Associations of Subtype and Isomeric Plasma Carotenoids with Prostate Cancer Risk in Low-Income African and European Americans

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ABSTRACT

Background: Various carotenoids in circulation, including isomers, may have different influences on cancer risk.

Methods: We conducted a nested case–control study including 343 incident prostate cancer cases and 640 controls individually matched on age, race, study site, and time of blood collection. Carotenoids investigated were carotene, cryptoxanthin, lycopene, dihydrolycopene, lutein, anhydrolutein, and zeaxanthin, including α versus β configurations and *cis* versus *trans* isomers. General linear model and conditional logistic regression were applied to evaluate associations for prostate cancer risk, with adjustment for potential confounders. We conducted additional analyses with further stratification by race, multivitamin use, and smoking status.

Results: Case–control differences were found in carotenoid subtype levels, although not all reached the multiple comparison adjusted threshold for significance. Plasma lycopene [$OR_{T1\ vs.\ T3} = 0.51$; 95% confidence interval (CI), 0.29–0.87; $P_{trend} = 0.014$], dihydrolycopene ($OR_{T1\ vs.\ T3} = 0.37$; 95% CI, 0.18–0.74; $P_{trend} = 0.006$), and *cis*-anhydrolutein ($OR_{T1\ vs.\ T3} = 0.57$; 95% CI, 0.33–0.96; $P_{trend} = 0.037$) were inversely, while β-*trans*-carotene ($OR_{T1\ vs.\ T3} = 2.13$; 95% CI, 1.32–3.43; $P_{trend} = 0.002$) and *trans*-lutein ($OR_{T1\ vs.\ T3}$, 1.86; 95% CI, 1.20–2.88; $P_{trend} = 0.006$) were positively associated with prostate cancer risk. Stratified analyses showed inverse associations of lycopene, dihydrolycopene, and *cis*-anhydrolutein with prostate cancer risk in subjects without multivitamin use; lycopene and dihydrolycopene in African-Americans and current smokers; and dihydrolycopene in nonsmokers. Positive associations of β-*trans*-carotene and *trans*-lutein were observed in African-Americans, nonsmokers, and multivitamin users.

Conclusions: The associations of carotenoids with risk of prostate cancer differed by carotenoid subtypes.

Impact: Public health recommendations on carotenoid intakes for prostate cancer prevention should take subtypes and isomers into consideration.

Introduction

The biological connection of retinoids and carotenoids with cancer development has been associated with their roles in immune system stimulation, gap junction communication (1), cell-cycle regulation and apoptosis, modulation of growth factors, cell differentiation (2), and various receptors and adhesion molecules (3), although results from epidemiologic studies remain controversial and inconsistent for some cancers, including prostate cancer. These inconsistencies could be due to characteristic differences in the population under study, such as race, multivitamin supplement usage, tumor stage, smoking status, and/or subtypes of retinoids and carotenoids.

One large pooled analysis, based on 15 prospective and intervention trial studies (4), found positive associations between circulating levels of lycopene and retinol with risk of aggressive prostate cancer. However, the associations were primarily driven by studies of Caucasians, including Finns, Norwegians, Australians, and European-Americans, with the exception of 1 cohort, which included a respective 218 and 438 African-American cases and controls. In the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) trial, consisting of 29,133 Finnish male smokers, β-carotene supplementation was not associated with prostate cancer incidence (5). The associations of retinoids and carotenoids with risk of prostate cancer have not been well-studied in low-income African-American and European-American populations. In a study of 1,079 European-Americans (164 high- and 915 low-aggressive prostate cancer) and 1,023 African-Americans (206 high- and 817 low-aggressive prostate cancer), Antwi and colleagues (6) reported that dietary lycopene and β–cryptoxanthin were inversely associated with aggressive status of prostate cancer in both races.

Carotenoid subtypes and isomeric arrangements may explain the reported inconsistent associations between circulating carotenoid levels and risk of prostate cancer. However, to our knowledge, no previous study has examined the associations by molecular configuration and/or isomeric occurrence. Overloading on antioxidant multivitamins, especially hepatic-storable lipid-soluble retinol and carotenoid antioxidants, can lead to prooxidant properties (7). Provitamin A carotenoids, including α-carotene, β-carotene, and β-cryptoxanthin, can be converted to retinol (5) but cannot lead to vitamin A toxicity due to enzymatic control over retinol synthesis in the body. Nonprovitamin A carotenoids, including lycopene, lutein, and lutein's isomer zeaxanthin, are not converted to retinol and have biological effects independent of

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vitamin A activity, particularly in the eyes, offering an advantage of chemoprevention without the associated toxicity from retinoids (6). In addition, the major circulating carotenoids in humans consist of hydrocarbons with (β-cryptoxanthin and lutein/zeaxanthin, which contain hydroxyl groups) or without (β-carotene, α-carotene, and lycopene) oxygen groups. Oxygen-containing carotenoids are more polar than carotenes, generally located on the surface of lipoproteins, and more likely to undergo rapid surface transfer (8). Most carotenoids occur in large varieties of *cis-trans* (or E/Z) geometrical configurations. However, it is not completely understood whether carotenoid configurations have specific biological properties (9).

This study is aimed at evaluating associations between plasma retinol and carotenoid levels with prostate cancer risk according to their bioactive forms, including α and β subtypes and *cis* and *trans* isomers, and exploring whether the associations differ by ethnicity, smoking status, and multivitamin supplementation.

Methods

Study population

We conducted a case–control study nested within the Southern Community Cohort Study (SCCS), a prospective cohort study investigating health disparities among predominantly low-income African-Americans and European-Americans across 12 Southeastern states. Details about the SCCS have been published previously (10). All participants provided written informed consent, and the study was approved by the Institutional Review Boards (IRB) at Vanderbilt University (Nashville, TN) and Meharry Medical College. Blood samples were collected at baseline recruitment in 10-mL EDTA tubes and 10-mL serum tubes. The vast majority (>95%) of blood samples were processed within 24 hours after collection and stored at −80°C. The plasma samples were used for all assays in this study.

Case identification and matched controls

Incident prostate cancer cases from nearly 34,000 male participants, aged 40 to 79 years at enrollment between 2002 and 2009, were identified through linkages with state tumor registries and/or National Death Index mortality records through January 2016. A total of 879 incident prostate cancer cases were identified from cohort participants. Among them, 347 had blood samples collected at baseline and were included for this study. Two controls per case were randomly selected from SCCS participants who provided a blood sample at the baseline survey. Matching criteria included race (African-American/European-American/others), enrollment site, age at enrollment +/− 2 years (relaxed to 5 years, when necessary), and date of blood sample collection +/- 6 months (relaxed to 9 months, when necessary). A total of 653 controls were included in the study. After exclusion of subjects without race information ($n = 2$) and other ethnicities ($n = 15$), a total of 343 cases and 640 controls (293 cases and 541 controls for African-Americans, and 50 cases and 99 controls for European-Americans) participated in this study. The average time between cohort entry and prostate cancer diagnosis was 4.4 years.

Plasma antioxidant vitamin analysis

Plasma concentrations of retinol and carotenoids were determined by high-pressure liquid chromatography (HPLC) with photo-diode array detection, as applied previously (11). Briefly, 0.15 mL plasma was mixed with an ethanolic solution of 3 internal standards (one each for retinoids, carotenoids, and tocopherols), followed by partitioning into hexane, drying, and redissolving in 0.15 mL of the HPLC mobile phase, as detailed previously (12, 13). Ten microliters were injected onto a Spherex C18 analytic column (150 mm × 3.2 mm, 3 μm), coupled to a Spherex C18 precolumn (4 mm × 3.0 mm, 10 μmol/L; Phenomenex), using isocratic elution with a mobile phase of 650 mL methanol, 250 mL dichloromethane, 100 mL acetonitrile, and 2 mL aqueous bis-tris propane (0.5 mol/L, pH 6.8; Sigma-Aldrich), and containing 0.25 g/L butylated hydroxytoluene (BHT; 1.14 mmol/L; Sigma-Aldrich), at an elution rate of 0.23 mL/minute. Retinol and carotenoid concentrations were determined by monitoring at 325 nm and 450 nm, respectively. Detection limits were approximately 1 ng/mL. Inter-assay variability was between 4% and 10% for the major analytes, at levels of 100 to 500 ng/mL. The assay was validated by participating in the quality assurance program for fat soluble vitamins, organized by the National Institute for Standards and Technologies (Gaithersburg, MD). The intraassay and interassay coefficients of variability were <7% and 12%, respectively, for all measurements.

Covariates

All information on covariates was based on self-reported data gathered at baseline. Education was categorized across five groups as <9 years, 9–11 years, 12 years, some college or training, and ≥graduated college. Annual household income was divided across three groups as <$15,000, $15,000–$24,999, and >$25,000. Family history of prostate cancer referred to first degree relatives, i.e., father and brother(s). The Nam-Powers-Boyd (NPB) occupational index, derived based on participants' longest-held occupation, was used to determine occupational status. For chronic disease history, the comorbidity index, a modified Charlson index (14) ranging from 0 to 12, was adopted. Scores were grouped as 0, 1, 2, 3, and ≥4. Body mass index (BMI) was categorized based on World Health Organization (WHO) criteria as <18.5, 18.5–24.9, 25–29.9, 30–34.9, and ≥35 kg/m^2. Lifestyle factors included smoking (never, former, and current), alcohol consumption (never and 3 groups by amount of daily use), total daily activity (TDA; quartile), and healthy eating index (HEI; quartile) scores. Dietary information was collected using a validated food frequency questionnaire (FFQ; ref. 15). To calculate HEI scores, SCCS FFQ data was linked to the MyPyramid Equivalents Database (version 2.0) to generate equivalent intakes (cup or ounce equivalent per 1,000 kcal) for food groups listed in the U.S. Dietary Guidelines for Americans. The equivalent intake for each food group was then scored using the standard HEI-2010 or HEI-2005. The reference SAS code used for calculating HEI scores was provided by the USDA (16). Multivitamin supplementation was based on any multivitamin use. To avoid a reduced number of informative covariates, we assigned missing values with the high frequency (for categorical variables) or median (for continuous variables).

Statistical analysis

After adjustment for potential confounders including education, family income, history of diabetes, family history of prostate cancer, NPB score, comorbidity index, BMI, smoking, alcohol consumption, TDA, HEI, and retinol and total carotenoid levels, associations of retinol and carotenoids with prostate cancer risk were assessed and presented by two approaches. We first compared geometric means of retinol and carotenoid plasma levels between cases and controls with adjustment for potential confounders using the general linear model (GLM), followed by conditional logistic regression to compute OR and 95% confidence intervals (CI). Trend test was conducted by assigning an ordinal score to each exposure level of a categorical variable and treating the ordinal score as continuous scale in the model. To minimize potential false positive findings due to multiple comparisons, we made a Bonferroni correction based on the number of items

examined. In total, we assessed 8 categories of retinol and carotenoids (carotene, cryptoxanthin, lycopene, dihydrolycopene, lutein, anhydrolutein, and zeaxanthin) and set P values of <0.006 (0.05/8) as statistically significant. Stratified analyses were also carried out to explore whether the associations varied by race (African-American vs. European-American), smoking status (never and former vs. current), and ever-users of multivitamins (yes vs. no). For analyses evaluating potential modifications of smoking status and multivitamin supplement use, we included an interaction term between each plasma vitamin level and smoking status or multivitamin supplement use (yes/no) by alternating the codes 0 and 1 for the stratifiers to generate stratum-specific estimates, to avoid the breakdown of matched sets in the conditional logistic regression model. Additional analyses were conducted among cases with stage II–IV prostate cancer, cases with stage III–IV prostate cancer, and by Gleason score, separately. All analyses were performed using SAS version 9.3 (SAS Institute).

Results

For prostate cancer cases included in the study, the distributions of tumor–node–metastasis (TNM) stages were: $T1_{a\text{-}c}$, 26.9%; $T2_{a\text{-}b}$, 7.9%; $T2_{a\text{-}b}$, 1.5%; N0, 31.2%; N1, 0.9%; M0, 32.6%; and M1, 2.0%. Gleason score percentages were: <6, 30.7%; 7, 31.5%; ≥8, 9.6%; and missing, 28.3%. There were only 39 stage III and IV prostate cancer cases, 33 of whom had a Gleason score ≥8, which is considered to be aggressive prostate cancer (17).

Sociodemographic factors including education, family income, occupational status, and comorbidity were not associated with prostate cancer risk. The lowest BMI (<18.5 kg/m^2) was associated with increased prostate cancer risk; however, the statistics were based on a small number of participants in this category. Smoking, alcohol drinking, and sleep duration were not significantly associated with prostate cancer risk. Higher scores for HEI ($OR_{Q1\ vs.\ Q3} = 0.63$; 95% CI, 0.42–0.95) were inversely associated with prostate cancer risk (**Table 1**). Associations of demographic and lifestyle factors with retinol and total carotenoids are shown in Supplementary Table S1.

Table 2 displays comparisons of case–control differences across 8 major categories of retinol and carotenoids, as well as in α, β subtypes and *cis* or *trans* isomeric occurrence within 4 carotenoid subcategories. Some case–control differences in geometric means were noted; however, not all reached multiple comparison adjusted significance ($P < 0.006$). Plasma levels of β-carotene and lutein, occurring predominantly as *trans* isomers ($P_{adj} = 0.001$ and 0.017, respectively), were higher in cases than controls and were positively associated with the risk of prostate cancer ($OR_{T1\ vs.\ T3} = 2.13$; 95% CI, 1.32–3.43; $P_{trend} = 0.002$ and $OR_{T1\ vs.\ T3} = 1.86$; 95% CI, 1.20–2.88; $P_{trend} = 0.006$, respectively). A similar positive association was observed for β-*cis*-carotene ($OR_{T1\ vs.\ T3} = 1.65$; 95% CI, 1.06–2.59; $P_{trend} = 0.030$). On the other hand, plasma levels of lycopene, both *trans* and *cis* isomers, were inversely associated with prostate cancer risk ($OR_{T1\ vs.\ T3} = 0.42$; 95% CI, 0.18–0.96; $P_{trend} = 0.040$; and $OR_{T1\ vs.\ T3} = 0.23$; 95% CI, 0.09–0.56; $P_{trend} = 0.001$, respectively). Dihydrolycopene levels were inversely associated with prostate cancer risk ($OR_{T1\ vs.\ T3} = 0.37$; 95% CI, 0.18–0.74; $P_{trend} = 0.006$), with average plasma levels of 54.4 ng/mL for cases and 57.8 ng/mL for controls ($P_{adj} = 0.016$). A similar inverse association was observed for *cis*-anhydrolutein ($OR_{T1\ vs.\ T3} = 0.57$; 95% CI, 0.33–0.96; $P_{trend} = 0.037$). The positive associations of β-carotene and lutein and inverse associations of lycopene and dihydrolycopene with prostate cancer risk persisted after excluding cases diagnosed within 1 or 2 years (Supplementary Table S2). In the analysis restricted to stage II to IV prostate cancer, positive associations were found for plasma levels of β-carotene ($P_{trend} = 0.008$), in both *trans* and *cis* isomers ($P_{trend} = 0.009$ and 0.032, respectively), and *trans*-lutein ($P_{trend} = 0.005$). Inverse associations were found for lycopene ($P_{trend} = 0.003$), in both *trans* and *cis* isomers ($P_{trend} = 0.013$ and 0.001, respectively), and dihydrolycopene ($P_{trend} = 0.004$; **Table 2**). We conducted additional analyses restricted to stage III and IV cases and found similar association patterns; however, none of the risk estimates reached statistical significance due to a small sample size (Supplementary Table S3). Results stratified by Gleason score are shown in Supplementary Table S4.

Subgroup analyses stratified by race showed that mean β–*trans*-carotene ($P_{adj} = 0.001$) and *trans*-lutein ($P_{adj} = 0.019$) levels were higher in African-American cases than controls, with $OR_{T1\ vs.\ T3} = 2.10$ (95% CI, 1.25–3.52) for β–*trans*-carotene and $OR_{T1\ vs.\ T3} = 1.89$ (95% CI, 1.18 to 3.02) for *trans*-lutein. In contrast, dihydrolycopene levels were inversely associated with prostate cancer risk in African-Americans ($OR_{T1\ vs.\ T3} = 0.27$; 95% CI, 0.12–0.59; $P_{trend} = 0.001$; ref. **Table 3**). No associations were observed among European-Americans. However, the sample size for European-Americans was small ($n = 50$ case–control sets). Tests for interaction were not significant.

Results of additional analyses stratified by smoking status and multivitamin supplement use are presented in **Tables 4** and **5**. β–*Trans- and* β–*cis*-carotene plasma levels were higher in cases than controls in never/former smokers ($P_{adj} = 0.019$ and 0.043, **Table 4**). Positive associations were observed for β-*trans*-carotene in both never/former smokers ($OR_{T1\ vs.\ T3} = 2.01$; 95% CI, 1.12–3.60; $P_{trend} = 0.017$) and current smokers ($OR_{T1\ vs.\ T3} = 2.44$; 95% CI, 1.30–4.56; $P_{trend} = 0.008$) and for β-*cis*-carotene and *trans*-lutein among never/former smokers ($OR_{T1\ vs.\ T3} = 2.00$; 95% CI, 1.11–3.60; $P_{trend} = 0.020$ and $OR_{T1\ vs.\ T3} = 2.33$; 95% CI, 1.32–4.12; $P_{trend} = 0.003$). Inverse associations were observed for *cis*-lycopene ($P_{trend} = 0.022$ and 0.001 in never/former and current smokers, respectively) and dihydrolycopene ($P_{trend} = 0.007$ and 0.032 in never/former and current smokers, respectively), regardless of smoking status (**Table 4**). *Cis*-anhydrolutein was negatively associated with the risk of prostate cancer in current smokers only ($OR_{T1\ vs.\ T3} = 0.52$; 95% CI, 0.27–1.02; $P_{trend} = 0.035$).

β-*trans*-carotene levels were higher in multivitamin users than in subjects without multivitamin supplementation, and a positive association of β-*trans*-carotene with risk of prostate cancer was observed regardless of multivitamin supplementation status ($OR_{T1\ vs.\ T3} = 1.94$; 95% CI, 1.13–3.32; $P_{trend} = 0.018$ in nonusers, $OR_{T1\ vs.\ T3} = 2.60$; 95% CI, 1.28–5.29; $P_{trend} = 0.005$ in users; **Table 5**). Plasma levels of *cis*-lycopene ($OR_{T1\ vs.\ T3} = 0.19$; 95% CI, 0.07–0.51; $P_{trend} = 0.001$), dihydrolycopene ($OR_{T1\ vs.\ T3} = 0.30$; 95% CI, 0.14–0.64; $P_{trend} = 0.002$), and *cis*-anhydrolutein ($OR_{T1\ vs.\ T3} = 0.50$; 95% CI, 0.28–0.90; $P_{trend} = 0.023$) were inversely associated, while plasma *trans*-lutein levels were positively associated with the risk of prostate cancer in subjects with no multivitamin supplementation ($OR_{T1\ vs.\ T3} = 1.97$; 95% CI, 1.16–3.33; $P_{trend} = 0.014$; **Table 5**).

Discussion

In this nested case–control study within the SCCS, we found associations of plasma β-*trans*-carotene, *cis*-lycopene, dihydrolycopene, and *trans*-lutein with prostate cancer risk after adjusting for multiple comparisons. *Trans* isomers for β-carotene were found to be nominally and positively associated with prostate cancer risk, predominantly in African-Americans and regardless of smoking status and multivitamin use. The positive association of plasma β-*trans*-lutein was observed in African-Americans, current and

Table 1. Associations of demographic and lifestyle factors with the risk of prostate cancer.

	Number of subjects		
	PC	Controls	Adjusted OR[a]
Age (mean ± SD)	57.8 ± 8.02	57.3 ± 8.08	
Race (African-American, %)	293 (35.1)	541 (64.9)	
Education			
<9 years	49 (14.29)	92 (14.38)	Reference
9–11 years	86 (25.07)	163 (25.47)	1.07 (0.68–1.67)
12 years	118 (34.40)	214 (33.44)	1.06 (0.68–1.65)
College or training	54 (15.74)	115 (17.97)	0.97 (0.58–1.62)
≥College graduate	36 (10.50)	55 (8.59)	1.37 (0.77–2.42)
Missing	0 (0.00)	1 (0.16)	
Family income ($)			
<15,000	188 (54.81)	385 (60.16)	Reference
15,000–24,999	81 (23.62)	135 (21.09)	1.19 (0.86–1.66)
≥25,000	69 (20.12)	113 (17.66)	1.27 (0.87–1.86)
Missing	5 (1.46)	7 (1.09)	
History of diabetes			
No	267 (77.84)	479 (74.84)	Reference
Yes	76 (22.16)	161 (25.16)	0.83 (0.61–1.15)
Family history of prostate cancer			
No	282 (82.22)	532 (83.13)	Reference
Yes	24 (7.00)	37 (5.78)	1.51 (0.65–2.70)
Unknown	37 (10.79)	71 (11.09)	
NPB_CAT			
Category 1	97 (28.28)	196 (30.63)	Reference
Category 2	90 (26.24)	166 (25.94)	0.98 (0.67–1.44)
Category 3	80 (23.32)	178 (27.81)	0.86 (0.59–1.25)
Category 4	48 (13.99)	63 (9.84)	1.39 (0.86–2.24)
Missing	28 (8.16)	37 (5.78)	
Comorbidity index (score)			
0	60 (17.49)	130 (20.31)	Reference
1	89 (25.95)	156 (24.36)	1.24 (0.80–1.92)
2	93 (27.11)	164 (25.63)	1.18 (0.77–1.82)
3	56 (16.33)	110 (17.19)	1.07 (0.66–1.74)
>4	38 (11.08)	73 (11.41)	1.12 (0.66–1.91)
Missing	7 (2.04)	7 (1.09)	
BMI			
<18.5	7 (2.04)	4 (0.63)	3.81 (1.08–13.4)
18.5–24.9	96 (27.99)	195 (30.47)	Reference
25.0–29.9	116 (33.82)	236 (36.88)	0.98 (0.69–1.39)
30.0–34.9	70 (20.41)	121 (18.91)	1.22 (0.82–1.83)
≥35	52 (15.16)	82 (12.81)	1.20 (0.75–1.91)
Missing	2 (0.58)	2 (0.31)	
Lifestyle factors			
Smoking			
Never	77 (22.45)	146 (22.80)	Reference
Former	116 (33.82)	172 (26.88)	1.17 (0.81–1.69)
Current	150 (43.73)	322 (50.31)	0.82 (0.58–1.16)
Less than 20 (pack-years)	128 (37.32)	255 (39.84)	0.91 (0.64–1.30)
20–39 (pack-years)	83 (24.20)	126 (60.29)	1.16 (0.78–1.73)
More than 40 (pack-years)	55 (16.03)	113 (17.66)	0.85 (0.54–1.32)
Alcohol use (g/week)			
Never	136 (39.65)	251 (39.22)	Reference
<3.5	77 (22.45)	149 (23.28)	0.91 (0.64–1.29)
3.5–14.0	40 (11.66)	80 (12.5)	0.83 (0.53–1.31)
≥14.0	84 (24.49)	154 (24.06)	1.00 (0.69–1.46)
Missing	6 (1.75)	6 (0.94)	
Sleep duration (per day)			
<6 h	49 (14.29)	103 (16.09)	0.91 (0.60–1.38)
6–7.99 h	133 (38.78)	261 (40.78)	Reference
≥8 h	159 (46.89)	272 (42.50)	1.16 (0.86–1.56)
Missing	2 (0.58)	4 (0.63)	

(Continued on the following page)

Table 1. Associations of demographic and lifestyle factors with the risk of prostate cancer. (Cont'd)

	Number of subjects		
	PC	Controls	Adjusted OR[a]
Total activity (h/day)			
Quartile 1 (<1.93)	94 (27.41)	148 (23.13)	Reference
Quartile 2 (<4.29)	84 (24.49)	163 (25.47)	0.84 (0.57–1.24)
Quartile 3 (<8.70)	89 (25.95)	155 (24.22)	0.91 (0.62–1.36)
Quartile 4 (≥8.70)	74 (21.57)	170 (26.56)	0.69 (0.46–1.05)
Missing	2 (0.58)	4 (0.63)	
Multivitamin use			
No	235 (68.51)	437 (68.28)	Reference
Yes	105 (30.61)	199 (31.09)	0.95 (0.70–1.28)
Missing	3 (0.87)	4 (0.63)	
HEI			
Quartile 1 (<47.93)	87 (25.36)	144 (22.50)	Reference
Quartile 2 (<55.48)	77 (22.45)	155 (24.22)	0.77 (0.52–1.15)
Quartile 3 (<62.89)	64 (18.66)	167 (26.09)	**0.63 (0.42–0.95)**
Quartile 4 (≥62.89)	89 (25.95)	143 (22.34)	1.00 (0.67–1.50)
Missing	26 (7.58)	31 (4.84)	

Note: Values in bold are statistically significant.

Abbreviations: h, hours; NPB_CAT, Categorize NPB (NPB scores in approximate quartiles); PC, prostate cancer.

[a]Adjusted for education, family income, history of diabetes, family history of prostate cancer, NPB score, comorbidity, and BMI using conditional multiple logistic analysis.

former smokers, and subjects without multivitamin supplement use. The inverse associations of plasma lycopene and dihydrolycopene with the risk of prostate cancer were more evident among African-Americans, never or former smokers, and subjects without multivitamin-supplement use. The inverse association of *cis*-anhydrolutein was restricted in subjects without multivitamin-supplement use. To our knowledge, this study is the first to examine associations between subtypes (α and β) and carotenoid isomers (*cis* and *trans)* on the risk of prostate cancer.

Retinol, the active form of vitamin A, has been proposed as a candidate for prostate cancer prevention because of its effect on regulating growth, differentiation, and apoptosis of normal and malignant cells through regulation of DNA transcription and DNA polymerase activity, or increasing levels of other antioxidants, such as selenium and α-tocopherol (18). However, retinol can also stimulate growth and dedifferentiation of prostate cells, thus negating any anticancer activity (19). Although some studies have reported positive (19, 20) or inverse (21) associations between circulating retinol and prostate cancer, we failed to find these associations after accounting for race, smoking status, and multivitamin use in a low socioeconomic and predominantly African-American population. Our findings are supported by results from a recent pooled analysis, showing a null association (4). Shiels and colleagues (22) found a smoking–retinol interaction on sex hormone levels, in which male smokers were shown to have higher levels of sex steroid hormones, such as testosterone, and suggested retinol may act synergistically with smoking through this mechanism. In our study, however, we did not observe an interaction between plasma retinol level and smoking status on prostate cancer risk. It is worth noting that circulating retinol levels are highly regulated by the liver, and thus, retinol may not be a good biomarker for vitamin A status. This may explain the large null results found in our and previous studies.

A pooled analysis reported positive associations for α- and β-carotene subtypes with prostate cancer risk in unadjusted analyses, but the associations were attenuated by sociodemographic and lifestyle factors (4). In our study, total carotene levels were higher, rather than lower, among prostate cancer cases compared with controls, consistent with results from the Multiethnic Cohort (MEC) study (23), which included less than 47% African-Americans, in which significant positive associations were found for overall and advanced prostate cancer. The significant positive associations we found for carotene, both *trans* and *cis* isomers, on prostate cancer risk were predominantly observed in the β but not α subtype. Positive associations for β-carotene were predominately seen in stage II–IV prostate cancer, consistent with findings from the Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial (24). Our findings support the claim that high carotene levels, particularly high β-carotene, may promote prostate cancer growth (25). When we examined isomers for β-carotene, the *trans* isomer was significantly higher among cases than controls in African-Americans. *Trans* isomers are more likely to be broken down into vitamin A compared with *cis* isomers, a possible explanation for the positive associations observed between provitamin A on prostate cancer risk (26, 27).

No association of cryptoxanthin with prostate cancer risk was observed in our study, regardless of subtype or isomer. Several prospective cohort studies, including the PLCO (24), European Prospective Investigation into Cancer and Nutrition (EPIC; ref. 28), and "Give Us a Clue to Cancer" (CLUE I and II; ref. 29) also reported null associations between plasma β-cryptoxanthin and prostate cancer risk, consistent with our results. Those studies, however, only analyzed the β-cryptoxanthin *trans* isomer, and not its *cis* isomer (30).

Among the various carotenoids, lycopene has been shown to be among those with the strongest antioxidant activities (31) and is regarded as potentially the most potent agent against risk of prostate cancer, especially the more lethal forms (32). The inhibitory effects of lycopene on carcinogenesis could involve free radical scavenging, upregulation of detoxification systems, interference with cell proliferation, induction of gap-junction communication, inhibition of cell-cycle progression, modulation of signal transduction pathways, and interaction with growth factors (33). The EPIC (27) and Physicians' Health Study (PHS; ref. 23), as well as a recent pooled analysis (4), showed inverse associations, especially with risk of aggressive prostate cancer, supporting the findings of our study. Approximately 80% of total lycopene occurs in the *cis* form in prostate tissue, compared with

Table 2. Geometric means and associations of plasma retinol and carotenoid subtypes and isomers with the risk of prostate cancer.

	All subjects (cases = 343, controls = 640)						Stage II–IV (cases = 238, controls = 640)					
	Geometric means (ng/mL)			Adjusted OR (95% CI)[a]			Geometric means (ng/mL)			Adjusted OR (95% CI)[a]		
	Case	Control	P_{adj}[a]	Tertile 2	Tertile 3	P_{trend}	Case	Control	P_{adj}[a]	Tertile 2	Tertile 3	P_{trend}
Retinol	680	688	*0.464*	0.91 (0.63–1.33)	1.17 (0.73–1.88)	*0.609*	683	690	*0.601*	0.98 (0.62–1.53)	1.00 (0.56–1.81)	*0.983*
Total carotenoid	930	931	*0.983*	1.03 (0.72–1.49)	1.03 (0.72–1.49)	*0.872*	927	934	*0.738*	1.02 (0.67–1.57)	1.10 (0.70–1.73)	*0.666*
Carotene	197	174	***0.002***	1.21 (1.36–3.47)	2.17 (1.36–3.47)	***0.001***	202	174	***0.002***	1.21 (0.75–1.94)	2.28 (1.29–4.02)	***0.004***
α-*trans*-Carotene	16.0	15.6	*0.581*	1.09 (0.75–1.58)	1.07 (0.68–1.68)	*0.766*	15.7	15.2	*0.521*	1.24 (0.76–2.01)	1.22 (0.70–2.13)	*0.485*
β-Carotene	88.7	78.0	***0.002***	1.17 (0.80–1.71)	2.13 (1.33–3.40)	***0.002***	91.7	78.2	***0.002***	1.16 (0.73–1.86)	2.11 (1.20–3.70)	***0.008***
β-*trans*-Carotene	79.1	69.0	***0.001***	1.15 (0.78–1.68)	2.13 (1.32–3.43)	***0.002***	81.5	69.2	***0.002***	1.10 (0.68–1.77)	2.11 (1.19–3.74)	***0.009***
β-*cis*-Carotene	8.54	7.83	***0.041***	1.17 (0.81–1.69)	1.65 (1.06–2.59)	***0.030***	8.76	7.71	***0.014***	1.40 (0.89–2.19)	1.82 (1.05–3.17)	***0.032***
Cryptoxanthin	93.8	95.4	*0.536*	0.93 (0.63–1.37)	0.86 (0.53–1.38)	*0.526*	96.5	99.3	*0.366*	1.18 (0.72–1.93)	1.06 (0.59–1.88)	*0.928*
α-*trans*-Cryptoxanthin	24.8	24.7	*0.824*	0.95 (0.65–1.40)	0.90 (0.55–1.47)	*0.679*	25.1	25.4	*0.739*	1.06 (0.65–1.71)	1.25 (0.68–2.30)	*0.458*
β-Cryptoxanthin	67.6	68.8	*0.520*	1.11 (0.76–1.62)	0.94 (0.59–1.50)	*0.768*	70.2	72.7	*0.316*	1.39 (0.87–2.23)	1.04 (0.59–1.84)	*0.947*
β-*trans*-Cryptoxanthin	50.5	50.8	*0.869*	1.12 (0.77–1.63)	1.11 (0.71–1.73)	*0.656*	52.5	53.8	*0.511*	1.45 (0.90–2.33)	1.49 (0.86–2.59)	*0.170*
β-*cis*-Cryptoxanthin	16.7	17.4	*0.139*	0.86 (0.57–1.28)	0.67 (0.39–1.12)	*0.127*	16.6	17.5	*0.118*	0.89 (0.54–1.45)	0.73 (0.38–1.41)	0.345
Lycopene	306	319	*0.138*	0.67 (0.45–0.99)	0.51 (0.29–0.87)	***0.014***	302	325	***0.036***	0.52 (0.31–0.86)	0.36 (0.18–0.72)	***0.003***
trans-Lycopene[b]	170	181	*0.325*	0.82 (0.44–1.53)	0.42 (0.18–0.96)	***0.040***	155	172	*0.165*	0.69 (0.32–1.50)	0.25 (0.09–0.74)	***0.013***
cis-Lycopene[b]	190	206	*0.059*	0.43 (0.23–0.84)	0.23 (0.09–0.56)	***0.001***	179	200	***0.032***	0.42 (0.19–0.93)	0.15 (0.05–0.48)	***0.001***
Dihydrolycopene	54.4	57.8	***0.016***	0.71 (0.46–1.09)	0.37 (0.18–0.74)	***0.006***	53.3	58.3	***0.004***	0.56 (0.33–0.96)	0.29 (0.12–0.67)	***0.004***
trans-Lutein	72.5	67.1	***0.017***	1.31 (0.90–1.92)	1.86 (1.20–2.88)	***0.006***	73.6	65.9	***0.006***	1.45 (0.91–2.31)	2.11 (1.26–3.54)	***0.005***
Anhydrolutein	62.7	63.1	*0.800*	1.35 (0.92–1.99)	1.21 (0.69–2.11)	*0.434*	63.9	64.4	*0.777*	1.22 (0.76–1.95)	0.99 (0.51–1.93)	*0.965*
trans-Anhydrolutein	39.4	38.8	*0.599*	1.22 (0.82–1.81)	1.33 (0.80–2.23)	*0.273*	40.4	39.7	*0.590*	1.07 (0.66–1.73)	1.24 (0.66–2.34)	*0.498*
cis-Anhydrolutein	22.1	23.1	*0.055*	0.96 (0.65–1.42)	0.57 (0.33–0.96)	***0.037***	22.3	23.6	*0.057*	1.09 (0.67–1.78)	0.60 (0.31–1.14)	*0.098*
trans-Zeaxanthin	45.2	44.5	*0.577*	1.15 (0.79–1.68)	1.17 (0.76–1.79)	*0.491*	44.4	43.1	*0.331*	1.22 (0.76–1.94)	1.21 (0.72–2.03)	*0.501*

Note: Values in bold are statistically significant; values in italics represent the isotonic form of each carotenoid.

[a]Adjusted for education, family income, history of diabetes, history of prostate cancer, NPB score, comorbidity index, BMI, smoking, alcohol consumption, physical activity, Healthy Eating Index, blood retinol and carotenoids, and time from meal to blood collection.

[b]41% of subjects had available serum *cis*- and *trans*-lycopene.

Table 3. Geometric means and associations of plasma retinol and carotenoid subtypes and isomers with the risk of prostate cancer according to race.

	African-American						European-American						
	Geometric means (ng/mL)			Adjusted OR (95% CI)[a]			Geometric means (ng/mL)			Adjusted OR (95% CI)[a]			
	Cases (293)	Con (541)	P_{adj}[a]	Tertile 2	Tertile 3	P_{trend}	Cases (50)	Con (99)	P_{adj}[a]	Tertile 2	Tertile 3	P_{trend}	$P_{interaction}$
Retinol	682	684	*0.874*	0.98 (0.66–1.48)	1.29 (0.76–2.18)	*0.403*	581	626	*0.174*	0.47 (0.16–1.44)	0.86 (0.25–2.97)	*0.864*	*0.420*
Total carotenoid	937	934	*0.827*	1.03 (0.70–1.52)	1.06 (0.71–1.57)	*0.774*	939	963	*0.642*	1.31 (0.49–3.49)	0.51 (0.15–1.77)	*0.449*	*0.259*
Carotene	199	174	***0.002***	1.00 (0.65–1.54)	2.19 (1.31–3.65)	***0.002***	187	186	*0.965*	3.76 (1.18–12.0)	1.63 (0.42–6.33)	*0.371*	*0.398*
α-*trans*-Carotene	15.9	15.5	*0.500*	0.90 (0.59–1.35)	1.02 (0.63–1.67)	*0.954*	17.1	17.8	*0.777*	4.03 (1.27–12.8)	2.32 (0.55–9.86)	*0.192*	*0.663*
β-Carotene	90.1	78.2	***0.002***	1.01 (0.66–1.54)	2.04 (1.22–3.39)	***0.006***	82.1	81.8	*0.978*	2.56 (0.84–7.78)	2.11 (0.53–8.50)	*0.233*	*0.662*
β-*trans*-Carotene	80.2	69.1	***0.001***	1.00 (0.66–1.53)	2.10 (1.25–3.52)	***0.005***	74.1	74.2	*0.988*	2.19 (0.75–3.39)	1.77 (0.45–3.99)	*0.334*	*0.543*
β-*cis*-Carotene	9.02	8.30	*0.060*	0.97 (0.65–1.45)	1.44 (0.89–2.31)	*0.148*	6.39	5.58	*0.369*	3.78 (1.22–11.8)	4.06 (0.79–20.7)	*0.046*	*0.542*
Cryptoxanthin	95.4	98.6	*0.242*	0.98 (0.63–1.53)	0.73 (0.43–1.23)	*0.196*	78.5	77.7	*0.897*	0.62 (0.22–1.79)	2.17 (0.53–8.82)	*0.379*	*0.675*
α–*trans*-Cryptoxanthin	25.0	25.2	*0.783*	0.89 (0.58–1.37)	0.82 (0.48–1.40)	*0.458*	23.4	23.3	*0.944*	1.47 (0.52–4.20)	1.96 (0.42–9.12)	*0.350*	*0.902*
β-Cryptoxanthin	68.6	71.4	*0.213*	1.04 (0.68–1.59)	0.77 (0.46–1.29)	*0.283*	54.2	53.8	*0.950*	1.21 (0.45–3.25)	2.17 (0.53–8.89)	*0.306*	*0.536*
β-*trans*-Cryptoxanthin	51.8	53.4	*0.383*	1.11 (0.72–1.71)	0.96 (0.59–1.58)	*0.841*	38.2	37.2	*0.790*	0.95 (0.36–2.52)	2.41 (0.63–9.12)	*0.272*	*0.748*
β-*cis*-Cryptoxanthin	16.9	17.8	*0.101*	0.73 (0.46–1.14)	0.51 (0.28–0.91)	***0.023***	14.0	14.8	*0.562*	2.29 (0.79–6.62)	2.43 (0.58–10.2)	*0.173*	*0.319*
Lycopene	306	319	*0.214*	0.62 (0.40–0.95)	0.44 (0.25–0.79)	***0.006***	305	333	*0.312*	1.86 (0.50–7.00)	3.08 (0.42–22.8)	*0.269*	*0.803*
trans-Lycopene[b]	155	171	*0.100*	0.71 (0.37–1.38)	0.33 (0.13–0.83)	***0.021***	NA	NA	*NA*	10.5 (0.06–999)	0.22 (0.01–11.4)	*0.981*	*NA*
cis-Lycopene[b]	184	199	*0.108*	0.39 (0.20–0.79)	0.27 (0.10–0.68)	***0.005***	NA	NA	*NA*	0.97 (0.03–32.4)	0.02 (0.00–8.98)	*0.535*	*NA*
Dihydrolycopene	54.2	57.4	***0.042***	0.66 (0.41–1.07)	0.27 (0.12–0.59)	***0.001***	58.8	63.9	*0.262*	1.44 (0.40–5.15)	2.72 (0.40–18.3)	*0.314*	*0.441*
trans-Lutein	78.0	71.7	***0.019***	1.30 (0.85–1.97)	1.89 (1.18–3.02)	***0.007***	58.7	55.1	*0.548*	2.08 (0.72–3.04)	1.14 (0.20–6.40)	*0.489*	*0.539*
Anhydrolutein	62.4	62.6	*0.901*	1.54 (1.00–2.36)	1.45 (0.79–2.65)	*0.195*	63.1	64.2	*0.795*	0.53 (0.18–1.63)	0.32 (0.06–1.84)	*0.189*	*0.077*
trans-Anhydrolutein	39.8	39.2	*0.623*	1.35 (0.87–2.08)	1.50 (0.86–2.63)	*0.153*	37.1	36.6	*0.868*	0.60 (0.20–1.77)	0.49 (0.10–2.35)	*0.354*	*0.127*
cis-Anhydrolutein	21.5	22.4	*0.103*	0.96 (0.62–1.48)	0.59 (0.33–1.07)	*0.076*	25.4	26.7	*0.434*	0.83 (0.28–2.51)	0.39 (0.09–1.69)	*0.231*	*0.342*
trans-Zeaxanthin	47.6	46.8	*0.581*	1.77 (0.77–1.79)	1.15 (0.73–1.83)	*0.587*	36.6	35.8	*0.752*	1.31 (0.49–3.49)	0.91 (0.19–4.28)	*0.843*	*0.523*

Note: Values in bold are statistically significant; values in italics represent the isotonic form of each carotenoid.

Abbreviation: Con, controls.

[a]Adjusted for education, family income, history of diabetes, history of prostate cancer, NPB score, comorbidity index, BMI, smoking, alcohol consumption, physical activity, Healthy Eating Index.

[b]41% of subjects had available serum *cis*-and *trans*-lycopene.

Table 4. Geometric means and associations of serum retinol and carotenoid subtypes and isomers with the risk of prostate cancer by smoking status.

	Never or former smoker (cases = 193, controls = 318)						Current smoker (cases = 150, controls = 322)						
	Geometric means (ng/mL)			Adjusted OR (95% CI)[a]			Geometric means (ng/mL)			Adjusted OR (95% CI)[a]			
	Cases	Con	P_{adj}[a]	Tertile 2	Tertile 3	P_{trend}	Cases	Con	P_{adj}[a]	Tertile 2	Tertile 3	P_{trend}	$P_{interaction}$
Retinol	667	677	*0.588*	1.13 (0.68–1.87)	1.54 (0.85–2.78)	*0.164*	735	701	*0.184*	0.75 (0.44–1.26)	0.86 0.48–1.56)	*0.527*	*0.080*
Total carotenoid	846	843	*0.869*	1.66 (0.99–2.80)	1.32 (0.79–2.20)	*0.437*	954	947	*0.854*	0.67 (0.41–1.09)	0.88 (0.52–1.48)	*0.449*	*0.241*
Carotene	210	182	***0.023***	1.33 (0.77–2.30)	2.13 (1.20–3.79)	***0.009***	226	204	*0.185*	1.19 (0.73–1.95)	2.36 (1.27–4.41)	***0.010***	*0.909*
α-*trans*-Carotene	16.0	15.8	*0.788*	1.53 (0.91–2.57)	1.06 (0.59–1.90)	*0.926*	17.7	18.4	*0.694*	0.77 (0.46–1.27)	1.17 (0.66–2.06)	*0.729*	*0.858*
β-Carotene	96.5	82.4	***0.018***	1.21 (0.70–2.11)	2.02 (1.12–3.62)	***0.015***	101	89.7	*0.143*	1.19 (0.72–1.95)	2.33 (1.25–4.31)	***0.010***	*0.853*
β-*trans*-Carotene	86.2	73.4	***0.019***	1.23 (0.72–2.12)	2.01 (1.12–3.60)	***0.017***	91.5	80.4	*0.124*	1.13 (0.66–1.87)	2.44 (1.30–4.56)	***0.008***	*0.745*
β-*cis*-Carotene	8.94	7.81	***0.043***	1.32 (0.78–2.23)	2.00 (1.11–3.60)	***0.020***	9.28	8.90	*0.615*	1.09 (0.67–1.76)	1.34 (0.74–2.42)	*0.360*	*0.270*
Cryptoxanthin	85.2	84.9	*0.647*	1.14 (0.67–1.92)	0.87 (0.49–1.57)	*0.605*	91.9	92.9	*0.842*	0.76 (0.45–1.28)	0.87 (0.47–1.60)	*0.610*	*0.915*
α-*trans*-Cryptoxanthin	23.4	23.8	*0.739*	1.11 (0.65–1.89)	1.01 (0.55–1.85)	*0.983*	23.8	24.1	*0.770*	0.83 (0.49–1.39)	0.82 (0.44–1.54)	*0.527*	*0.529*
β-Cryptoxanthin	60.0	59.1	*0.719*	1.43 (0.85–2.41)	1.05 (0.59–1.87)	*0.953*	66.7	67.3	*0.885*	0.89 (0.53–1.48)	0.84 (0.45–1.56)	*0.582*	*0.564*
β-*trans*-Cryptoxanthin	45.2	44.3	*0.716*	1.44 (0.85–2.45)	1.32 (0.75–2.31)	*0.383*	50.2	49.7	*0.900*	0.92 (0.55–1.51)	0.93 (0.51–1.68)	*0.782*	*0.323*
β-*cis*-Cryptoxanthin	16.9	16.6	*0.530*	1.09 (0.64–1.85)	0.71 (0.38–1.33)	*0.279*	15.5	16.2	*0.485*	0.68 (0.40–1.16)	0.65 (0.34–1.24)	*0.169*	*0.783*
Lycopene	265	256	*0.227*	0.85 (0.51–1.42)	0.59 (0.32–1.10)	*0.096*	312	327	*0.432*	0.52 (0.30–0.90)	0.40 (0.20–0.80)	***0.008***	*0.270*
trans-Lycopene[b]	123	104	*0.867*	0.90 (0.39–2.07)	0.49 (0.19–1.30)	*0.131*	153	150	*0.920*	0.81 (0.35–1.87)	0.37 (0.13–1.10)	*0.092*	*0.717*
cis-Lycopene[b]	141	138	*0.482*	0.48 (0.20–1.17)	0.32 (0.12–0.85)	***0.022***	226	229	*0.852*	0.41 (0.17–0.97)	0.14 (0.04–0.46)	***0.001***	*0.133*
Dihydrolycopene	51.7	53.2	*0.573*	0.71 (0.40–1.24)	0.34 (0.15–0.73)	***0.007***	54.1	55.6	*0.626*	0.71 (0.41–1.25)	0.41 (0.18–0.90)	***0.032***	*0.494*
trans-Lutein	71.2	68.9	*0.293*	1.48 (0.89–2.46)	2.33 (1.32–4.12)	***0.003***	63.5	60.6	*0.447*	1.21 (0.71–2.05)	1.54 (0.88–2.68)	*0.131*	*0.212*
Anhydrolutein	55.4	57.6	*0.170*	2.08 (1.23–3.50)	1.61 (0.83–3.12)	*0.146*	68.4	67.4	*0.755*	0.91 (0.54–1.54)	0.97 (0.49–1.93)	*0.991*	*0.151*
trans-Anhydrolutein	35.5	36.4	*0.610*	1.69 (0.99–2.87)	1.86 (1.01–3.43)	*0.052*	43.8	41.9	*0.403*	0.93 (0.55–1.56)	1.00 (0.52–1.93)	*0.975*	*0.062*
cis-Anhydrolutein	19.0	20.6	***0.028***	1.57 (0.94–2.63)	0.70 (0.37–1.32)	*0.203*	23.2	23.9	*0.531*	0.58 (0.34–0.99)	0.52 (0.27–1.02)	***0.035***	*0.364*
trans-Zeaxanthin	42.3	43.3	*0.576*	1.13 (0.68–1.88)	1.25 (0.73–2.14)	*0.419*	44.1	43.2	*0.696*	1.19 (0.72–1.99)	1.06 (0.60–1.88)	*0.828*	*0.597*

Note: Values in bold are statistically significant; values in italics represent the isotonic form of each carotenoid.

Abbreviation: Con, controls.

[a]Adjusted for education, family income, history of diabetes, history of prostate cancer, NPB score, comorbidity index, BMI, smoking, alcohol consumption, physical activity, Healthy Eating Index.

[b]41% of subjects had available serum *cis*-and *trans*-lycopene.

Table 5. Geometric means and associations of serum retinol and carotenoid subtypes and isomers with the risk of prostate cancer by multivitamin supplement status.

	Without multivitamin supplement (cases = 229, controls = 433)						With multivitamin supplement (cases = 114, controls = 207)						
	Geometric means (ng/mL)			Adjusted OR (95% CI)[a]			Geometric means (ng/mL)			Adjusted OR (95% CI)[a]			
	Cases	Con	P_{adj}[a]	Tertile 2	Tertile 3	P_{trend}	Cases	Con	P_{adj}[a]	Tertile 2	Tertile 3	P_{trend}	$P_{interaction}$
Retinol	689	689	*0.980*	0.94 (0.61–1.45)	1.21 (0.71–2.07)	*0.582*	767	748	*0.635*	0.85 (0.45–1.63)	1.09 (0.54–2.19)	*0.837*	*0.845*
Total carotenoid	964	982	*0.425*	1.09 (0.72–1.63)	1.02 (0.66–1.59)	*0.917*	1146	1143	*0.956*	0.89 (0.46–1.73)	1.03 (0.57–1.86)	*0.874*	*0.946*
Carotene	204	175	***0.004***	1.28 (0.81–2.01)	2.07 (1.21–3.54)	***0.009***	266	255	*0.723*	1.09 (0.56–2.13)	2.39 (1.20–4.79)	***0.009***	*0.591*
α-*trans*-Carotene	16.8	16.6	*0.815*	1.08 (0.70–1.66)	1.13 (0.66–1.92)	*0.644*	29.6	29.5	*0.965*	1.12 (0.58–2.17)	0.99 (0.51–1.93)	*0.951*	*0.705*
β-Carotene	92.5	78.0	***0.003***	1.25 (0.79–1.96)	1.92 (1.13–3.28)	***0.019***	113	108	*0.752*	1.06 (0.55–2.03)	2.60 (1.29–5.25)	***0.005***	*0.342*
β-*trans*-Carotene	82.5	68.9	***0.002***	1.20 (0.76–1.89)	1.94 (1.13–3.32)	***0.018***	104	99.8	*0.722*	1.08 (0.56–2.08)	2.60 (1.28–5.29)	***0.005***	*0.361*
β-*cis*-Carotene	9.60	8.74	*0.101*	1.10 (0.71–1.70)	1.69 (1.01–2.84)	*0.054*	7.18	7.11	*0.934*	1.34 (0.71–2.52)	1.64 (0.85–3.16)	*0.158*	*0.919*
Cryptoxanthin	96.0	96.5	*0.888*	0.73 (0.46–1.15)	0.97 (0.57–1.66)	*0.836*	106	106	*0.970*	1.54 (0.79–2.99)	0.76 (0.38–1.53)	*0.332*	*0.461*
α-*trans*-Cryptoxanthin	25.6	26.1	*0.562*	0.93 (0.60–1.44)	0.88 (0.51–1.52)	*0.639*	26.6	26.8	*0.949*	1.00 (0.52–1.93)	0.95 (0.47–1.92)	*0.874*	*0.843*
β-Cryptoxanthin	68.9	69.1	*0.958*	0.93 (0.60–1.43)	1.03 (0.61–1.76)	*0.896*	78.9	78.4	*0.954*	1.62 (0.85–3.09)	0.82 (0.41–1.65)	*0.434*	*0.421*
β-*trans*-Cryptoxanthin	52.6	51.9	*0.770*	0.98 (0.63–1.51)	1.19 (0.71–1.51)	*0.525*	57.5	56.9	*0.915*	1.51 (0.79–2.90)	1.02 (0.52–1.99)	*0.936*	*0.606*
β-*cis*-Cryptoxanthin	16.4	17.0	*0.298*	0.78 (0.49–1.22)	0.71 (0.40–1.26)	*0.215*	20.6	20.4	*0.954*	1.04 (0.54–1.99)	0.62 (0.29–1.31)	*0.189*	*0.739*
Lycopene	312	345	***0.011***	0.59 (0.37–0.94)	0.43 (0.24–0.79)	***0.005***	388	421	*0.330*	0.89 (0.47–1.70)	0.69 (0.33–1.46)	*0.326*	*0.210*
trans-Lycopene[b]	150	173	*0.102*	0.79 (0.38–1.64)	0.38 (0.15–1.00)	*0.050*	NA	NA	NA	0.92 (0.34–2.49)	0.54 (0.18–1.62)	*0.284*	*0.545*
cis-Lycopene[b]	177	212	***0.004***	0.32 (0.15–0.71)	0.19 (0.07–0.51)	***0.001***	NA	NA	NA	0.79 (0.29–0.21)	0.40 (0.13–1.31)	*0.140*	*0.139*
Dihydrolycopene	58.4	62.5	***0.050***	0.65 (0.39–1.07)	0.30 (0.14–0.64)	***0.002***	53.7	60.8	*0.081*	0.88 (0.45–1.71)	0.54 (0.23–1.28)	*0.171*	*0.164*
trans-Lutein	72.6	68.9	*0.224*	1.15 (0.73–1.80)	1.97 (1.16–3.33)	***0.014***	100	84.5	*0.159*	1.80 (0.95–3.43)	1.86 (0.97–3.57)	*0.075*	*0.835*
Anhydrolutein	63.5	67.5	***0.047***	1.22 (0.77–1.91)	1.10 (0.60–2.02)	*0.664*	73.9	67.6	*0.298*	1.69 (0.90–3.17)	1.47 (0.69–3.12)	*0.294*	*0.477*
trans-Anhydrolutein	39.6	41.5	*0.213*	1.16 (0.74–1.82)	1.19 (0.67–2.11)	*0.540*	42.8	37.6	*0.199*	1.39 (0.74–2.61)	1.66 (0.82–3.38)	*0.164*	*0.382*
cis-Anhydrolutein	22.5	24.7	***0.002***	0.87 (0.55–1.37)	0.50 (0.28–0.90)	***0.023***	28.9	28.5	*0.839*	1.21 (0.64–2.29)	0.72 (0.35–1.51)	*0.353*	*0.384*
trans-Zeaxanthin	47.3	47.4	*0.951*	1.19 (0.76–1.87)	1.25 (0.75–2.05)	*0.339*	40.5	38.8	*0.619*	1.08 (0.58–2.03)	1.03 (0.54–1.99)	*0.933*	*0.621*

Note: Values in bold are statistically significant; values in italics represent the isotonic form of each carotenoid.

Abbreviation: Con, controls.

[a]Adjusted for education, family income, history of diabetes, history of prostate cancer, NPB score, comorbidity index, BMI, smoking, alcohol consumption, physical activity, Healthy Eating Index.

[b]41% of subjects had available serum *cis*-and *trans*-lycopene.

50% in blood (34) and 5% to 10% in foods (35), although the biological significance of this isomeric composition is not known. The *cis*-lycopene isomer differs slightly in molecular shape than the all-*trans* form, and, therefore, may have a different pharmacologic effect and metabolism (36). In the PHS, the inverse association of lycopene was particularly apparent in aggressive prostate cancer and in men who did not consume β-carotene supplements (37). In our study, total lycopene was associated with the risk of prostate cancer, in both *trans* and *cis* isomers, particularly among African-Americans. *Cis*-lycopene plasma levels were associated with prostate cancer risk in never-users of multivitamin supplements and in both never/former and current smokers. Vogt and colleagues suggested that serum lycopene concentrations were significantly lower in African-Americans than in European-Americans, suggesting that differences in lycopene exposure may contribute to incident prostate cancer racial disparities (28). Our study, including 83% African-American and 17% European-American participants, also showed lower lycopene levels among African-American controls than European-American controls.

The plasma lycopene metabolite 5,6-dihydroxy-5,6-dihydrolycopene, resulting from *in vivo* oxidation of lycopene to an intermediate lycopene epoxide product, undergoes metabolic reduction to form the above metabolite. It has been shown that tomatoes and tomato-based food products are major dietary sources of this compound (38). The correlation between 5,6-dihydroxy-5,6-dihydrolycopene and lycopene was as high as 0.70 in this study, suggesting that the variation in 5,6-dihydroxy-5,6-dihydrolycopene level was largely due to lycopene levels. Plasma dihydrolycopene showed an inverse association with prostate cancer risk in our study, predominantly for stage II–IV prostate cancer. This association was mainly seen in African-Americans, never or former smokers, and subjects without supplementation.

In vivo oxidation of lutein and zeaxanthin is a key reaction in the metabolism of these nonprovitamin A active dihydroxycarotenoids (8). When free-radical mechanisms are involved in the initiation and promotion of carcinogenesis, carotenoids such as lutein and zeaxanthin may participate in quenching peroxides and protecting cells from oxidative damage (39). Our study showed a significant positive association with plasma *trans*-lutein on prostate cancer risk, predominantly among African-Americans, in subjects without multivitamin supplementation, and in advanced prostate cancer, inconsistent with the reduction in risk claimed for these carotenoid compounds. On the other hand, anhydrolutein, or 3′-hydroxy-3,4-dehydro-ss-carotene, is lutein's dehydrated metabolite. The presence of anhydrolutein I and II in human plasma is potentially due to acid catalyzed dehydration of dietary lutein as it passes through the stomach (40). In our study, *cis*-type anhydrolutein concentrations differed between prostate cancer cases and controls and were inversely associated with prostate cancer risk, particularly among those with no vitamin supplementation. No association was observed between plasma zeaxanthin and prostate cancer risk in the present study, consistent with findings from most previous cohort studies (24, 28, 29, 41). A pooled analysis (4) suggested heterogeneity in lutein- and zeaxanthin–prostate cancer associations; an inverse association between lutein and prostate cancer risk was only observed for prostate cancer diagnosed before the age of 60 years, and zeaxanthin was positively associated with prostate cancer risk only among men with a BMI of less than 25 kg/m^2.

Our study has many strengths. First, this prospective study included prediagnostic plasma samples, a large sample size of African-Americans, and detailed baseline data, which allowed for simultaneous adjustment for multiple confounders and evaluation of effect modifiers. Second, circulating plasma vitamin concentrations were measured using standardized procedures at one laboratory, with extensive experience in measuring lipid-phase micronutrients, especially carotenoids, in only a few batches and in a relatively short timeframe (thus, attenuating any potential technical measurement errors). Third, our report is the first study to consider various subtypes and isomers for each carotenoid simultaneously among the same population.

Our study also has some potential limitations. First, single measurements of plasma retinol and carotenoids may not be sufficient in detecting meaningful differences in long-term exposures between prostate cancer cases and controls. However, several reports (19, 42, 43) have suggested that single measurements of these biomarkers provide moderately reliable information on usual concentrations over a few years, although the true associations with prostate cancer risk are likely to be underestimated. Second, we did not have adequate power to investigate the associations for late-stage or aggressive-type of prostate cancers. Because of regular PSA screening, most prostate cancer cases are diagnosed at an early stage, so assembling large numbers of advanced cases has become increasingly difficult (our study had 11% stage III–IV cases), which hinged analyses for late-stage or aggressive prostate cancer in our study. Third, despite adjusting for HEI, we could not completely rule out confounding from healthier diets, as this is associated with higher screening rates. Fourth, the relatively short follow-up time raises a concern of reverse causation. However, excluding cases diagnosed within 1 or 2 years after blood sample collection showed a similar association pattern. Fifth, our study had low statistical power for subgroup analyses, particularly for aggressive prostate cancer and white participants. Sixth, 45.8% of prostate cancer patients were included in the current study, raising a potential concern on selection bias. We found that patients included in our study and those excluded had a similar Gleason score but differed significantly in several sociodemographic characteristics. Patients included in this study were less educated, more likely to have a lower family income, to be in lower NPB category and to have a lower HEI score, and less likely to take multivitamin supplement than patients excluded (Supplementary Table S5). However, all these factors were adjusted in the multivariate analyses. Finally, the generalizability of these results may be restricted, as study participants were predominantly of low socioeconomic status. Considering that risk factors for prostate cancer may differ by race, future studies investigating the effects of carotenoid subtypes and isomeric arrangements should incorporate men of different ethnicities and sociodemographic backgrounds into their study design.

In conclusion, in a low-income study population, we found positive associations of *trans* isomers, particularly β-carotene and lutein, and inverse associations of lycopene, dihydrolycopene, and *cis*-anhydrolutein with prostate cancer risk, even after adjusting for multiple comparisons. The associations were restricted by subgroups of individuals defined by race, smoking status, and multivitamin supplement use. Nevertheless, it should be noted that case–control differences in mean measured concentrations for all carotenoids were relatively small. The clinical significance for these findings remains to be determined.

Authors' Disclosures

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Disclaimer

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Authors' Contributions

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