Inverse Associations between Plasma Lycopene and Other Carotenoids and Prostate Cancer[1]

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Abstract

Although dietary intake of tomatoes and tomato products containing lycopene has been reported to reduce the risk of prostate cancer, few studies have been done on the relationship between plasma lycopene and other carotenoids and prostate cancer. This case-control study was conducted to investigate the effects of plasma lycopene, other carotenoids, and retinol, as well as α- and γ-tocopherols on the risk of prostate cancer. The study included 65 patients with prostate cancer and 132 cancer-free controls; all of them were interviewed using a standard epidemiological questionnaire at the Memorial Sloan-Kettering Cancer Center from 1993 to 1997. Plasma levels of carotenoids, retinol, and tocopherols were measured by high performance liquid chromatography. An unconditional logistic regression model was used in bivariate and multivariate analyses using Statistical Analysis System (SAS). After adjusting for age, race, years of education, daily caloric intake, pack-years of smoking, alcohol consumption, and family history of prostate cancer, significantly inverse associations with prostate cancer were observed with plasma concentrations of the following carotenoids: lycopene [odds ratio (OR), 0.17; 95% confidence interval (CI), 0.04–0.78; *P* for trend, 0.0052] and zeaxanthin (OR, 0.22; 95% CI, 0.06–0.83; *P* for trend, 0.0028) when comparing highest with lowest quartiles. Borderline associations were found for lutein (OR, 0.30; 95% CI, 0.09–1.03; *P* for trend, 0.0064) and β-cryptoxanthin (OR, 0.31; 95% CI, 0.08–1.24; *P* for trend, 0.0666). No obvious associations were found for α- and β-carotenes, retinol, and α- and γ-tocopherols. Our study confirmed the inverse associations between lycopene, other carotenoids such as zeaxanthin, lutein, and β-cryptoxanthin, and prostate cancer. This study provides justification for further research on the associations between lycopene and other antioxidants and the risk of prostate cancer.

Introduction

Prostate cancer is the most common cancer and second leading cause of cancer mortality in men in the United States (1). Studies indicate that prostate cancer incidence and mortality rates may be associated with dietary factors (2–5). A diet high in total fat probably increases the risk for occurrence of prostate cancer (5). It was hypothesized that dietary vitamins and carotenoids may reduce proliferation and provide protection of DNA and membrane lipids from oxidative damage (6), which may lead to reduced risk of prostate cancer.

Epidemiological studies have conflicted on the associations between intake of vitamins and carotenoids and risk of prostate cancer. An estimated intake of vitamin A and β-carotene has been associated with increased risk (7–10) or with the decreased risk (11) of prostate cancer. Other studies (12–13) revealed no associations. A prospective cohort study showed that dietary intake of tomato products may reduce prostate cancer risk by 35% (11).

Most epidemiological studies have used the dietary history to identify possible associations between antioxidants and prostate cancer, yet very few studies have analyzed the association between plasma antioxidant levels and prostate cancer risk. It has been reported that serum retinol levels were significantly lower in prostate cancer cases than in controls (14). An inverse association of serum vitamin A and prostate cancer was found in a cohort study (15). Plasma lycopene was significantly lower in cases than in matched controls in two case-control studies (16, 17). Other studies (18, 19) found no association with vitamin A, vitamin E, and carotenoids. Few studies (18, 20) have evaluated associations of plasma levels of lutein, zeaxanthin, and β-cryptoxanthin with the risk of prostate cancer.

In this study, we measured plasma concentrations of six major types of carotenoids including lycopene, α-carotene, β-carotene, lutein, zeaxanthin, and β-cryptoxanthin plus retinol and two tocopherols such as α- and γ-tocopherol in 65 prostate cancer patients and 132 cancer-free controls. We evaluated the possible role of carotenoids, retinol, and vitamin E on the risk of prostate cancer when controlling for potential confounding factors, including age, gender, education, pack-years of smoking, alcohol consumption, family history of prostate cancer, and dietary caloric intake.

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Table 1 Demographic characteristics of prostate cancer cases and controls[a]

	No. of cases	%	No. of controls	%	*P*
Total	65		130		
Age					
<60	31	47.7	117	90.0	
60–69	29	46.6	11	8.5	
≥70	5	7.7	2	1.5	0.001
Mean (SD)	59.98 (±6.19)		41.9 (±13.64)		0.001
Race					
White	60	92.3	126	96.9	
Non-white	5	7.7	4	3.1	0.148
Education (yr)					
≥12	23	35.9	22	17.2	
13–16	19	29.7	64	50.0	
>16	22	34.4	42	32.8	0.005
Mean (SD)	14.94 (±4.20)		15.72 (±2.37)		0.170
Smoking					
No	27	41.5	80	60.6	
Yes	38	58.5	52	39.4	0.012
Pack-yr of smoking					
No	27	41.5	80	60.6	
≤30	18	27.7	35	29.6	
>30	20	30.8	13	9.8	0.001
Alcohol consumption					
No	3	4.6	31	23.5	
Yes	62	95.4	101	76.5	0.001
Family history of prostate cancer					
No	53	81.5	120	90.9	
Yes	12	18.5	12	9.1	0.059

[a] *P*s were obtained by Student's *t*-test for continuous variable and Fisher's exact test or χ^2 test for categorical variables.

Table 2 Comparison of mean levels (μmol/l) of plasma carotinoids, retinol, and tocopherols in prostate cancer cases with controls

	Cases		Controls		*P* (*t*-test)
	Mean	SD	Mean	SD	
Lycopene	0.223	0.1226	0.307	0.1925	0.0003
Zeaxanthin	0.048	0.0351	0.065	0.0455	0.0040
α-Carotene	0.097	0.1816	0.086	0.0937	0.6425
β-Carotene	0.361	0.4786	0.267	0.2436	0.1422
Lutein	0.131	0.1067	0.158	0.1084	0.0915
β-Cryptoxanthin	0.081	0.0565	0.107	0.1047	0.0233
Retinol	1.313	0.4778	1.521	0.7804	0.0221
α-Tocopherol	20.02	8.4173	20.17	12.032	0.9181
γ-Tocopherol	2.502	1.6558	3.343	2.4783	0.0053

Materials and Methods

Study Population. A hospital-based case-control study was conducted at MSKCC.[3] The study was approved by the Institutional Research Board on Human Subjects of MSKCC, and study participants were asked to sign an informed consent form stating they agreed to participate in the study.

Cases. Eligible cases were patients seen at MSKCC from August, 1993 to July, 1995 with pathologically confirmed diagnoses of prostate adenocarcinoma. Cases were either newly diagnosed or undergoing prostectomy. We approached 165 consecutive patients with prostate cancer, and 147 consented to be interviewed. Among these, 144 had completed dietary data, and 65 had adequate plasma samples for nutritional analyses. Patients had consented in writing to participate in the study. Informed consent forms were obtained, and 20-ml blood samples were collected upon preadmission from patients to be admitted into the Urology Service at MSKCC. A research nurse interviewed patients when admitted in the medical/surgical ward. The process of obtaining informed consent and interview took between 2–4 days. Interviews were conducted before surgeries and subsequent pathological diagnoses. Pathology reports were obtained after surgery or upon discharge. All of the cases with pathologically confirmed diagnoses were included in the study.

Controls. Eligible controls were healthy and cancer-free males, recruited from the blood bank at MSKCC. They were approached and interviewed in the same manner as cases and met the same criteria, except for a cancer history or diagnosis. The nurse interviewer approached blood donors, explained the study, and asked them to read the study description and sign the informed consent form if they agreed to participate. Controls also donated a 20-ml blood sample. A total of 163 potential male participants were approached, and 153 individuals consented to be interviewed for the study. Among these, 150 controls had complete dietary data, and 132 had sufficient plasma samples.

Epidemiological Data Collection. A nurse interviewer approached and interviewed participants using a standardized questionnaire and asked participants to donate blood and tissue samples for biomarker measurements. Using a detailed questionnaire, we collected the following: (*a*) demographic information (name, gender, race, birth date and birthplace, marital status, education, address, telephone number, etc); (*b*) occupational titles held and occupational and environmental exposures with detailed timeline of exposures; (*c*) personal habits summary (cigarette smoking, passive smoking, alcohol consumption, coffee and tea consumption, with detailed time-frame of exposures); (*d*) family history of cancer; (*e*) dietary factors using a NCI HHHQ short food frequency questionnaire that collected usual dietary patterns a year before diagnosis for cases and a year ago for controls; (*f*) physical activity report (2 year ago, 10 year ago, 20 year ago and at age 16); (*g*) history of sexually transmitted diseases; and (*h*) past medical history. An epidemiological database was established and maintained to store patient data. Pathological and laboratory data were collected, recorded, and linked to the epidemiological database. Medical charts and pathology reports were examined to insure a control did not have a prior history of cancer. Tumor and normal tissues were collected and stored in a −70°C freezer. Blood samples (20 ml) were collected from each case and control for laboratory assays, and plasma samples were stored at −70°C.

Nutrient Estimates. A NCI short HHHQ dietary questionnaire, including food items, frequency of consumption, usual portion size, and a restaurant-use section, was used to collect dietary histories (21–23). The estimates of nutrients were calculated using a NCI algorithm (22, 23). Briefly, nutrient indices were obtained from the Second National Health and Nutrition Examination Survey conducted from 1976 to 1980, based on a United States Department of Agriculture food composition data tape (24, 25), as well as industry and other sources. Nutrient composition values for vitamin A are based on Revised Handbook 8 values (26). The food database includes three parts: portion sizes, food in grams, and nutrients/100 g for each food

[3] The abbreviations used are: MSKCC, Memorial Sloan-Kettering Cancer Center; NCI, National Cancer Institute; UCLA, University of California at Los Angeles; OR, odds ratio; CI, confidence interval; HHHQ, Health Habits and History Questionnaire.

Table 3 The cutoff points for quartile distributions of controls for plasma carotenods, retinol, and tocopherols, as well as dietary nutrients

Measures	Cut-off points		
	25%	50%	75%
Plasma levels (μmol/l)			
Lycopene	0.178700	0.275150	0.400980
Zeaxanthin	0.030990	0.052853	0.083088
α-Carotene	0.034547	0.058654	0.106860
β-Carotene	0.124210	0.174780	0.341080
Lutein	0.083994	0.132480	0.194740
β-Cryptoxanthin	0.044962	0.070330	0.132610
Retinol	0.939490	1.312860	2.026660
α-Tocopherol	11.23290	16.89760	27.00730
γ-Tocopherol	1.873190	2.717580	3.991640
Dietary level			
Vitamin A (IU)	6153.50	8666.25	13781.58
Pro-A Carotene (μg)	2914.59	4108.44	7028.67
α-Carotene (μg)	385.765	699.293	1142.31
β-Carotene (μg)	2381.23	3495.32	5851.36
Lycopene (μg)	1458.21	2370.58	3450.01
Lutein (μg)	1009.78	1666.75	2916.75
Cryptoxanthin (μg)	23.0516	71.1566	120.847
Retinol (μg)	255.976	384.817	646.468
Vitamin B1 (mg)	0.78511	1.04804	1.40824
Vitamin B2 (mg)	0.84394	1.23072	1.66504
Vitamin B6 (mg)	0.94857	1.21174	1.66276
Vitamin C (mg)	85.3116	127.444	187.508
Vitamin E (a-TE)[a]	5.15199	6.79295	9.49193
Folate (μg)	170.557	251.965	354.408
Niacin (mg)	10.3535	14.3908	18.9274
Fiber (g)	7.89965	10.2227	13.7164

[a] a-TE, α-Tocopherol Equivalents.

item. Each nutrient was calculated by the following equation: (reported food frequency × gram-portion size × the nutrient content/100 g × seasonality factor)/100. Nutrients were then summed over all of the food and beverage items, including restaurant foods, to obtain the average intake/day. During calculation, vegetables and fruits were adjusted for seasonal consumption by multiplying the computed yearly intake of each food item by the seasonal index (proportion of year during which seasonal consumption of food occurred). Restaurant food frequency was compared with frequency of corresponding food items in the main food list, and appropriate adjustment was done before calculation of estimates according to the NCI algorithm (22, 23).

Blood Sample Collection and Storage. After blood samples were collected from both cases and controls, they were transported immediately to the laboratory at 4°C. About 10 ml of blood were collected in EDTA tubes. The blood was centrifuged at 200 × *g* for 30 min at 4°C. The plasma was then divided into several 1-ml aliquots and stored in a −70°C freezer. Blood samples were packed in styrofoam containers with a large amount of dry ice and shipped from MSKCC to UCLA in 1997. All of the blood specimens were finally stored in a −70°C freezer at the Molecular Epidemiology Laboratory of the UCLA Jonsson Comprehensive Cancer Center.

Laboratory Analysis. Individual tocopherols, retinol, and carotenoids were measured by high-performance liquid chromatography at the Biomarker Laboratory in the UCLA Center for Human Nutrition, Department of Medicine. Case and control samples were prepared simultaneously. Quality control procedures included analyses of a pooled plasma sample daily and National Institute of Standards and Technology Reference Material periodically. A modification of the procedures described by Epler *et al.* (27) was used in our study. Briefly, after thawing, an aliquot of 250 μl of plasma was deproteinated with an equal volume of ethanol solution containing tocopherol acetate as an internal standard and butylated hydroxytoluene as an antioxidant. The mixture was extracted twice with hexane, and the combined hexane was evaporated under a stream of nitrogen. The residue was dissolved in a 1:1 mixture of ethanol: ethyl acetate. Plasma concentrations of retinol, tocopherols, and carotenoids were determined by reverse-phase analytic high-performance liquid chromatography. The system is validated against standard reference sera from the National Institute of Standards and Technology Micronutrients Measurement Quality Assurance Program two to three times annually. Coefficients of variation for intra-assay pool plasma sample were 7.4 for lutein, 8.7 for retinol, 8.8 for α-tocopherol, 10.3 for β-carotene, 10.6 for γ-tocopherol, 11.5 for β-cryptoxanthin, 12.2 for lycopene, and 14.2 for α-carotene.

Statistical Analysis. For categorical data, Fisher's exact test or χ^2 tests were used for association between cases and controls. For continuous variables, Student's *t* test was used to compare means of these variables between cases and controls. The associations of plasma carotenoids, retinol, and tocopherols, as well as dietary intake of carotenoids, vitamins, and antioxidants with prostate cancer, were estimated with ORs and their 95% CIs derived from logistic regression analysis. Continuous variables were first analyzed and then divided into four groups according to the quartile distribution of each variable of the control group. The cutoff points and units for each variable are presented in Table 3. Dummy variables were used in logistic regression analysis to estimate ORs for each category of exposure. Trend tests for ordered variables were performed by assigning the score *j* to the *j*th exposure level of a categorical variable (where $j = 1, 2\ldots$) and treating the categorical variable as an interval predictor in unconditional logistic regression. Two steps were used to assess associations: (*a*) statistical adjustment for age (continuous variable); and (*b*) statistical adjustment for age, race, and education, plus daily caloric intake (continuous variable), pack-years of smoking (continuous variable), alcohol consumption, and family history of prostate cancer.

Results

The demographic characteristics of 65 prostate cancer patients and 132 controls are shown in Table 1. Briefly, at the time of study admission, the mean age was 59.98 years for cases and 41.9 years for controls (*P*, 0.001). The proportion of nonwhites was slightly higher in cases (7.7%) than in controls (3.1%), which was not statistically significant. There were significantly more ever-smokers among cases (58.5%) than among controls (39.4%; *P*, 0.012). Furthermore, the prevalence of heavy smokers (>30 pack-years) was higher in cases (30.8%) than in controls (9.8%; *P*, 0.001). More cases than controls were ever-alcohol drinkers (95.4%; *P*, 0.001), and a higher percentage of cases had family histories of prostate cancer (18.5%) in comparison with controls (9.1%; *P*, 0.059).

Using Student's *t* test, we compared differences of mean plasma concentrations of six carotenoids, retinol, and two tocopherols between cases and controls (Table 2). The mean plasma concentrations of lycopene, zeaxanthin, retinol, β-cryptoxanthin, and γ-tocopherol were significantly higher in controls than in cases. Although the mean concentrations of α-carotene and β-carotene appeared higher in cases than in controls, and the mean level of lutein and α-tocopherol were

Table 4 ORs and 95% CIs for plasma carotinoids, retinol and, tocopherols in 65 prostate cancer cases and 132 controls by quartile of plasma concentrations of controls

	No. of cases	No. of controls	OR[a] (adjusted)	95% CI	*P*	OR[b] (adjusted)	95% CI	*P*
Lycopene								
0	28	33	1.00		0.0057	1.00		0.0052
1	21	33	0.67	0.25–1.76		0.64	0.23–1.75	
2	12	33	0.26	0.09–0.78		0.29	0.09–0.90	
3	4	33	0.24	0.06–0.90		0.17	0.04–0.78	
Continuous[c]	65	132	0.70	0.52–0.94	0.0165	0.67	0.49–0.92	0.0127
Zeaxanthin								
0	23	33	1.00		0.0006	1.00		0.0028
1	23	33	0.81	0.29–2.32		0.97	0.31–3.07	
2	11	33	0.18	0.06–0.57		0.18	0.05–0.64	
3	8	33	0.18	0.05–0.61		0.22	0.06–0.83	
Continuous[c]	65	132	0.16	0.05–0.52	0.0020	0.20	0.06–0.65	0.0074
α-Carotene								
0	14	33	1.00		0.0620	1.00		0.1617
1	20	33	0.62	0.20–1.89		0.57	0.18–1.80	
2	20	33	0.76	0.26–2.26		0.94	0.29–3.04	
3	11	33	0.24	0.07–0.86		0.26	0.07–1.05	
Continuous[c]	65	132	1.09	0.84–1.41	0.5276	1.05	0.77–1.44	0.7388
β-Carotene								
0	15	33	1.00		0.2299	1.00		0.4541
1	6	33	0.22	0.06–0.81		0.23	0.60–0.88	
2	23	33	0.65	0.23–1.90		0.70	0.23–2.16	
3	21	33	0.36	0.12–1.11		0.43	0.13–1.49	
Continuous[c]	65	132	1.02	0.93–1.12	0.6112	1.04	0.94–1.16	0.4577
Lutein								
0	23	33	1.00		0.0012	1.00		0.0064
1	20	33	0.65	0.22–1.93		0.96	0.29–3.14	
2	9	33	0.18	0.06–0.58		0.17	0.05–0.59	
3	13	33	0.21	0.07–0.64		0.30	0.09–1.03	
Continuous[c]	65	132	0.60	0.40–0.91	0.0149	0.65	0.43–0.97	0.0330
β-Cryptoxanthin								
0	17	33	1.00		0.0187	1.00		0.0666
1	21	33	1.45	0.51–4.17		1.66	0.51–5.37	
2	19	33	0.65	0.23–1.83		0.82	0.25–2.65	
3	8	33	0.23	0.07–0.84		0.31	0.08–1.24	
Continuous[c]	65	132	0.42	0.22–0.79	0.0073	0.44	0.23–0.85	0.0153
Retinol								
0	10	33	1.00		0.0667	1.00		0.1318
1	26	33	1.11	0.35–3.46		1.10	0.35–3.49	
2	26	33	1.01	0.33–3.11		1.10	0.35–3.42	
3	3	33	0.18	0.04–0.91		0.19	0.03–1.00	
Continuous[c]	65	132	0.93	0.87–0.99	0.0324	0.94	0.88–1.01	0.0743
α-Tocopherol								
0	5	33	1.00		0.0532	1.00		0.1162
1	27	33	4.27	1.09–16.7		6.67	1.47–30.3	
2	21	33	1.50	0.38–5.95		2.24	0.51–9.94	
3	12	33	0.70	0.17–2.87		1.02	0.23–4.62	
Continuous[c]	65	132	0.995	0.991–0.999	0.0102	1.00	0.99–1.00	0.0208
γ-Tocopherol								
0	26	33	1.00		0.3905	1.00		0.4136
1	15	33	1.04	0.35–3.05		1.23	0.40–3.78	
2	12	33	0.85	0.29–2.46		0.81	0.26–2.49	
3	12	33	0.65	0.24–1.78		0.66	0.22–1.97	
Continuous[c]	65	132	0.99	0.97–1.01	0.2077	0.99	0.97–1.01	0.2696

[a] ORs were adjusted for age only.
[b] ORs were adjusted for age, race, years of education, family history of prostate cancer, pack-years of smoking, alcohol consumption, and daily caloric intake.
[c] Continuous variable with 10 units of increment.

higher in controls than in cases, these differences were not statistically significant.

Table 3 presents the units and cutoff points for quartile distributions of plasma levels of carotenoids, retinol, and tocopherols and of dietary carotenoids and antioxidants. Table 4 presents adjusted ORs and 95% CIs for plasma measurements based upon logistic regression analyses. Plasma concentrations of lycopene, zeaxanthin, lutein, and β-cryptoxanthin were associated with a reduced risk of prostate cancer in multivariate analysis when only adjusting for age, as well as further adjusting for additional potential confounding factors including race, education, pack-years of smoking, alcohol drinking, family

Table 5 Stratified analysis by age on the relationship between plasma lycopene, zeaxanthin, lutein, and β-crytoxanthin and prostate cancer in 65 cases and 132 controls[a]

	OR (all of the cases)	OR (age < 60)	OR (age ≥ 60)
Lycopene			
0	1.00	1.00	1.00
1	0.64, 0.23–1.75	0.53, 0.13–2.18	0.53, 0.08–3.37
2	0.29, 0.09–0.90	0.02, 0.01–0.37	1.02, 0.15–7.10
3	0.17, 0.04–0.78	0.05, 0.005–0.51	0.10, 0.003–3.31
Trend-test	$P = 0.0052$	$P = 0.0015$	$P = 0.6133$
Zeaxanthin			
0	1.00	1.00	1.00
1	0.97, 0.31–3.07	0.27, 0.05–1.40	29.0, 1.29–652
2	0.18, 0.05–0.64	0.02, 0.002–0.20	3.69, 0.37–37.0
3	0.22, 0.06–0.83	0.09, 0.01–0.59	0.81, 0.08–7.82
Trend-test	$P = 0.0028$	$P = 0.0014$	$P = 0.3957$
Lutein			
0	1.00	1.00	1.00
1	0.96, 0.29–3.14	0.33, 0.05–2.04	5.16, 0.43–62.3
2	0.17, 0.05–0.59	0.04, 0.01–0.25	0.86, 0.09–8.57
3	0.30, 0.09–1.03	0.09, 0.01–0.80	1.16, 0.15–9.16
Trend-test	$P = 0.0064$	$P = 0.0025$	$P = 0.5341$
β-Cryptoxanthin			
0	1.00	1.00	1.00
1	1.66, 0.51–5.37	1.17, 0.34–3.96	8.09, 0.76–85.7
2	0.82, 0.25–2.65	1.67, 0.49–5.63	1.66, 0.24–11.8
3	0.31, 0.08–1.24	0.15, 0.02–1.38	2.01, 0.25–16.1
Trend-test	$P = 0.0666$	$P = 0.2724$	$P = 0.8584$

[a] ORs were adjusted for age, race, years of education, family history of prostate cancer, pack-years of smoking, alcohol consumption, and daily caloric intake.

history of prostate cancer, and daily caloric intake. The adjusted OR for plasma lycopene was 0.17 (95% CI, 0.04–0.78) for the highest quartile, in comparison with the lowest quartile (*P* for trend, 0.0052). With plasma lycopene treated as a continuous variable, the *P* was 0.0165. The adjusted OR for zeaxanthin was 0.22 (95% CI, 0.06–0.83) for the highest quartile when compared with the lowest quartile (*P* for trend, 0.0280), and the *P* for continuous variable is 0.0020. Borderline associations with prostate cancer were observed between plasma concentrations of lutein and β-cryptoxanthin. No obvious relationships were found for plasma α-carotene, β-carotene, α-tocopherol, γ-tocopherol, and retinol with prostate cancer after adjusting for all of the potential confounding factors.

Additional analyses were conducted for plasma levels of lycopene, zeaxanthin, lutein, and β-cryptoxanthin, stratifying age into two groups: less than 60 years and 60 years or older. The observed inverse associations were confirmed for lycopene, zeaxanthin, and lutein in those who were less than 60 years of age because a higher proportion of controls ($n = 118$) were in this age group (Table 5).

Adjusted ORs and 95% CIs for selected dietary intake of carotenoids, vitamins, and antioxidants in the 65 cases and 132 controls are shown in Table 6. Although dietary intakes of lycopene, lutein, vitamin A, Pro-A carotene, β-carotene, α-carotene, and vitamin B1 showed an inverse relationship with prostate cancer, no obvious significant relationship was observed.

Discussion

In this study, we found that a plasma level of lycopene was inversely associated with prostate cancer after adjusting for potential confounding factors such as age, race, years of education, pack-years of smoking, alcohol consumption, family history of prostate cancer, and average daily caloric intake. An 83% reduction of prostate cancer risk was observed in the group with the highest plasma concentration of lycopene in comparison with individuals with the lowest concentration. The strength of association was basically the same, whether adjusted or unadjusted for the confounding factors we considered. The association was dose-responsive; with increased concentration of plasma lycopene, the risk of prostate cancer was decreased. Furthermore, in the analysis of dietary lycopene among same subjects, adjusting for age, race, education, total caloric intake, pack-years of smoking, alcohol drinking, and family history of cancer, we observed a similar dose-response pattern with an OR of 0.69 (95% CI, 0.23–2.08) for those in the highest quartile as compared with those in the lowest quartile, although it was not statistically significant (Table 6). Our observation of inverse association of plasma lycopene with prostate cancer is consistent with two recent studies (16, 17) on plasma lycopene level and prostate cancer. A prospective cohort study (11) found that intake of lycopene or other tomato products was associated with reduced prostate cancer risk. In addition to our findings in plasma lycopene, we observed inverse associations of plasma lutein, zeaxanthin, and β-cryptoxanthin with prostate cancer. When the data were categorized into quartiles, dose-response relationships were observed for all of the three carotenoids.

Although the participation rate was 87% for cases and 92% for controls, a potential selection bias needs to be noted because the proportion of cases with blood samples was substantially low (45% in cases and 88% in controls). Patients with blood samples may be a selected population, which may affect the association under study. We have analyzed our data to explore whether there is a potential selection bias for both cases and controls, with and without blood samples; however, no obvious differences were found in cases in terms of age, race, education, smoking, alcohol drinking, and family history of prostate cancer. Similar results were found in controls, except a borderline difference was found for alcohol drinking. Controls with blood samples were relatively less alcohol drinkers than those who did not provide samples (*P*, 0.05). A recent study (28) has shown that relatively few lifestyle and demographic factors were important determinants of plasma lycopene levels, with plasma cholesterol, marital status, and lycopene intake being of greatest importance. The borderline difference of alcohol drinking between those with and without blood samples might not affect the observed association.

The possible confounding effect of age needs to be considered. The age difference in cases and controls was substantial in this study. If the plasma levels of lycopene decline with age, the observed inverse association might be spurious, although age was adjusted in the data analysis. To exclude the possibility of age effect on lycopene levels, the correlation between age and plasma levels of lycopene was analyzed among controls. The results showed that there was no obvious correlation between plasma lycopene level and age in controls (*r*, 0.0342; *P*, 0.6971), so that we are confident that the potential influence of age on plasma level of lycopene and prostate cancer is minimal. Because prostate cancer cases were much older than the controls in this study, we tried to minimize the possible confounding effect attributable to age by controlling for age as a continuous variable in data analyses. However, the potential residual confounding effect might not be excluded because a total of 90% of controls and 48% of cases were less than 60 years old. We did additional analyses stratified by age (<60 years old and ≥60 years old). The ORs from stratified analyses for both individuals younger than 60 years of age and

Table 6 ORs and 95% CIs for dietary intake of carotenoids, other vitamins, and antioxidants in 65 cases and 132 controls

Nutrients (quartile)	OR[a]	95% CI	OR[b]	95% CI
Vitamin A				
0	1.00		1.00	
1	0.64	0.21–1.92	0.59	0.18–1.91
2	0.67	0.22–2.06	0.66	0.19–2.29
3	0.69	0.24–2.01	0.58	0.17–1.98
Trend-test	$P = 0.5554$		$P = 0.4524$	
Carotene				
0	1.00		1.00	
1	0.27	0.07–0.98	0.20	0.05–0.81
2	0.78	0.29–2.13	0.66	0.22–1.99
3	0.48	0.15–1.46	0.35	0.10–1.27
Trend-test	$P = 0.4364$		$P = 0.3356$	
β-Carotene				
0	1.00		1.00	
1	0.34	0.09–1.22	0.28	0.07–1.12
2	0.68	0.25–1.88	0.60	0.20–1.81
3	0.56	0.19–1.65	0.44	0.13–1.52
Trend-test	$P = 0.4458$		$P = 0.3519$	
α-Carotene				
0	1.00		1.00	
1	1.06	0.35–3.21	0.87	0.26–2.90
2	0.90	0.30–2.65	0.73	0.23–2.33
3	0.59	0.19–1.81	0.47	0.14–1.66
Trend-test	$P = 0.3329$		$P = 0.2273$	
Lycopene				
0	1.00		1.00	
1	1.07	0.37–3.16	1.14	0.36–3.62
2	0.78	0.25–2.45	0.91	0.27–3.11
3	0.63	0.23–1.76	0.69	0.23–2.08
Trend-test	$P = 0.3197$		$P = 0.4689$	
Lutein				
0	1.00		1.00	
1	0.57	0.18–1.84	0.54	0.16–1.83
2	0.67	0.23–1.91	0.62	0.20–1.89
3	0.75	0.25–2.22	0.55	0.16–1.88
Trend-test	$P = 0.6519$		$P = 0.3721$	
Cryptoxanthin				
0	1.00		1.00	
1	0.73	0.23–2.28	0.70	0.22–2.27
2	1.35	0.48–3.83	1.21	0.41–3.59
3	1.11	0.35–3.50	0.92	0.26–3.20
Trend-test	$P = 0.5832$		$P = 0.8116$	
Retinol				
0	1.00		1.00	
1	0.83	0.28–2.48	0.97	0.29–3.30
2	1.83	0.66–5.07	3.55	1.05–12.0
3	1.07	0.33–3.47	1.24	0.30–5.18
Trend-test	$P = 0.4778$		$P = 0.2217$	
Vitamin B1				
0	1.00		1.00	
1	1.00	0.33–3.06	1.09	0.33–3.54
2	1.23	0.44–3.48	0.99	0.30–3.35
3	0.63	0.20–1.97	0.50	0.13–1.98
Trend-test	$P = 0.5795$		$P = 0.3496$	
Vitamin B2				
0	1.00		1.00	
1	1.91	0.66–5.57	2.28	0.68–7.63
2	1.41	0.48–4.16	1.44	0.40–5.22
3	0.75	0.24–2.28	0.71	0.17–2.95
Trend-test	$P = 0.6052$		$P = 0.4986$	
Vitamin B6				
0	1.00		1.00	
1	1.23	0.41–3.70	1.53	0.45–5.27
2	1.44	0.50–4.15	1.45	0.41–5.08
3	0.93	0.32–2.75	1.03	0.25–4.19
Trend-test	$P = 0.9941$		$P = 0.9916$	
Vitamin C				
0	1.00		1.00	
1	0.52	0.15–1.84	0.53	0.15–1.89
2	1.47	0.55–3.99	1.17	0.40–3.41
3	0.96	0.31–2.98	0.88	0.25–3.02
Trend-test	$P = 0.6122$		$P = 0.8918$	
Vitamin E				
0	1.00		1.00	
1	0.76	0.26–2.25	0.68	0.20–2.26
2	2.26	0.75–6.78	1.88	0.54–6.56
3	0.50	0.15–1.60	0.38	0.09–1.68
Trend-test	$P = 0.7174$		$P = 0.5858$	
Folate				
0	1.00		1.00	
1	3.29	0.97–11.2	3.72	1.05–13.2
2	2.85	0.82–10.0	3.32	0.83–13.4
3	2.28	0.65–8.02	2.66	0.61–11.5
Trend-test	$P = 0.7112$		$P = 0.3723$	
Fiber				
0	1.00		1.00	
1	2.45	0.73–8.20	2.60	0.72–9.38
2	0.78	0.24–2.58	0.68	0.19–2.45
3	1.72	0.58–5.05	1.81	0.55–5.96
Trend-test	$P = 0.6749$		$P = 0.6517$	
Niacin				
0	1.00		1.00	
1	2.57	0.85–7.83	3.38	0.89–12.6
2	3.02	0.95–9.63	3.84	0.99–14.8
3	1.02	0.33–3.20	1.32	0.30–5.83
Trend-test	$P = 0.9509$		$P = 0.8990$	

[a] Adjusted for age.
[b] Adjusted for age, race, education, alcohol drinking, pack-years of smoking, family history of prostate cancer, and total dietary caloric intake.

individuals 60 years of age or older were slightly lower then the adjusted OR for whole study population. The result indicates that the possible residual confounding effect might exist, which is probably directed toward null (Table 5).

An inverse association between serum lycopene and prostate cancer was found in this study. However, it is difficult to infer a causal relationship. Considering the long duration of cancer development, plasma measurements might not be a good index for long-term dietary intake. Plasma levels of carotenoids only reveal a short-term dietary intake, because the half-life of carotenoids in blood ranges only a few days. The measured levels may only represent the dietary changes after prostate cancer diagnosis in cases. If patients changed their lifestyles and dietary habits by increasing intake of dietary antioxidants after cancer diagnosis, the plasma concentrations of these factors should be higher than before their diagnoses. This would lead to an underestimation of associations of lycopene and other antioxidants with prostate cancer and would bias the association toward null. Despite this potential limitation, we have found a significant inverse association between plasma lycopene, other carotenoids, and prostate cancer, which indicates that our estimates are relatively conservative. Another possibility is that after the diagnosis or treatment of prostate cancer, the metabolic process of prostate cancer might influence the level of plasma carotenoids in cases. The observed inverse associations might be affected by metabolic consequences of cancer process or by treatments for prostate cancer. Although the possibility may exist that the disease process or treatment may reduce

plasma levels of retinol (29), no direct evidence in the literature could be identified to support the hypothesis that these factors would alter the level of plasma lycopene. In addition, a similar dose-response pattern was found in the same study subjects between usual dietary intake of lycopene and prostate cancer, which further supports the observed inverse association between plasma level of lycopene and prostate cancer.

The small sample size may have reduced the power of the study, resulting in imprecision of the measurements and limiting our ability to estimate the association precisely. However, despite limited statistical power because of a small sample size, we have achieved statistical significance with lycopene and other carotenoids. We believe that the limitation of small sample size will not affect the conclusion of the study.

Lycopene is a predominant carotenoid in blood and tissues (30–33). It is more efficient at quenching singlet oxygen and scavenging free radicals than other common carotenoids (34). Oxidative damage, to either DNA or membranes, might play a role in the development of prostate and other cancers. Lycopene was found to be very active in suppressing certain human cancer cell proliferation (35, 36). *In vivo* studies (37) have shown a tumor-suppressive activity of lycopene. The suppression of tumor incidence was associated with a decrease in mammary gland activity and diminished serum levels of free fatty acids and prolactin. Lutein, zeaxanthin, and β-cryptoxanthin are xanthophyll carotenoids, which are characterized by the presence of functional groups such as hydroxyl and carbonyl. Studies on the protective effects of lutein, zeaxanthin, and β-cryptoxanthin are scarce and mostly limited to the macular membrane of human retina. Plasma β-cryptoxanthin in humans was found to correlate inversely with indices of oxidative DNA damage and lipid peroxidation in humans (38). In animal studies (39), a significant suppression of azoxymethane-induced colon carcinogenesis by mandarin juices rich in β-cryptoxanthin was observed in male F344 rats.

In conclusion, our study supports previous studies that indicate lycopene may be inversely associated with prostate cancer. In addition, we found that other plasma carotenoids, such as lutein, zeaxanthin, and β-cryptoxanthin, may also be negatively related to prostate cancer risk. This study provides justification for further research on the associations between lycopene and other antioxidants and the risk of prostate cancer. It is important to confirm the patterns observed in this and previous studies and to evaluate whether these associations are causal.

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