Associations of Serum Carotenoids With Risk of Cardiovascular Mortality Among Individuals With Type 2 Diabetes: Results From NHANES

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OBJECTIVE

Although carotenoids have been suggested to exhibit antioxidant properties, some experimental studies reported that β-carotene may show pro-oxidant effects under certain conditions. Current evidence regarding the cardiovascular effects of carotenoids among patients with type 2 diabetes (T2D) is scarce. This study aimed to prospectively examine the associations of individual serum carotenoid concentrations with cardiovascular mortality among adults with T2D.

RESEARCH DESIGN AND METHODS

This analysis included 3,107 individuals with T2D from the Third National Health and Nutrition Examination Survey (NHANES III) and NHANES 2001–2006. Cardiovascular mortality was ascertained by linkage to National Death Index records through 31 December 2015. Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95% CIs.

RESULTS

During an average of 14 years of follow-up, 441 cardiovascular deaths occurred. After multivariate adjustment including lifestyles, dietary factors, glucose control, and other major carotenoids, higher serum β-carotene concentrations were significantly associated with an elevated risk of cardiovascular mortality in a dose-response manner. When extreme quartiles of β-carotene were compared, the multivariable-adjusted HR was 2.47 (95% CI 1.62, 3.76) for cardiovascular mortality ($P_{trend} = 0.002$); and per one-unit increment in natural log-transformed serum β-carotene was associated with a 46% higher risk of cardiovascular mortality ($P = 0.001$). Other individual carotenoids (α-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin) were not significantly associated with the risk of cardiovascular mortality. Consistent results were observed when stratifying by age, sex, race, BMI, smoking status, diabetes duration, and glycated hemoglobin A_{1c} levels.

CONCLUSIONS

Higher concentrations of serum β-carotene, but not other individual carotenoids, were significantly associated with an increased risk of cardiovascular mortality among individuals with T2D. Our findings, if replicated, underscore the need to estimate the optimal serum β-carotene concentrations in individuals with T2D.

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Type 2 diabetes (T2D) has become a public health problem worldwide. It is estimated that people living with diabetes will increase to 700 million in 2045 (1). Cardiovascular disease (CVD) is the major cause of mortality among patients with diabetes (2). Accumulating evidence has suggested that dietary factors play an important role in the prevention of CVD mortality among patients with diabetes (3).

Carotenoids, known as common natural antioxidants, are produced by plants and some microorganisms (4). More than 700 carotenoids have been identified so far, and 6 of them (α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin) account for >95% of total carotenoids in circulation (5). Of note, the health effect of carotenoids, especially β-carotene, on CVD risk has been controversial. Although some observational studies suggested inverse or null associations between serum carotenoids and the risk of CVD in general populations (6,7), several (8–10), but not all (11), interventional trials of β-carotene supplementation showed an increased risk of developing CVD and cancer, especially in smokers. Some in vitro and in vivo studies suggested that β-carotene itself usually acts as an antioxidant, whereas its oxidized products might exhibit pro-oxidant properties (12,13). However, among patients with diabetes, who have heightened oxidative stress status and higher risk of developing CVD (14,15), the evidence regarding the cardiovascular effects of carotenoids is scarce. In addition, whether sex, race/ethnicity, and smoking status could modify the association of interest remains unclear.

To fill these knowledge gaps, this study aimed to prospectively examine the associations between serum carotenoids concentrations and risk of CVD mortality in a nationally representative sample of U.S. adults with T2D.

RESEARCH DESIGN AND METHODS

Study Population

The National Health and Nutrition Examination Survey (NHANES) is an ongoing program of study that provides population estimates related to nutrition and health of adults and children in the U.S. The survey used a stratified, multistage probability design to recruit a representative sample of the U.S. population. Data were obtained via personal structured interviews at home, health examinations at a mobile examination center, and specimen analyses in the laboratory (16).

We used the data from NHANES III and continuous NHANES 2001–2006, which provided information on serum carotenoids. At first, patients with T2D aged ≥20 years at enrollment were included, which resulted in a study sample of 4,323 subjects. T2D was defined as self-reported doctor diagnosis of diabetes, use of insulin or oral hypoglycemic medication, fasting blood glucose ≥7.0 mmol/L (126 mg/dL), postprandial 2-h plasma glucose ≥11.1 mmol/L (200 mg/dL) from an oral glucose tolerance test, or glycated hemoglobin A_{1c} (HbA_{1c}) ≥6.5% (48 mmol/mol). We excluded participants who were self-reported as pregnant ($n = 23$), who had CVD ($n = 794$) or cancer ($n = 391$), or with no follow-up information ($n = 8$), resulting in 3,107 participants with T2D being included in the final analysis (Supplementary Fig. 1).

Assessment of Carotenoids

Serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene were measured using high-performance liquid chromatography (HPLC) in NHANES III and NHANES 2001–2002 and 2005–2006, while in NHANES 2003–2004, these carotenoids were measured using a comparable HPLC method. Data from NHANES 2003–2004 were then converted by a regression method to equivalent carotenoids measurements from the HPLC method. These carotenoids were available in NHANES III and three NHANES cycles (2001–2006), except for total lycopene (not available in NHANES 2001–2002). The laboratory procedures and quality control methods for serum carotenoids measurements were described in detail elsewhere (17,18). Total serum carotenoids concentrations were derived by summing the serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene.

Ascertainment of Mortality

Mortality was ascertained by linkage to National Death Index records through 31 December 2015 (19). ICD-10 was used to determine the causes of death. Death due to CVD included rheumatic heart diseases, hypertensive heart and renal disease, ischemic heart disease, heart failure, and cerebrovascular disease (ICD-10 codes I00–I09, I11, I13, I20–I51, and I60–I69).

Assessment of Covariates

Standardized questionnaires obtained information on sociodemographic characteristics, smoking status, alcohol consumption, physical activity, dietary intake, diabetes duration, diabetes medication use, and presence of hypertension. Never smokers were classified as those who reported smoking <100 cigarettes during their lifetime. Those who smoked >100 cigarettes in their lifetime were considered as current smokers, and those who smoked >100 cigarettes and had quit smoking were considered as former smokers. Drinking status was classified as nondrinker, low-to-moderate drinker (<2 drinks/day in men and <1 drink/day in women), or heavy drinker (≥2 drinks/day in men and ≥1 drinks/day in women). Physical activity was categorized as inactive group (no leisure-time physical activity), insufficiently active group (leisure-time moderate activity 1–5 times per week with MET ranging from 3 to 6 or leisure-time vigorous activity 1–3 times per week with MET >6), or active group (those who had more leisure-time moderate-or-vigorous activity than above) (20). BMI was calculated from weight/height^2 (kg/m^2). The Healthy Eating Index (HEI) was calculated to indicate overall diet quality (HEI-1995 for NHANES III and HEI-2010 for continuous NHANES).

In addition, strict laboratory analyses were performed, including the assessment of triglycerides and total cholesterol at baseline. Further details of these measurements were documented in the NHANES Laboratory Medical Technologists Procedures Manual (21).

Statistical Analysis

All analyses incorporated sample weights, strata, and primary sampling units to produce accurate national estimates. Sample characteristics are reported as means (SEs) for normally distributed continuous variables, medians (interquartile ranges) for nonnormally distributed continuous variables, and numbers (percentages) for categorical variables. Quartiles of serum carotenoids levels were determined based on the distribution in the study

population. The difference between the four groups was compared by one-way ANOVA tests (continuous variables with normal distribution), Kruskal-Wallis test (continuous variables with nonnormal distribution), and χ^2 test (categorical variables). The partial Spearman rank correlation coefficient was used to test the correlations between dietary and serum carotenoids. Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% CIs of CVD mortality in relation to serum carotenoids. Person-time was calculated as the interval between the NHANES interview date and the date of death or the end of the follow-up (31 December 2015), whichever occurred first.

We fitted two statistical models. Model 1 was adjusted for age (continuous), sex (male or female), race/ethnicity (non-Hispanic White or other), BMI (continuous), education level (less than high school, high school or equivalent, or college or above), family income-to-poverty ratio (≤1.0, 1.0–3.0, or >3.0), drinking status (nondrinker, low-to-moderate drinker, or heavy drinker), smoking status (never smoker, former smoker, or current smoker), leisure-time moderate-to-vigorous physical activity (inactive group, insufficiently active group, or active group), HEI (in quartiles), total energy intakes (in quartiles), supplement use (yes or no), and other individual carotenoids (natural log-transformed). Model 2 was further adjusted for diabetes medication use (yes or no), diabetes duration (<3, 3–10, or >10 years), HbA_{1c} (<7 or ≥7% [<53 or ≥53 mmol/mol]), self-reported hypertension (yes or no), serum total cholesterol (in quintiles), and triglycerides (in quintiles). The linear trend was calculated by assigning a median value to each category as a continuous variable. To minimize sample size reduction due to missing covariates, multiple imputation was performed.

To investigate dose-response associations between serum carotenoids concentrations and CVD mortality, we used a restricted cubic spline regression model with three knots at the 5th, 50th, and 95th percentiles of the serum carotenoids distribution, excluding the most extreme 5% values to reduce the potential influence of outliers. Tests for nonlinearity were performed using the likelihood ratio test.

Stratified analyses were conducted by age (≤60 or >60 years), sex (male or female), race/ethnicity (non-Hispanic White or other), smoking status (current or never/past), BMI (<30 or ≥30 kg/m^2), diabetes duration (<3 or ≥3 years), and HbA_{1c} (<7 or ≥7% [<53 or ≥53 mmol/mol]). Potential modifying effects were examined by testing the corresponding multiplicative interaction terms.

Several sensitivity analyses were also performed to test the robustness of the findings. First, to minimize the potential reverse causation bias, we excluded participants who died within the first 2 years of follow-up (n = 120). Second, dietary β-carotene intake was additionally adjusted. Third, instead of HEI, we further adjusted for individual foods or nutrients, including intakes of vegetables, fruits, total polyunsaturated fatty acids, total monounsaturated fatty acids, total saturated fatty acids, cholesterol, fiber, vitamin A, vitamin E, vitamin C, and fiber (all in quintiles). In addition, we further adjusted for other dietary biomarkers, including serum vitamin A, vitamin E, vitamin C, and vitamin D levels (all in quintiles). Fourth, given the moderate correlations between some carotenoids, we reperformed the analyses without mutual adjustment of carotenoids. Fifth, we further stratified analyses by therapy method (insulin or others) and liver disease (yes or no). Lastly, as additional analyses, we also explored the relationship between dietary carotenoid intakes and CVD mortality among patients with T2D.

All analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC). Two-sided $P < 0.05$ was considered statistically significant. The Bonferroni correction threshold was used to account for multiple comparisons and define statistical significance (0.05/5 = 0.01 for primary analyses and 0.05/7 = 0.007 for the subgroup interaction tests).

Data and Resource Availability

The data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval from the corresponding author.

RESULTS

Among 3,107 participants with diabetes (mean age, 56.1 years; 46.8% men), the median (interquartile range) serum concentration was 60.0 (30.0, 90.0) nmol/L for α-carotene, 240.0 (148.4, 424.9) nmol/L for β-carotene, 140.0 (90.0, 240.0) nmol/L for β-cryptoxanthin, 370.0 (240.0, 580.0) nmol/L for lycopene, and 320.0 (229.0, 470.0) nmol/L for lutein/zeaxanthin. The baseline characteristics according to quartiles of serum β-carotene are summarized in Table 1. Participants with higher serum β-carotene concentrations were older, more likely to be women and never smokers, less likely to be obese, and tended to have greater family income, and higher leisure-time physical activity and HEI. In addition, those patients seemed to experience longer diabetes duration and to more often use dietary supplements. There were weak to modest correlations between dietary and serum carotenoids (r_s ranged from 0.12 to 0.27).

During an average of 14 years of follow-up, 441 deaths due to CVD were identified. After multivariable adjustment, higher serum β-carotene concentrations were significantly associated with elevated risk of CVD mortality among patients with T2D (Table 2). The multivariable-adjusted HRs (95% CIs) across quartiles of serum β-carotene concentrations were 1.00 (reference), 1.43 (0.85, 2.42), 2.16 (1.24, 3.75), and 2.47 (1.62, 3.76) (P_{trend} = 0.002). A linear dose-response relationship of serum β-carotene concentrations (ranged from 65.0 to 950.0 nmol/L) with CVD mortality was also demonstrated (P = 0.001) (Fig. 1). Per one-unit increment in the natural log-transformed β-carotene concentrations was associated with a 46% (HR 1.46, 95% CI 1.17, 1.82) increased risk of CVD mortality. In addition, when extreme quartiles were compared, serum α-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin were not significantly associated with risk of CVD mortality (Table 2). Similarly, no significant association was observed between serum total carotenoids and CVD mortality among individuals with T2D (Supplementary Table 1).

Consistent results were observed between serum β-carotene and CVD mortality when analyses were stratified by age (≤60 or >60 years), sex (male or female), race/ethnicity (non-Hispanic White or other), BMI (<30 or ≥30 kg/m^2), smoking status (current or never/past), diabetes duration (<3 or ≥3 years), and HbA_{1c} (<7 or ≥7% [<53 or ≥53 mmol/mol]) (Table 3). No significant interactions

Table 1—Baseline characteristics of patients with type 2 diabetes in NHANES III and NHANES 2001–2006

		Serum β-carotene (nmol/L)				
Characteristics	Total	Quartile 1 <148.4	Quartile 2 148.4–240.0	Quartile 3 240.1–424.9	Quartile 4 >424.9	*P*
Patients, *n*	3,107	776	778	776	777	
Age, years	56.1 ± 0.4	51.0 ± 0.7	54.9 ± 0.8	57.3 ± 0.6	62.1 ± 1.0	<0.001
Male sex	1,454 (46.8)	429 (55.3)	392 (50.4)	339 (43.7)	294 (37.8)	<0.001
Non-Hispanic White	1,086 (35.0)	247 (31.8)	274 (35.2)	271 (34.9)	294 (37.8)	0.319
Educational attainment						0.140
Less than high school	1,476 (49.3)	345 (46.1)	376 (49.7)	364 (49.3)	391 (52.0)	
High school or equivalent	772 (25.8)	212 (28.3)	201 (26.6)	198 (26.8)	161 (21.4)	
College or above	748 (25.0)	191 (25.5)	180 (23.8)	177 (24.0)	200 (26.6)	
Family income-to-poverty ratio						0.004
≤1.0	730 (24.1)	218 (29.2)	176 (22.2)	183 (24.2)	153 (20.0)	
1.1–3.0	1,325 (43.8)	312 (41.8)	347 (45.7)	323 (42.7)	343 (44.9)	
>3.0	971 (32.1)	217 (29.1)	236 (31.1)	250 (33.1)	268 (35.1)	
Leisure-time physical activity						<0.001
Inactive	704 (22.7)	149 (19.2)	173 (22.2)	194 (25.0)	188 (24.2)	
Insufficiently active	1,864 (60.0)	527 (67.9)	478 (61.4)	438 (56.4)	421 (54.2)	
Active	539 (17.4)	100 (12.9)	127 (16.3)	144 (18.6)	168 (21.6)	
Smoking status						<0.001
Never smoker	1,486 (47.9)	317 (40.9)	335 (43.1)	393 (50.6)	441 (56.8)	
Former smoker	1,014 (32.7)	219 (28.3)	276 (35.5)	261 (33.6)	258 (33.3)	
Current smoker	604 (19.5)	239 (30.8)	166 (22.4)	122 (15.7)	77 (9.9)	
Drinking status						<0.001
Nondrinker	892 (30.0)	179 (23.9)	215 (28.4)	243 (32.8)	255 (35.0)	
Low-to-moderate drinker	1,886 (63.4)	468 (62.6)	495 (65.5)	476 (64.2)	447 (61.4)	
Heavy drinker	195 (6.6)	101 (13.5)	46 (6.1)	22 (3.0)	26 (3.6)	
BMI, kg/m^2	30.9 ± 0.2	33.5 ± 0.5	31.9 ± 0.4	30.0 ± 0.3	28.0 ± 0.3	<0.001
HbA_{1c}, % (mmol/mol)						<0.001
<7.0 (<53.0)	1,798 (58.0)	404 (52.1)	439 (56.6)	467 (60.3)	488 (63.1)	
≥7.0 (≥53.0)	1,302 (42.0)	372 (47.9)	337 (43.4)	307 (39.7)	286 (37.0)	
Diabetes medication use	1,375 (45.7)	354 (46.9)	352 (47.0)	328 (43.4)	341 (45.5)	0.131
Diabetes duration						0.309
<3 years	1,601 (55.5)	400 (55.6)	411 (57.5)	404 (55.6)	386 (53.4)	
3–10 years	577 (20.0)	158 (22.0)	142 (19.9)	147 (20.2)	130 (18.0)	
>10 years	706 (24.5)	161 (22.4)	162 (22.7)	176 (24.2)	207 (28.6)	
Self-reported hypertension	1,561 (50.4)	395 (51.6)	393 (50.6)	384 (49.6)	389 (50.3)	0.161
Supplement use	1,257 (40.5)	226 (29.1)	313 (40.2)	324 (41.8)	394 (50.8)	<0.001
HEI						<0.001
Quartile 1 (<50.6)	746 (25.0)	272 (36.8)	195 (25.9)	166 (22.2)	113 (15.3)	
Quartile 2 (50.6–61.5)	742 (25.0)	205 (27.7)	191 (25.4)	176 (23.5)	170 (23.0)	
Quartile 3 (61.6–71.8)	747 (25.0)	177 (23.9)	190 (25.3)	207 (27.6)	173 (23.4)	
Quartile 4 (>71.8)	745 (25.0)	86 (11.6)	176 (23.4)	200 (26.7)	283 (38.3)	
Total energy intakes, kcal						0.010
Quartile 1 (<1,227.0)	744 (24.8)	163 (22.2)	191 (25.4)	194 (25.9)	196 (26.5)	
Quartile 2 (1,227.0–1,659.0)	746 (25.8)	157 (21.2)	163 (21.7)	212 (28.3)	214 (29.0)	
Quartile 3 (1,659.1–2,247.0)	745 (24.6)	198 (26.8)	182 (24.2)	180 (24.0)	185 (25.0)	
Quartile 4 (>2,247.0)	745 (24.8)	222 (29.8)	216 (28.7)	163 (21.8)	144 (19.5)	
Serum triglycerides, mg/dL	155.9 (108.5, 236.8)	156.3 (114.5, 245.4)	167.2 (119.3, 238.6)	153.4 (105.0, 242.9)	141.8 (99.6, 210.6)	0.001
Serum total cholesterol, mg/dL	207.1 (178.1, 239.5)	192.9 (166.0, 217.8)	207.5 (179.8, 234.1)	211.8 (185.5, 248.0)	222.6 (190.2, 255.6)	<0.001
α-Carotene, nmol/L	59.6 (26.7, 90.0)	20.0 (16.9, 36.5)	51.7 (33.5, 70.0)	69.9 (53.1, 108.9)	109.6 (69.9, 178.5)	<0.001
β-Cryptoxanthin, nmol/L						<0.001

Continued on p. 1457

Table 1—Continued

Characteristics	Total	Serum β-carotene (nmol/L) Quartile 1 <148.4	Quartile 2 148.4–240.0	Quartile 3 240.1–424.9	Quartile 4 >424.9	*P*
	129.0 (77.0, 199.8)	70.0 (49.9, 109.5)	110.0 (86.2, 159.4)	139.8 (104.8, 239.1)	199.9 (129.9, 283.5)	
Lycopene, nmol/L	432.1 (279.5, 685.7)	405.7 (238.5, 676.0)	389.2 (259.6, 644.1)	498.2 (337.9, 709.8)	449.1 (287.6, 729.9)	<0.001
Lutein/zeaxanthin, nmol/L	300.0 (203.5, 431.1)	192.3 (141.2, 277.6)	300.0 (208.8, 388.2)	339.3 (259.7, 487.3)	418.5 (299.6, 594.5)	<0.001

Normally distributed continuous variables are described as means ± SEs, and continuous variables without a normal distribution are described as medians (interquartile ranges). Categorical variables are presented as numbers (percentages). All estimates accounted for complex survey designs.

were detected after accounting for multiple testing.

In sensitivity analyses, the positive association of serum β-carotene concentrations with CVD mortality was not materially changed when participants who died within first 2 years of follow-up were excluded (Supplementary Table 2). After additional adjustment for dietary β-carotene intake, the results remained largely unchanged (Supplementary Table 3). Similar results were observed when further adjusting for intakes of vegetables, fruits, total polyunsaturated fatty acids, total monounsaturated fatty acids, total saturated fatty acids, cholesterol, vitamin A, vitamin E, vitamin C, and fiber; or serum vitamin A, vitamin E, vitamin C, and vitamin D levels (Supplementary Table 3); or when we did not mutually adjust for individual carotenoids (Supplementary Table 4). When we stratified analyses by therapy method and liver disease, consistent results were observed, although the association of some subgroups did not reach statistical significance, largely due to limited sample sizes. In addition, dietary carotenoids intakes were not associated with CVD mortality among patients with T2D, except for a borderline inverse association for dietary α-carotene (Supplementary Table 5).

CONCLUSIONS

To the best of our knowledge, this is the first study to prospectively examine the associations between major serum carotenoids concentrations and CVD mortality among patients with T2D. We found that higher serum β-carotene concentrations were associated with an increased risk of CVD mortality in a dose-response manner. This association was independent of traditional risk factors, including dietary and lifestyle factors, diabetes duration, and serum lipid levels. There were no significant associations of concentrations of serum α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin with CVD mortality among individuals with T2D. A variety of stratified analyses and sensitivity analyses demonstrated the robustness of our findings.

Carotenoids have various important biological functions, among which antioxidation is particularly important to human health (22). However, the associations between carotenoids and CVD risk among general populations are inconsistent, with several randomized controlled trials (RCTs) raising concerns on the cardiovascular safety of high β-carotene supplements (7–11). For example, an interventional trial conducted in a general healthy population found that supplementation with β-carotene yielded neither benefit nor harm on CVD incidence (11). However, two other large interventional trials that were launched mainly in smokers found potentially adverse cardiovascular effects of β-carotene supplementation (8–10). The results may be due to the instability of the β-carotene molecule in the free radical-rich environment in smokers (13,23). In addition, one meta-analysis of 131,551 participants from six large RCTs demonstrated that β-carotene supplementation (15–50 mg/day, 1.4–12 years) resulted in a small but significantly excess risk of CVD mortality (odds ratio 1.10, 95% CI 1.03, 1.17) (24). Recently, the U.S. Preventive Services Task Force announced against the use of β-carotene supplements to prevent heart disease in a general population (25).

However, among patients with diabetes who have elevated oxidative stress status and higher risk of developing CVD, whether carotenoids status, especially β-carotene, would influence the cardiovascular outcomes of this particular population remains unclear. A secondary analysis of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study among 1,700 participants with T2D suggested that β-carotene supplements (20 mg/day, 3 years) did not significantly increase the risk of major coronary events and total mortality (26). However, by restricting the analyses to 699 patients who took diabetes medication at baseline, the risk of stroke doubled (relative risk 2.06, 95% CI 1.00, 4.27), although no significant interaction was observed (26). Another large-scale, randomized, placebo-controlled trial among 20,536 high-risk individuals (more than a quarter with diabetes) found that daily supplementation of antioxidant vitamins (combination of vitamin E, vitamin C, and β-carotene for 5 years) had no effect on incidence or mortality of any vascular events, but resulted in increased levels of plasma triglycerides and LDL cholesterol (27). The discrepancy of these studies may be due to different sample size (ranged from 699 to 20,536), intervention durations (ranged from 3 to 5 years), forms of supplements (isolated or used in combination), or characteristics of study participants (smokers or not).

Using a relatively large multiethnic cohort with a long follow-up duration, we found a positive dose-response relationship between serum β-carotene and CVD mortality among patients with T2D. After further adjustment of dietary β-carotene intakes, the association

Table 2—CVD mortality according to quartiles of serum carotenoids concentrations among patients with T2D in NHANES III and NHANES 2001–2006

Carotenoids	Quartiles of serum levels (nmol/L)				P_{trend}
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
α-Carotene					
Range	<30.0	30.0–60.0	60.1–90.0	>90.0	
No. deaths/total	99/771	99/871	118/712	125/753	
Crude	1	0.85 (0.54, 1.34)	1.25 (0.81, 1.93)	1.16 (0.83, 1.61)	0.207
Model 1	1	0.61 (0.39, 0.95)	0.96 (0.62, 1.45)	0.73 (0.46, 1.15)	0.659
Model 2	1	0.59 (0.37, 0.96)	0.93 (0.54, 1.61)	0.76 (0.44, 1.32)	0.874
β-Carotene					
Range	<148.4	148.4–240.0	240.1–424.9	>424.9	
No. deaths/total	78/776	95/778	118/776	150/777	
Crude	1	1.43 (0.83, 2.47)	1.83 (1.04, 3.22)	2.23 (1.49, 3.34)	<0.001
Model 1	1	1.50 (0.89, 2.51)	1.88 (1.05, 3.38)	2.21 (1.38, 3.55)	0.015
Model 2	1	1.43 (0.85, 2.42)	2.16 (1.24, 3.75)	2.47 (1.62, 3.76)	0.002
β-Cryptoxanthin					
Range	<90.0	90.0–139.9	140.0–240.0	>240.0	
No. deaths/total	97/684	125/816	113/867	106/740	
Crude	1	1.09 (0.76, 1.56)	0.98 (0.64, 1.49)	0.99 (0.67, 1.46)	0.753
Model 1	1	0.98 (0.68, 1.42)	0.85 (0.56, 1.30)	0.91 (0.57, 1.46)	0.680
Model 2	1	0.92 (0.64, 1.32)	0.75 (0.49, 1.14)	0.81 (0.50, 1.30)	0.401
Lycopene					
Range	<240.0	240.0–370.0	370.1–580.0	>580.0	
No. deaths/total	149/664	104/696	77/660	72/658	
Crude	1	0.66 (0.46, 0.93)	0.58 (0.42, 0.80)	0.57 (0.40, 0.80)	0.009
Model 1	1	0.86 (0.60, 1.24)	0.95 (0.70, 1.29)	1.08 (0.73, 1.60)	0.569
Model 2	1	0.77 (0.53, 1.12)	0.87 (0.66, 1.16)	0.90 (0.57, 1.42)	0.872
Lutein/zeaxanthin					
Range	<229.0	229.0–320.0	320.1–470.0	>470.0	
No. deaths/total	87/776	111/791	103/749	140/791	
Crude	1	1.04 (0.71, 1.52)	1.14 (0.78, 1.66)	1.37 (0.88, 2.14)	0.129
Model 1	1	0.88 (0.58, 1.34)	1.09 (0.71, 1.67)	1.31 (0.77, 2.25)	0.148
Model 2	1	0.84 (0.53, 1.35)	0.99 (0.63, 1.55)	1.23 (0.69, 2.17)	0.209

Data are presented as HR (95% CI) unless indicated otherwise. Model 1: Adjusted for age (continuous), sex (male or female), race/ethnicity (non-Hispanic White, or other), BMI (continuous), education level (less than high school, high school or equivalent, or college or above), family income-to-poverty ratio (≤1.0, 1.0–3.0, or >3.0), drinking status (nondrinker, low-to-moderate drinker, or heavy drinker), smoking status (never smoker, former smoker, or current smoker), leisure-time moderate-to-vigorous physical activity (inactive group, insufficiently active group, or active group), HEI (in quartiles), total energy intakes (in quartiles), supplement use (yes or no), and other individual carotenoids (natural log-transformed). Model 2: Model 1 + diabetes medication use (yes or no), diabetes duration (<3, 3–10, >10 years), HbA_{1c} (<7% or ≥7%), self-reported hypertension (yes, or no), serum total cholesterol (in quintiles), and triglycerides (in quintiles).

between serum β-carotene concentrations and CVD mortality remained largely unchanged, suggesting that the observed association seems unlikely to be dietary in origin but may be mainly attributed to β-carotene in supplements. We also observed a low correlation between serum β-carotene levels and dietary β-carotene intakes (r_s = 0.19), which was in line with some previous studies (28,29). Thus, this result should not keep us from recommending consumption of fruits and vegetables, which are the major dietary sources of β-carotene. We did not further investigate the relationship between β-carotene supplement intake and CVD mortality among patients with diabetes, because only a subgroup of participants had data on specific supplement use in NHANES III and NHANES 2001–2006. However, caution should be taken with the use of β-carotene supplements in the prevention of heart disease among individuals with T2D, given some evidence has raised concerns about it (25). Of note, the possibility could not be excluded that serum β-carotene may be only a risk marker that is primarily affected by several host-related factors (i.e., disease state or genetic makeup) (30). Hence, the complex and multistep metabolic pathways of β-carotene and its relation to health effects should be explored further.

Our findings were inconsistent with the findings of observational studies that were conducted in the general population (31,32). An important explanation for this discrepancy may be the different study population (general population vs. patients with diabetes). Some experiments have suggested that β-carotene is prone to exert a pro-oxidant effect rather than an antioxidant effect under certain conditions, such as a highly oxidative environment (23). An RCT conducted in smokers with myocardial infarction who tended to have heightened oxidative

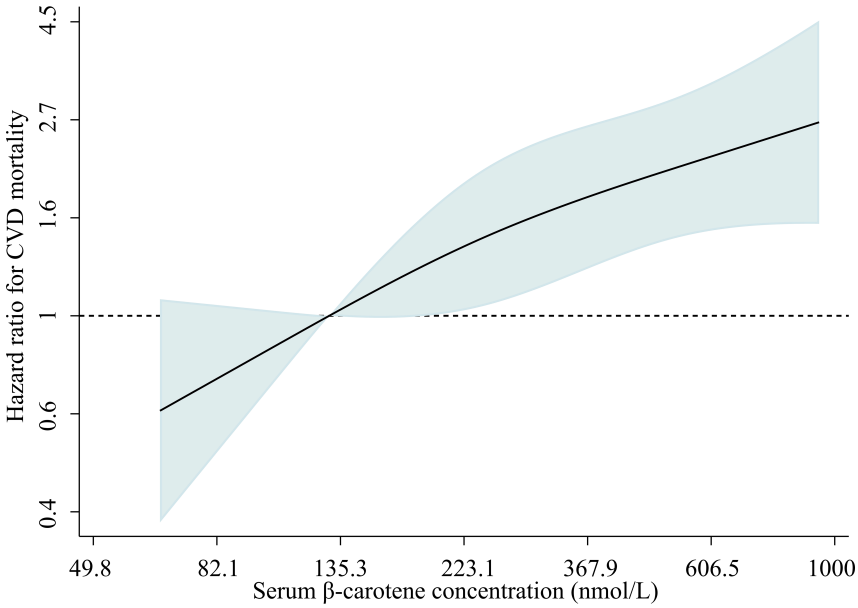

Figure 1—Association between serum β-carotene concentrations with CVD mortality among patients with type 2 diabetes in NHANES III and NHANES 2001–2006. Serum β-carotene concentrations were natural log-transformed in a restricted cubic splines model and then converted. HRs were adjusted for age (continuous), sex (male or female), and race/ethnicity (non-Hispanic White or other), BMI (continuous), education level (less than high school, high school or equivalent, or college or above), family income-to-poverty ratio (≤1.0, 1.0–3.0, or >3.0), drinking status (nondrinker, low-to-moderate drinker, or heavy drinker), smoking status (never smoker, former smoker, or current smoker), leisure-time moderate-to-vigorous physical activity (inactive group, insufficiently active group, or active group), HEI (in quartiles), total energy intakes (in quartiles), supplement use (yes or no), serum total cholesterol (in quintiles), triglycerides (in quintiles), diabetes medication use (yes or no), diabetes duration (<3, 3–10, or >10 years), HbA_{1c} (<7% or ≥7%), self-reported hypertension (yes or no), and serum α-carotene, β-cryptoxanthin, lutein + zeaxanthin, and lycopene (all natural log-transformed). A serum level of 130 nmol/L was used as the reference to estimate all HRs. The shaded areas indicate the 95% CI. *P* linearity = 0.001.

stress status also found that high β-carotene supplement intakes significantly increased cardiovascular risk (10). Compared with the general population, patients with diabetes have higher oxidative stress status and risk of CVD (14,33), in whom therefore β-carotene may change from an anti- to a pro-oxidant, with increased risk of CVD. Additionally, various degrees of measurement error are inevitable in dietary and biomarker studies, which may obscure the association, although the coefficient of variance of serum β-carotene was <5% in our study. More studies are needed to confirm the association between serum β-carotene and CVD mortality in patients with T2D.

To date, little is known about the effects of other major individual carotenoids (i.e., α-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene) on CVD risk among individuals with T2D. Only one cross-sectional study of 105 patients with diabetes suggested that plasma lycopene was inversely related to atherosclerotic burden (34). In the current study, we did not observe any significant association between other serum individual carotenoids and CVD mortality among individuals with T2D. Further prospective studies with large sample sizes are needed to investigate the associations.

Although biological mechanisms underlying the potential adverse cardiovascular effect of β-carotene among patients with T2D remain unclear, several in vitro and animal experiments have indicated that the presence of a highly oxidative environment, elevated β-carotene concentrations, or oxygen tension will alter β-carotene metabolism and result in a significant increase in its oxidized form or its oxidative metabolites (13,23). These metabolites could act as pro-oxidants (23), causing DNA damage (35), enhancing lipid peroxidation (36), and interfering with some other compounds that are important to redox relations (8). Thus, in patients with diabetes, who usually have an oxidative and proinflammatory state (14,37), β-carotene may be prone to exert pro-oxidant effects. In addition, β-carotene located in adipose tissue may be metabolized to thrombogenic or atherogenic derivatives, leading to increased risk of CVD (10,38). Nevertheless, more studies are warranted to clarify the underlying mechanisms between β-carotene and CVD mortality among patients with diabetes.

Based on a prospective cohort over a long follow-up period, the current study is among the first to evaluate the associations of serum major carotenoids with CVD mortality in a nationally representative sample of U.S. adults with diabetes. Moreover, multiple potential confounders were carefully adjusted, including lifestyle and dietary factors, diabetes duration, glucose control, and lipid levels.

Despite the strengths, several limitations should also be considered. First, because of the observational study design, causality could not be established. Second, because we relied on single baseline measures of serum carotenoids concentrations, we were not able to evaluate the time-varying association of interest. Third, the current study did not have detailed information on the severity of diabetes, although the results did not substantially change when further adjusting for diabetes duration, diabetes medication use, and HbA_{1c} levels. Fourth, these results are based on U.S. adults with diabetes, which may limit the generalizability to other populations. Fifth, genetic risk factors affecting β-carotene metabolism were not analyzed. Thus, it would be interesting to investigate the potential diet-gene interaction on CVD mortality in future studies. Sixth, the statistical power is limited to detect weak or moderate differences in the subgroup analyses, and the results should be interpreted with caution. Finally, the possibility of residual and unknown confounding cannot be entirely excluded.

Conclusion

In a nationally representative sample of U.S. adults with T2D, we found that higher concentrations of serum β-carotene, but not other individual carotenoids, were linearly associated with elevated risk of CVD mortality. Further

Table 3—Stratified analyses of the associations between serum β-carotene concentrations and CVD mortality among patients with T2D in NHANES III and NHANES 2001–2006

	Serum β-carotene (nmol/L)					
Characteristics	Quartile 1 <148.4	Quartile 2 148.4–240.0	Quartile 3 240.1–424.9	Quartile 4 >424.9	P_{trend}	$P_{interaction}$
Age, years						0.822
≤60 (n = 1,589)	1	1.56 (0.62, 3.89)	1.92 (0.70, 5.29)	3.75 (1.32, 10.65)	0.014	
>60 (n = 1,518)	1	1.49 (0.83, 2.67)	2.68 (1.61, 4.46)	2.88 (1.78, 4.65)	0.003	
Sex						0.790
Male (n = 1,454)	1	1.64 (1.02, 3.12)	2.63 (1.24, 5.61)	2.97 (1.33, 6.66)	0.054	
Female (n = 1,653)	1	1.03 (0.46, 2.32)	1.86 (0.85, 4.09)	1.96 (0.88, 4.36)	0.048	
Race/ethnicity						0.155
Non-Hispanic White (n = 1,086)	1	1.49 (0.72, 3.10)	2.28 (1.14, 4.58)	2.88 (1.58, 5.26)	0.011	
Other (n = 2,021)	1	1.36 (0.78, 2.37)	2.06 (1.04, 4.08)	1.95 (1.26, 3.03)	0.022	
BMI, kg/m^2						0.598
<30 (n = 1,398)	1	1.34 (0.58, 3.09)	2.46 (1.18, 5.14)	2.53 (1.40, 4.57)	0.008	
≥30 (n = 1,647)	1	1.38 (0.63, 3.04)	1.29 (0.56, 3.01)	2.21 (0.86, 5.64)	0.105	
Smoking status						0.592
Current (n = 604)	1	0.58 (0.15, 2.25)	2.56 (0.99, 6.61)	3.62 (1.57, 8.34)	<0.001	
Never/past (n = 2,500)	1	1.70 (0.94, 3.06)	2.13 (1.14, 4.01)	2.35 (1.22, 4.52)	0.135	
Diabetes duration, years						0.144
<3 (n = 1,601)	1	1.55 (0.86, 2.80)	2.48 (1.09, 5.71)	3.94 (1.69, 9.21)	0.004	
≥3 (n = 1,283)	1	1.43 (0.72, 2.85)	2.07 (1.06, 4.06)	1.76 (0.98, 3.19)	0.229	
HbA_{1c}, % (mmol/mol)						0.214
<7.0 (<53) (n = 1,798)	1	1.72 (0.77, 3.87)	3.02 (1.37, 6.70)	4.16 (1.88 9.42)	0.001	
≥7.0 (≥53) (n = 1,302)	1	1.38 (0.67, 2.83)	1.52 (0.84, 3.21)	1.35 (0.66, 2.77)	0.849	

Data are presented as HR (95% CI). Adjusted for age (continuous), sex (male or female), race/ethnicity (non-Hispanic White, or other), BMI (continuous), education level (less than high school, high school or equivalent, or college or above), family income-to-poverty ratio (≤1.0, 1.0–3.0, or >3.0), drinking status (nondrinker, low-to-moderate drinker, or heavy drinker), smoking status (never smoker, former smoker, or current smoker), leisure-time moderate-to-vigorous physical activity (inactive group, insufficiently active group, or active group), HEI (in quartiles), total energy intakes (in quartiles), supplement use (yes or no), serum total cholesterol (in quintiles), triglycerides (in quintiles), diabetes medication use (yes or no), diabetes duration (<3, 3–10, or >10 years), HbA_{1c} (<7% or ≥7%), self-reported hypertension (yes or no), serum α-carotene, β-cryptoxanthin, lutein + zeaxanthin, and lycopene (all natural log-transformed). The strata variable was not included in the model when stratifying by itself. The missing values were n = 62 for BMI, n = 3 for smoking status, n = 223 for diabetes duration, and n = 7 for HbA_{1c}.

studies are warranted to confirm these findings.

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