-smoking adults is consistent with the hypothesis that carotenoids are decreased in protecting against oxidative stress generated by adipose tissue, while smokers maintain a minimal level of serum carotenoids independent of adiposity. The results for lycopene were, however, discordant from those of the other carotenoids.

### BMI: CARDIA study: Carotenoids

Several epidemiological studies have reported an independent and inverse association between BMI and carotenoid concentrations in blood even when adjusted for other factors associated with carotenoid concentration, such as intake of fruit and vegetables, fat, fibre, alcohol, supplement use, smoking, gender and lipid concentrations (Brady *et al.* 1996; Yeum *et al.* 1998; Neuhauser *et al.* 2001; Wallstrom *et al.* 2001; Ford *et al.* 2002). This association may be important in the development of chronic diseases related to BMI as high concentrations of blood constituents that have antioxidant properties, such as carotenoids, have been prospectively shown to be associated with a low incidence of CVD and several cancers (Comstock *et al.* 1992; Gey, 1993; Ito *et al.* 2003; Sesso *et al.* 2004).

The relationship between BMI and carotenoid concentration may reflect three pathways. First, BMI and serum carotenoids may be correlated because of dietary and other lifestyle factors that affect them both. Second, serum carotenoids are partially fat soluble and are spread throughout both blood and an

enlarged mass of fat tissue; they may be reduced in obesity because relatively fewer are located in the blood (Brady *et al.* 1996; Wallstrom *et al.* 2001). Third, adipose tissue may generate oxidative stress (Davi *et al.* 2002; Higdon & Frei, 2003; Morrow, 2003), and the carotenoids may be reduced because of defending against this stress. In support of this 'stress defence' hypothesis are earlier Coronary Artery Risk Development in Young Adults (CARDIA) findings of a positive association between BMI and  $\gamma$ -glutamyl transferase (GGT), both cross-sectionally (Lee *et al.* 2003) and longitudinally (Lee *et al.* 2004b), and an inverse association between GGT and serum carotenoids (Lee *et al.* 2004b).

With the increase in obesity observed worldwide (Darnton-Hill *et al.* 2004), it is important to have a more complete understanding of the association between carotenoids and BMI. Most of the previous studies investigating the association between BMI and serum carotenoid concentrations have been cross-sectional. The purpose of the present study was to examine, in a prospective fashion, the association

between BMI and the serum concentration of five carotenoids in the large and biracial CARDIA study, expanding on our earlier work (Lee *et al.* 2003, 2004a,b). Our primary hypothesis was that BMI would independently predict future carotenoid level.

### Subjects and methods

The Young Adult Longitudinal Trends in Antioxidants study measured serum antioxidant concentrations in frozen samples as an ancillary study to the CARDIA study. CARDIA is a longitudinal, multicentre epidemiological study of lifestyle and other factors on the evolution of CHD risk factors during young adulthood; no interventions are included in the study. Study design, recruitment of participants and methods have been described elsewhere (Friedman *et al.* 1988). In 1985–6, 5115 black and white men and women aged 18–30 years were recruited and examined at four field centres in the USA: Birmingham, AL; Chicago, IL; Minneapolis, MN; Oakland, CA. Participants were re-examined at 2, 5, 7, 10 and 15 years after baseline, with re-examination rates among surviving cohort members of 91%, 86%, 81%, 79% and 74%, respectively.

Standard questionnaires were used to maintain consistency in the assessment of demographic and behavioural information across CARDIA examination visits. Sex, race, date of birth and cigarette smoking were determined by structured interview or by 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 (Jacobs *et al.* 1989). Body weight with light clothing was measured to the nearest 0.2 kg, and height without shoes was measured to the nearest 0.5 cm. BMI was computed as weight divided by height squared ( $\text{kg/m}^2$ ).

The CARDIA Diet History, an interviewer-administered quantitative food-frequency questionnaire identified 1609 distinct food items at years 0 and 7. Intake of energy, fibre, alcohol, total fruit (including fruit juice) and total vegetables (including vegetable juice) was computed from the Diet History (Liu *et al.* 1994).

Blood samples were drawn after an overnight fast by venepuncture into vacutainers. After plasma or serum separation, aliquots were stored at  $-70^\circ\text{C}$  until shipped on dry ice to a central laboratory. Year 0 and 7 plasma lipids were measured at the University of Washington Northwest Lipid Research Clinic Laboratory, USA. Total plasma cholesterol levels were determined using enzymatic procedures. HDL-cholesterol was determined after precipitation of LDL-containing lipoproteins with dextran sulphate/ $\text{MgCl}_2$  (Warnick *et al.* 1982). LDL-cholesterol was calculated using the Friedewald equation (Friedewald *et al.* 1972); the few individuals with triacylglycerol levels above 4.52 mmol/l (400 mg/dl) were excluded from this calculation.

Serum obtained at CARDIA years 0 and 7 was used to assay the carotenoids  $\alpha$ - and  $\beta$ -carotene, lycopene, zeaxanthin/lutein, and  $\beta$ -cryptoxanthin in year 8 at the Molecular Epidemiology and Biomarker Research Laboratory, University of Minnesota, USA. The carotenoids were measured by an HPLC-based assay based on a modification of the method of Bieri *et al.* (1985) with calibration as described by Craft

*et al.* (1988) and sample-handling as described by Gross *et al.* (1995). The primary modification was a simultaneous detection of tocopherols and carotenoids. Calibration was performed against pure compounds (Hoffman-LaRoche, Basel, Switzerland; Sigma Chemical Co., St Louis, MO, USA). Quality-control procedures included the routine analysis of plasma and serum control pools containing high and low concentrations of each analyte. In addition, the laboratory routinely analysed National Institutes of Standards and Technology reference sera and was a participant in the National Institutes of Standards and Technology Fat-Soluble Vitamin Quality Assurance Group. The coefficients of variation were less than 10% for all analytes and control pools. We have previously shown that the intraclass correlation coefficients (ratio of between-person variance to between- plus within-person variance) were 0.93 for  $\alpha$ -carotene, 0.98 for  $\beta$ -carotene, 0.73 for lutein/zeaxanthin, 0.97 for  $\beta$ -cryptoxanthin and 0.73 for lycopene (Iribarren *et al.* 1997).

Serum GGT was measured at year 0 using a SMAC II continuous-flow analyser (Technicon Instruments Corp. Tarrytown, NY, USA) at American Bio-science Laboratories (now Smith-Kline Beecham King of Prussia, Pennsylvania, USA), and at year 10 colorimetrically with nitroanilide methodology on a Roche Cobas Indianapolis, Indiana, USA. Mira Plus chemistry instrument at Linco Research Inc St Louis, Missouri, USA. As previously described (Lee *et al.* 2004b), we corrected for a lack of comparability between year 0 and year 10 measurements using the formula: recalibrated year 0 GGT values =  $2.7618 + (1.9004 \times \text{original year 0 values})$ .

A supplement user was defined as any person consuming  $\beta$ -carotene or vitamins A, E or C in excess of his or her intake from foods alone. In the present paper a supplement user was a person who used one or more of these supplements at either year 0 or year 7, and a non-supplement user was a person who did not use supplements at both years 0 and 7.

For some of the analyses, BMI was classified into BMI  $< 22 \text{ kg/m}^2$ ,  $22\text{--}24.9 \text{ kg/m}^2$ ,  $25\text{--}29.9 \text{ kg/m}^2$  (overweight) and  $\geq 30 \text{ kg/m}^2$  (obese); the lowest cut-off point is approximately the median of those with normal weight.

This paper includes non-pregnant participants who attended both the year 0 and 7 examinations and for whom we had both BMI and serum carotenoid assessments, and who were either smokers at both year 0 and year 7 or non-smokers at both exams. We excluded pregnant women, including seven at year 0 and sixty-two at year 7. A total of 1029 of the CARDIA study participants did not return for the year 7 follow-up examination. BMI was missing in seventeen of all participants at year 0 and in ninety-nine of those who returned at year 7. All five serum carotenoid assessments were missing in 305 of all participants at year 0 and in 321 of those who returned at year 7. A total of forty-three of the participants attending both examinations were missing smoking information at either examination, and 459 had variable smoking patterns. Many of these exclusions overlapped, with the result that 3174 people were available for analysis. After excluding people who had missing covariate data in the fully adjusted model described below under statistical analysis, the final sample was reduced from 3174 to 3071. For the analyses including year 10 GGT, the sample was reduced to 2730.

### Statistical analysis

We examined associations between serum carotenoids and BMI in several ways:

1. the cross-sectional association between year 0 serum carotenoids (dependent variables) and year 0 BMI (independent variable), and year 7 serum carotenoids (dependent variables) and year 7 BMI (independent variable);
2. the longitudinal association between year 7 serum carotenoids (dependent variables) and year 0 BMI (independent variable);
3. the longitudinal association between the changes in serum carotenoids (year 7 – year 0; dependent variable) and changes in BMI (year 7 – year 0; independent variables).

Results were presented for long-term smokers and long-term non-smokers separately as there was a significant interaction of smoking and BMI with each serum carotenoid except lycopene in both cross-sectional and longitudinal analyses.

Adjustment for potential confounding was analysed by linear regression. Two models were run. The first included minimal adjustment for field centre, race, sex and year 0 values of age (years), plasma cholesterol and HDL-cholesterol (we additionally adjusted for changes in total cholesterol and HDL-cholesterol between year 0 and 7 in Tables 3 and 4 later). We adjusted for lipids because several of the carotenoids are lipophilic (Gross *et al.* 2003).

The second fully adjusted model also included possibly confounding behaviours at year 0: alcohol consumption (ml/d); physical activity (continuous); energy intake (kcal); vegetable and fruit intake (eating occasions/week); and vitamin supplement use at either year 0 or year 7. Replacement of the dichotomous supplement use covariate with the continuous amounts of each of four supplements at both years did not greatly alter the results. In an earlier study, supplement use, alcohol intake and physical activity were significantly correlated with the serum concentrations of carotenoids in the CARDIA study (Lee *et al.* 2004b). The fully adjusted model also included year 0 carotenoids in the model that predicted year 7 carotenoids from year 0 BMI, thereby estimating the evolution of carotenoids over the 7-year period.

The age-, race- and sex-adjusted correlation of the year 0 physical activity score with the year 0 sum of four serum carotenoids was 0.10, whereas the corresponding correlation for serum lycopene was –0.01. The age-, race- and sex-adjusted correlation of the year 0 total intake of fresh fruit, juice and vegetables with the year 0 sum of the serum concentrations of four carotenoids was  $r = 0.22$ , whereas the intake of these foods was unrelated to year 0 serum lycopene ( $r = -0.06$ ).

We considered alternative adjustment for diet in sensitivity analyses, given the complexity of the association of diet with serum carotenoids. Specifically, starting with 195 food groups, we used a stepwise series of regressions to identify the foods explaining the most between-person variance of the sum of four serum carotenoids. *A priori* hypotheses in these regressions were that carotenoid-containing fruits and vegetables would relate positively to the levels of circulating carotenoids, as would other plant foods that more generally reduce oxidative stress. Red meat and deep-fried foods were hypothesised *a priori* to increase oxidative stress and therefore

to reduce circulating carotenoids. With these hypotheses in mind, and adjusting for total energy intake, we performed regressions of specific food subgroups within each major food group (vegetables, fruit, grains, dairy, fish, red meat, poultry, fats and oils, legumes, nuts, and non-alcoholic beverages). We dropped food subgroups that were not significant, and then ran a combined regression across food groups of statistically significant food subgroups. Finally, we dropped non-significant food subgroups from the combined regression. A dietary score including twenty-eight food groups (prominently featuring carotenoid-containing fruit and vegetables) was developed from this procedure; this had a high correlation with the sum of four serum carotenoids ( $r = 0.5$ ). Similarly, we devised a dietary score that included eleven food groups (prominently featuring tomato products) and had a correlation with serum lycopene of  $r = 0.26$ . Adjustment for these two dietary scores did not alter the findings presented; these more complex analyses are not shown.

We examined associations between BMI and GGT by looking at the association between year 10 GGT (dependent variable) and year 0 BMI (independent variable), holding year 0 GGT constant. In the adjusted model, we included meat in addition to the fully adjusted model described above, as meat has been shown to be associated with GGT (Lee *et al.* 2004c).

In the present paper, only the fully adjusted models are presented. There were only small differences between the minimally and fully adjusted models. Statistical analyses were performed with SAS software, version 8.2 (SAS Institute, Inc, Cary, NC, USA).

## Results

### Cross-sectional and tracking

At baseline, the distribution of gender was almost equal between non-smokers and smokers. No differences were observed between smokers and non-smokers in relation to age and BMI. Non-smokers reported a significantly higher physical activity and had significantly higher serum concentrations (17–100%) of  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin and zeaxanthin/lutein compared with smokers (Table 1). Moreover, the non-smokers had a significantly higher percentage of white participants and supplement users compared with the smokers.

The serum carotenoids are mostly intercorrelated. Year 0 age-, race- and sex-adjusted correlations are:  $\alpha$ -carotene and  $\beta$ -carotene,  $r = 0.72$ ;  $\beta$ -cryptoxanthin with  $\alpha$ -carotene,  $\beta$ -carotene and zeaxanthin/lutein,  $r = 0.40$ ,  $0.42$  and  $0.43$ , respectively; zeaxanthin/lutein with  $\alpha$ -carotene,  $\beta$ -carotene and lycopene,  $r = 0.29$ ,  $0.30$  and  $0.30$ , respectively. Serum lycopene shows little correlation with  $\alpha$ -carotene,  $\beta$ -carotene and cryptoxanthin ( $r = -0.04$ ,  $0.06$  and  $0.08$ , respectively); although small, these correlations achieve statistical significance given that  $n = 3071$ ). We present associations with BMI for the sum of four carotenoids to emphasise this correlation structure and the similarity of associations described below between each of  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin and zeaxanthin/lutein, and BMI, whereas lycopene followed a distinct pattern. This sum also simplifies the statistical discussion. The variables of interest also display strong tracking

**Table 1.** Characteristics of the sample by smoking at year 0 (Means and standard deviations, or percentages)

	Non-smokers (n 2310)		Smokers (n 761)		P-value	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	25.0	3.6	25.3	3.7	0.09	
Male (%)	45		48		0.15	
White race (%)	56		42		<0.001	
Physical activity (exercise units)	432	301	404	295	0.03	
BMI (kg/m <sup>2</sup> )	24.4	4.8	24.5	5.1	0.55	
Supplement user (%)	52		40		<0.001	
Dietary intake						
Energy (kcal)	2700	1432	3404	1904	<0.001	
Alcohol (g)	8.6	14.8	20.3	30.8	<0.001	
Crude fibre (g)	5.7	3.5	5.9	3.7	0.17	
β-Carotene (μg)	4540	6263	3991	4484	0.009	
Vitamin E (mg)	20.6	69.5	16.1	24.2	0.008	
Vitamin C (mg)	277	384	237	245	<0.001	
Fruit (times/week)	8.3	7.7	6.9	7.6	<0.001	
Vegetable (times/week)	17.9	12.1	17.7	11.4	0.65	
Serum concentration (μg/dl)						
α-Carotene	3.2	4.1	1.6	1.6	<0.001	
β-Carotene	17.3	15.3	10.7	7.9	<0.001	
β-Cryptoxanthin	9.3	6.0	6.4	3.8	<0.001	
Zeaxanthin/lutein	19.2	8.9	16.4	8.5	<0.001	
Lycopene	29.6	14.2	30.2	14.9	0.30	

correlations between year 0 and year 7 values, namely 0.87 for BMI and 0.45–0.62 for the five carotenoids. GGT correlation between year 0 and 10 was 0.61.

In cross-sectional analyses restricted to year 0 data, serum concentrations of carotenoids showed significant inverse associations with BMI. Among long-term non-smokers, obese subjects had 24–37% lower carotenoid concentrations than were seen among subjects with a BMI of less than

22 kg/m<sup>2</sup>, except for serum lycopene, for which the difference was only 9% (Table 2). Among smokers, the differences between obese subjects and subjects with a BMI of less than 22 kg/m<sup>2</sup>, although statistically significant, were generally smaller (14–24%), except for serum α-carotene, for which the difference was 39%. Cross-sectional analyses restricted to year 7 data showed the same pattern (data not shown).

**Table 2.** Adjusted† means and their standard errors of year 0 serum carotenoids by classification of year 0 BMI\*

	BMI category								P <sub>trend</sub>	% Difference low – obese
	<22.0 kg/m <sup>2</sup> (low)		22.0–24.9 kg/m <sup>2</sup> (normal)		25.0–29.9 kg/m <sup>2</sup> (overweight)		≥30.0 kg/m <sup>2</sup> (obese)			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Non-smokers										
	n 794		n 711		n 568		n 237			
α-Carotene (μg/l)	3.8	0.14	3.3	0.14	2.7	0.16	2.4	0.25	<0.0001	37
β-Carotene (μg/l)	19.9	0.53	17.2	0.54	15.4	0.61	13.4	0.98	<0.0001	33
β-Cryptoxanthin (μg/l)	10.2	0.20	9.6	0.21	8.7	0.24	7.1	0.38	<0.0001	30
Zeaxanthin/lutein (μg/l)	20.7	0.30	19.2	0.31	18.5	0.35	15.8	0.56	<0.0001	24
Sum of carotenoids (μg/l)‡	54.6	0.86	49.3	0.88	45.3	0.99	38.6	1.60	<0.0001	29
Lycopene (μg/l)	30.5	0.48	29.2	0.49	29.5	0.56	27.8	0.89	0.006	9
Smokers										
	n 256		n 251		n 156		n 98			
α-Carotene (μg/l)	1.8	0.09	1.6	0.09	1.5	0.11	1.1	0.15	0.001	39
β-Carotene (μg/l)	11.8	0.48	10.7	0.48	10.2	0.60	9.0	0.79	0.002	24
β-Cryptoxanthin (μg/l)	6.8	0.23	6.5	0.22	5.9	0.28	5.7	0.37	0.01	16
Zeaxanthin/lutein (μg/l)	17.6	0.51	16.0	0.50	15.8	0.64	15.2	0.83	0.004	14
Sum of carotenoids (μg/l)†	38.0	1.02	34.8	1.01	33.4	1.28	31.1	1.68	0.0002	18
Lycopene (μg/l)	31.8	0.90	30.8	0.89	28.7	1.13	27.0	1.48	0.006	15

\* P-values for interaction with smoking and BMI: 0.03 with α-carotene, 0.016 with β-carotene, 0.003 with β-cryptoxanthin, 0.12 with zeaxanthin/lutein, 0.002 with sum of carotenoids and 0.20 with lycopene.

† Adjustment was made by linear regression. Adjustment included field centre, race, sex, and year 0 age, alcohol consumption, physical activity, energy intake, fruit intake, vegetable intake and plasma concentration of cholesterol and HDL-cholesterol plus year 0 or 7 supplement use.

‡ Sum of α-carotene, β-carotene, β-cryptoxanthin and zeaxanthin/lutein.

**Table 3.** Adjusted† means and their standard errors of year 7 serum carotenoids by classification of year 0 BMI\*

	BMI category								<i>P</i> <sub>trend</sub>	>% Difference low – obese
	< 22.0 kg/m <sup>2</sup> (low)		22.0–24.9 kg/m <sup>2</sup> (normal)		25.0–29.9 kg/m <sup>2</sup> (overweight)		≥ 30.0 kg/m <sup>2</sup> (obese)			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Non-smokers	<i>n</i> 794		<i>n</i> 711		<i>n</i> 568		<i>n</i> 237			
α-Carotene (μg/l)	4.6	0.14	4.5	0.14	4.0	0.16	3.4	0.25	<0.0001	26
β-Carotene (μg/l)	22.6	0.56	21.5	0.58	18.4	0.65	14.9	1.02	<0.0001	34
β-Cryptoxanthin (μg/l)	9.9	0.18	9.4	0.18	9.0	0.21	7.7	0.32	<0.0001	22
Zeaxanthin/lutein (μg/l)	20.8	0.25	20.8	0.26	19.9	0.29	18.4	0.46	<0.0001	12
Sum of carotenoids (μg/l)‡	57.8	0.81	56.1	0.83	51.4	0.94	44.8	1.47	<0.0001	22
Lycopene (μg/l)	33.2	0.45	33.0	0.47	32.2	0.53	33.1	0.82	0.53	0
Smokers	<i>n</i> 256		<i>n</i> 251		<i>n</i> 156		<i>n</i> 98			
α-Carotene (μg/l)	2.0	0.11	2.1	0.11	2.1	0.14	2.0	0.19	0.92	0
β-Carotene (μg/l)	11.8	0.50	12.6	0.50	11.6	0.64	10.8	0.82	0.50	8
β-Cryptoxanthin (μg/l)	6.1	0.22	6.7	0.22	6.7	0.28	6.1	0.36	0.97	0
Zeaxanthin/lutein (μg/l)	16.0	0.36	16.4	0.37	16.7	0.46	14.9	0.59	0.34	7
Sum of carotenoids (μg/l)†	35.9	0.84	37.8	0.85	37.2	1.07	33.8	1.38	0.47	6
Lycopene (μg/l)	31.6	0.78	31.0	0.79	34.6	1.00	30.9	1.27	0.76	2

\* *P*-values for interaction with smoking and BMI: 0.03 with α-carotene, 0.001 with β-carotene, 0.001 with β-cryptoxanthin, 0.002 with zeaxanthin/lutein, <0.001 with sum of carotenoids and 0.56 with lycopene.

† Adjustment was made by linear regression. Adjustment included field centre, race, sex, and year 0 age, alcohol consumption, physical activity, energy intake, vegetable intake, fruit intake, plasma carotenoids year 0 (e.g. α-carotene in its row, β-carotene in its row, etc.) and plasma concentration of cholesterol and HDL-cholesterol plus year 0 and 7 supplement use.

‡ Sum of α-carotene, β-carotene, β-cryptoxanthin and zeaxanthin/lutein.

### Prospective

Longitudinal associations predicting the evolution of carotenoids showed the same general pattern as observed in the cross-sectional analysis. The year 0 BMI strongly and inversely predicted the year 7 serum concentrations of carotenoids among non-smokers. Obese subjects at year 0 had 12–34% lower carotenoid concentrations at year 7 than subjects with a BMI below 22 kg/m<sup>2</sup> at year 0, except for lycopene, for which no differences were observed. No associations were observed among the smokers (Table 3). These findings indicate that the cross-sectional inverse association between year 0 BMI and the year 0 sum of carotenoids (Table 2) evolved during 7 years of follow-up. No such evolution was seen when the variables were reversed; that is, the year 0 sum of carotenoids did not predict the year 7 BMI, holding the year 0 BMI constant (data not shown).

The change from year 0 to year 7 in the serum concentrations of carotenoids except for lycopene showed significant inverse associations with changes in BMI among non-smokers, but no association was found among smokers except for serum β-carotene and the sum of carotenoids (Table 4).

### Race interaction

In the cross-sectional analyses, there was no interaction of gender and BMI (that is, BMI–carotenoid relations were similar in both sexes), but there was a significant interaction of race and BMI with each serum carotenoid except lycopene. A significant interaction between race and BMI was observed for serum α-carotene, β-carotene and β-cryptoxanthin in the longitudinal analyses. In the analysis of the data separately for black and white participants, qualitatively similar associations to those presented in Tables 2 and 3 were observed in both groups, but the carotenoid concentrations except for

lycopene were generally lower and the size of the association was weaker for the black compared with the white participants.

### γ-Glutamyl transferase

We assessed the extent to which BMI at year 0 was associated with year 10 serum GGT, holding year 0 GGT constant (Table 5). Year 0 BMI strongly and inversely predicted year 10 serum GGT among non-smokers, indicating an evolution of the previously reported (Lee *et al.* 2003) cross-sectional association between BMI and GGT during 10 years of follow-up. Among smokers, no association between year 0 BMI and year 10 GGT was observed, but GGT levels were considerably higher among smokers than among non-smokers at every BMI level. Baseline GGT did not predict the evolution of BMI during follow-up (data not shown).

### Discussion

The present study indicated that a strong inverse relationship exists between BMI and all measured serum carotenoids, with the exception of lycopene, among non-smokers, both cross-sectionally and longitudinally.

Several studies have reported inverse associations between carotenoid concentrations and BMI, but most of these studies have looked at the associations cross-sectionally, and several have examined only a single carotenoid (Zhu *et al.* 1997; Rock *et al.* 1999, 2002; Neuhouser *et al.* 2001; van Kappel *et al.* 2001; Wallstrom *et al.* 2001; Ford *et al.* 2002). In a 3-year follow-up study among fifty-six American women with breast cancer who received advice to increase their fruit and vegetable intake, a marginally significant inverse relationship between changes in BMI and changes in plasma lutein, cryptoxanthin and lycopene ( $P < 0.09$ ) was found; the magnitude of

**Table 4.** Adjusted† means and their standard errors of changes in serum carotenoids (year 7 – year 0) by quintiles (Q1–Q5) of changes in BMI (year 7 – year 0)\*

	Q1 (–22.2– < 0.1 kg/m <sup>2</sup> )		Q2 (0.1– < 1.2 kg/m <sup>2</sup> )		Q3 (1.2– < 2.3 kg/m <sup>2</sup> )		Q4 (2.3– < 4.0 kg/m <sup>2</sup> )		Q5 (4.0–15.8 kg/m <sup>2</sup> )		<i>P</i> <sub>trend</sub>
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
<b>Non-smokers (n 2310)</b>											
α-Carotene (μg/l)	1.5	0.21	1.4	0.20	1.0	0.20	1.0	0.20	0.4	0.21	<0.0001
β-Carotene (μg/l)	6.3	0.87	4.2	0.83	2.7	0.80	2.8	0.82	0.2	0.85	<0.0001
β-Cryptoxanthin (μg/l)	0.9	0.29	0.1	0.27	0.1	0.27	–0.6	0.27	–0.5	0.28	0.02
Zeaxanthin/lutein (μg/l)	2.1	0.38	1.1	0.36	1.3	0.35	0.8	0.36	0.4	0.37	0.005
Sum of carotenoids (μg/l)†	10.8	1.23	6.8	1.17	5.0	1.14	4.0	1.16	0.4	1.21	<0.0001
Lycopene (μg/l)	1.9	0.71	3.5	0.67	4.0	0.65	3.6	0.66	3.4	0.69	0.77
	Q1 (–10.1– < 0.1 kg/m <sup>2</sup> )		Q2 (0.1– < 1.2 kg/m <sup>2</sup> )		Q3 (1.2– < 2.3 kg/m <sup>2</sup> )		Q4 (2.3– < 4.0 kg/m <sup>2</sup> )		Q5 (4.0–14.8 kg/m <sup>2</sup> )		
<b>Smokers (n 761)</b>											
α-Carotene (μg/l)	0.4	0.15	0.6	0.15	0.6	0.16	0.2	0.16	0.4	0.17	0.59
β-Carotene (μg/l)	2.9	0.66	1.2	0.68	1.3	0.69	0.7	0.69	–0.6	0.74	0.0002
β-Cryptoxanthin (μg/l)	0.4	0.32	–0.9	0.33	0.3	0.34	–0.1	0.34	0.5	0.36	0.47
Zeaxanthin/lutein (μg/l)	0.1	0.56	–1.0	0.57	–0.2	0.58	0.5	0.59	–0.7	0.62	0.41
Sum of carotenoids (μg/l)‡	3.7	1.19	–0.1	1.23	1.9	1.25	1.3	1.25	–0.4	1.33	0.02
Lycopene (μg/l)	1.7	1.13	0.8	1.16	3.2	1.18	1.4	1.18	1.4	1.26	0.90

\* *P*-values for interaction smoking and BMI: 0.02 for α carotene, 0.13 for β-carotene, 0.07 for β-cryptoxanthin, 0.06 for zeaxanthin/lutein, 0.01 for sum of carotenoids and 0.95 for lycopene.

† Adjustment made done by linear regression. Adjustment included field centre, race, sex, and year 0 age, alcohol consumption, physical activity, fruit and vegetable intake and plasma concentration of cholesterol and HDL-cholesterol plus year 0 and 7 supplement use.

‡ Sum of α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin/lutein.

**Table 5.** Adjusted† means and their standard errors of year 10 serum γ-glutamyl transferase (GGT) by classification of year 0 BMI

	BMI category				<i>P</i> <sub>trend</sub>	% Difference low – obese
	< 22.0 kg/m <sup>2</sup> (low)	22.0–24.9 kg/m <sup>2</sup> (normal)	25.0–29.9 kg/m <sup>2</sup> (overweight)	≥ 30.0 kg/m <sup>2</sup> (obese)		
<b>Year 0 BMI</b>						
Non-smokers	<i>n</i> 722		<i>n</i> 637		<i>n</i> 514	
GGT (μg/l)	21.3	0.66	22.7	0.68	25.2	0.77
Smokers	<i>n</i> 212		<i>n</i> 226		<i>n</i> 131	
GGT (μg/l)	39.9	3.77	38.2	3.57	36.5	4.67
					38.0	6.00
					<i>P</i> for interaction	
					0.001	

† Adjustment was made by linear regression. Adjustment included field centre, race, sex, and year 0 age, alcohol consumption, physical activity, energy intake, meat intake, vegetable intake, fruit intake, serum GGT year 0 and plasma concentration of cholesterol and HDL-cholesterol and triacylglycerols, plus year 0 and 7.

the relationship was not stated (McEligot *et al.* 1999). No such relation was observed for plasma α- and β-carotene. Serum lycopene showed little relation to BMI, as has also been observed by others (Brady *et al.* 1996; Zhu *et al.* 1997; Neuhouser *et al.* 2001; van Kappel *et al.* 2001; Ford *et al.* 2002).

At least three reasons for the different behaviour of lycopene compared with other carotenoids exist. Lycopene concentration responds differently to fruit and vegetable intake than does that of other carotenoids; its response is almost specific to the intake of tomato products (Smith-Warner *et al.* 2000; Brevik *et al.* 2004). In the present study, serum lycopene concentrations were only weakly related to fruit and vegetable intake (partial correlation coefficients adjusted for sex, race, field centre and energy intake: *r* = –0.09 and –0.04, respectively). Moreover, serum lycopene levels may reflect

a different lifestyle compared with other carotenoids: in our study, for example, lycopene level was almost unrelated to smoking. Finally, some have suggested that the association between serum carotenoids and BMI results from adipose tissue acting as a reservoir that actively takes up lipoprotein-associated carotenoids (Brady *et al.* 1996; Wallstrom *et al.* 2001). In individuals with a higher fat mass, a larger proportion of the newly absorbed carotenoids would enter this reservoir, with the result that fewer of the total body carotenoids would exist in the serum. A small controlled study (*n* 31) by Zhu *et al.* (1997) suggested that fat-free mass might also serve as a dynamic reservoir that actively takes up carotenoids from plasma. Lycopene is highly fat-soluble and may easily disperse through adipose tissue as well as blood. Blood values may therefore be proportionately less

influenced by the intake of lycopene than are those of other carotenoids. We cannot rule out the possibility that this mechanism plays a role for other carotenoids.

Oxidative stress might also explain how an increased BMI might cause a decrease in carotenoid levels (Higdon & Frei, 2003; Keaney *et al.* 2003; Morrow, 2003; Suzuki *et al.* 2003). Oxidative stress, measured mainly by urinary F2-isoprostanes, has been positively associated with BMI (Davi *et al.* 2002; Keaney *et al.* 2003; Morrow, 2003; Suzuki *et al.* 2003), and carotenoid concentrations may be reduced with increasing BMI owing to their use in defence against this oxidative stress (Lasheras *et al.* 2002). In the present study, serum GGT, which has been asserted to be a marker of oxidative stress (Lee *et al.*, 2004a), was measured at year 10. A positive association was observed between year 10 serum GGT and year 0 BMI, holding year 0 GGT constant, among non-smokers but not among smokers. However, the considerably higher GGT levels among smokers than among non-smokers correspond to the considerably lower sum of carotenoids among smokers than among non-smokers, both these phenomena occurring at every BMI level. The data are consistent with the idea that the oxidative stress caused by smoking is so large that any association of BMI with carotenoids or GGT among smokers is masked.

Another explanation could be that BMI may be reduced as a consequence of the consumption of specific foods, diet generally or physical activity at the same time as these lifestyle factors elevate serum carotenoid levels. Although diet and physical activity were closely related to the values for serum carotenoids, except lycopene, little association was found in this study between BMI and fruit and vegetable intake or physical activity. Our ability to assess this hypothesis fully is compromised by difficulty in reliably assessing lifestyle. The failure of this adjustment to explain the relation could be the result of high within-person variation in both the diet and the physical activity measures relative to the sum of serum carotenoids or BMI.

Smoking causes oxidative stress, against which serum carotenoids are thought to defend (Morrow *et al.* 1995; Kelly, 2002; Dietrich *et al.* 2003). In the present study, we found lower levels of serum carotenoids except for lycopene at all levels of BMI among smokers compared with non-smokers. This has been observed in several other studies (Stryker *et al.* 1988; Ross *et al.* 1995; Marangon *et al.* 1998; Dietrich *et al.* 2003). At the same time, smoking is associated with reduced fruit and vegetable intake, physical activity and increased alcohol consumption, factors associated with reduced carotenoid levels. We do not, however, know what aspect of smoking might limit the relationship between BMI and serum carotenoids. It may be that, under enough oxidative stress, obese smokers induce antioxidant enzymes such as superoxide dismutase or catalase to maintain a minimum carotenoid level.

In summary, these results showed that the strong inverse association between BMI and serum carotenoids, except lycopene, observed by others cross-sectionally also exists over a 7-year follow-up in the CARDIA study among non-smokers but not among smokers.

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