
REPORTS

Relationship Between Plasma Carotenoids and Prostate Cancer

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Abstract: Carotenoids, particularly lycopene, are thought to decrease prostate cancer risk, but the relationship between plasma carotenoid concentrations and risk in various populations has not been well characterized. Comparing 118 non-Hispanic Caucasian men mainly from southeast Texas with nonmetastatic prostate cancer with 52 healthy men from the same area, we conducted a case-control analysis evaluating associations between risk and plasma levels of total carotenoids, β -cryptoxanthin, α - and trans- β -carotene, lutein and zeaxanthin, total lycopenes, trans-lycopene, total cis-lycopenes, and cis-lycopene isoforms 1, 2, 3, and 5. Risk for men with high plasma levels of α -carotene, trans- β -carotene, β -cryptoxanthin, and lutein and zeaxanthin was less than half that for those with lower levels. In contrast, we observed no significant associations for total lycopenes, all-trans-lycopene, and cis-lycopene isomer peaks 2, 3, and 5, although high levels of cis-lycopene isomer peak 1 were inversely associated with risk. Analysis of men with aggressive disease (Gleason scores of ≥ 7 , $n = 88$) vs. less aggressive cases (Gleason scores of < 7 , $n = 30$) failed to reveal significant associations between carotenoid levels and the risk of diagnosis with aggressive disease. These findings suggest that, in these men, higher circulating levels of β -cryptoxanthin, α -carotene, trans- β -carotene, and lutein and zeaxanthin may contribute to lower prostate cancer risk but not to disease progression.

Introduction

Despite much effort spent investigating the role of carotenoids in prostate carcinogenesis, whether and how carotenoids influence cancer risk are still somewhat ambiguous. In general, dietary studies have consistently suggested an inverse association between prostate cancer risk and consumption of carotenoids in tomato products (1). At the same time, studies using biological measures of tomato-based carotenoids have been less uniform in their findings (2–8). Such inconsistency may result in part from differences between studies. Closer investigation of carotenoids, including consideration of their biochemical structures, may help resolve some of these discrepancies, particularly for lycopene, one of the primary carotenoids in tomatoes.

Measuring the quantities of carotenoids that are relevant to prostate carcinogenesis is complicated. For example, dietary assessment of lycopene intake may capture biological exposure inaccurately because lycopene concentrations in foods can vary by the period of cultivation and method of food processing (9,10). Dietary assessment methods also do not easily accommodate variations in carotenoid bioavailability; individual differences in digestion, metabolism, and even patterns of food intake can all influence lycopene absorption. Consuming lycopene-containing foods

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with fat, for instance, can increase the absorption and bioavailability of lycopene (8,11).

Capturing the pertinent internal exposure to lycopene is also complicated. Concentrations of lycopene are lower in blood than in prostate tissues (9); however, whereas the former is easier to obtain for research purposes, it may not reflect the relevant exposure to lycopene as well as specimens collected from the prostate gland. The distributions of isomeric forms of lycopene also differ between raw and heat-processed tomatoes, serum, and prostate tissue (9,12); the all-*trans* form is the most prevalent form in raw tomato-based products, whereas *cis*-isomers, several of which exist *in vivo* (9), are predominant in human tissues. Of eight prostate cancer studies published since 1990 that measured lycopene in biological samples (2–8,13), none have evaluated the association between individual isomeric forms of lycopene and risk. Because individual lycopene isomers may be more strongly associated with risk than all isomeric types of lycopene considered together, investigating individual isoforms may reveal new information that can help address the discrepancies in lycopene findings between studies.

We analyzed a limited number of plasma samples available from a case-control analysis to evaluate associations between prostate cancer risk and plasma carotenoid levels in 118 men with nonmetastatic prostate cancer and 52 healthy men. In addition to reporting on plasma levels of total carotenoids, α - and *trans*- β -carotene, β -cryptoxanthin, lutein, zeaxanthin, and total lycopenes, we evaluated the association with risk of prostate cancer for *trans*- and *cis*-lycopenes separately as well as for four *cis*-lycopene isomers. As with previous studies, our hypothesis was that higher concentrations of these carotenoids would be associated with lower risk of prostate cancer, particularly for the *cis*-isomer. In addition, we predicted that, among only the men with prostate cancer, high carotenoid levels would be associated with lower risk of diagnosis with more aggressive prostate cancer than less aggressive disease according to Gleason score.

Materials and Methods

Study Population and Data Collection

For this analysis, 118 men with prostate cancer and 52 healthy age-matched controls were drawn from the non-Hispanic Caucasian subjects enrolled from 1996 to 1998 in an ongoing study of prostate cancer being conducted at The University of Texas M. D. Anderson Cancer Center and Baylor College of Medicine in Houston, Texas, described more fully elsewhere (14). Inclusion in this analysis was based on the availability of blood serum for high-pressure liquid chromatography (HPLC) analysis. In the parent case-control analysis, cases were defined as men with histological evidence of either organ-confined or extraprostatic extension of their prostate cancer without metastatic disease as determined after radical prostatectomy according to a published protocol (15). Controls were men who

had participated in a prostate cancer-screening program at M. D. Anderson from August to March, had prostate specific antigen (PSA) concentrations of <4 ng/ml, and had negative digital rectal exams. Healthy control subjects were frequency matched to cases by age (± 5 yr) and year at recruitment (± 1 yr). In a subset analysis, we compared 30 prostate cancer patients with low Gleason scores (that is, Gleason scores of <7) with 88 patients with higher scores (that is, Gleason scores of ≥ 7), which were used as a proxy for aggressive prostate cancer.

At study enrollment, trained interviewers obtained informed consent and a blood sample, measured the height and weight of all subjects, and administered a standardized questionnaire to collect information, including age, smoking status, and family history of prostate cancer. We checked questionnaires for completeness, accuracy, unusual responses, and errors, contacting and querying the subjects when necessary. Data were double entered by two research staff members trained in data entry, and an edit check was performed to confirm the accuracy of data entry. Blood was collected post-operatively from cases (93% collected at least 6 wk after surgery) and at the time of PSA testing from healthy controls.

We calculated body mass index (BMI) as weight (kg)/height (m²) and categorized subjects by smoking status as never smokers, current smokers, and former smokers. We defined former smokers as men who had quit smoking for at least 12 mo prior to study enrollment. We defined having a family history of prostate cancer as reporting a father, brother, or son diagnosed with prostate cancer.

HPLC Analysis of Plasma Carotenoids

Blood samples were collected and processed within 2 h of collection, and plasma was stored in individual aliquots at -80°C until analysis. Analyses were conducted under yellow light, and preparations were stored on ice whenever possible throughout the procedure. Plasma was thawed on ice, sonicated for 5 min at room temperature, and returned to an ice bath. Ethanol (0.5 ml) containing 1.0 g/l butylated hydroxytoluene (Sigma Chemical Company, St. Louis, MO) and echinenone internal standard (gift of F. Hoffmann—La Roche Ltd., Basel, Switzerland) were added to duplicate portions. Carotenoids were extracted three times with 1.0 ml with hexane (Fisher Scientific, Fair Lawn, NJ), and preparations were dried under vacuum. Extracts were reconstituted in methyl-*tert*-butyl ether (Fisher Scientific) and analyzed by reverse-phase HPLC using the method of Yeum et al. (16). Peak identifications were made by comparison of retention times with known standards and spectral analysis using photodiode array detection (Waters model 996, Millipore). For lycopene, putative identification of *cis*-isomers was based upon the spectra and relative retention times reported by Yeum et al. (16). The laboratory (JWE) participates quarterly in the National Institutes of Standards in Technology micronutrient measurement proficiency testing program. The coefficient of variance for lycopene analysis was <12% and for other carotenoids was <10%.

The chromatographic system consisted of a Varian model 410 autosampler (Varian, Walnut Creek, CA) with sample cooling tray maintained at 4°C, Varian model SD 200 dual pumps, an analytical guard column (Upchurch Scientific, Oak Harbor, WA) packed with C-18 pellicular material (Alltech Associates, Inc., Deerfield, IL), a Waters/YMC carotenoid column (150-mm length × 4.6-mm internal diameter, 3-µm particle size, C30 bonded phase, Waters Corporation, Milford, MA), a Dynamax model UV D II absorbance detector set at 450 nm (Rainin Instrument Company, Woburn, MA), and Rainin Dynamax software run on a Macintosh Quadra (Apple Computer, Inc.). The elution gradient system was described by Yeum et al. (16). Calculations were made with plasma carotenoid peak areas, correcting with echinenone internal standard peak areas, and utilizing standard curves. Plasma was measured for concentrations of β-cryptoxanthin; α-carotene; *trans*-β-carotene; all *trans*-lycopene; *cis*-lycopene isomers 1, 2, 3, and 5; and lutein and zeaxanthin, whose concentrations were presented together because their peaks were indistinguishable (Fig. 1). We also evaluated levels of total carotenoids [sum of β-cryptoxanthin, α-carotene, *trans*-β-carotene, lutein and zeaxanthin, and total lycopenes (sum of *trans*-lycopene and sum of total *cis*-lycopene isomers 1, 2, 3, and 5)]. Because the samples were from a larger study, the quantity of plasma available for analysis was limited; thus, carotenoid values presented are unadjusted for plasma cholesterol.

Statistical Analysis

For descriptive analyses, after evaluating distributions for normality, we used Student's *t*-tests, Fisher's exact tests, and standard χ^2 tests to compare means and distributions of several characteristics between case-control groups and between less aggressive and aggressive prostate cancer cases. To calculate odds ratios as estimates of the relative risks

and to adjust for other factors, we conducted unconditional logistic regression analysis using the Statistical Analysis System package (version 6.12, SAS Institute, Cary, NC) (17). We categorized plasma carotenoid levels as lower than or equal to the median plasma value (that is, "low") and higher than the median value (that is, "high") among the controls. We used men with values below the medians as the reference groups. In the subset analyses among only men with prostate cancer, we estimated the risk of aggressive prostate cancer (Gleason score of ≥ 7) relative to development of less aggressive disease (Gleason score of < 7) for high carotenoid levels.

We derived overall models predicting independent effects of carotenoids considered simultaneously, building from the individual carotenoids we identified as having significant associations in multivariate models. Additional carotenoids were added in a stepwise fashion, including only those that contributed significantly to the overall model. All multivariate models were adjusted for the effects of age, smoking (current smoker vs. other), and height, and the potential confounding effects of BMI and family history were also evaluated. We provided 95% confidence intervals where appropriate and all statistical tests were two-sided.

Results

Selected Characteristics Among Prostate Cancer Cases and Healthy Controls

In this analysis, prostate cancer cases were similar to healthy controls in age, weight, BMI, smoking status, and report of family history of prostate cancer (Table 1). Men with prostate cancer were shorter than healthy men were ($P = 0.01$). Among cases, men with aggressive disease (Gleason score of ≥ 7) compared with those with less aggressive pros-

Table 1. Means and Distributions of Selected Characteristics Among Prostate Cancer Cases and Controls^a

Characteristic	Mean (SD)		P Value ^b
	Controls, n = 52	Cases, n = 118	
Age (yr)	62.8 (6.6)	63.9 (7.0)	0.37
Height (m)	1.80 (0.06)	1.77 (0.06)	0.01
Weight (kg)	86.9 (13.2)	86.9 (11.6)	1.00
BMI (kg/m ²)	26.9 (4.6)	27.7 (3.5)	0.29
	n (%)	n (%)	
Smoking status			0.10 ^c
Never	20 (43.5)	37 (33)	
Former	20 (43.5)	68 (61)	
Current	6 (13)	7 (6)	
Family history of prostate cancer			0.41 ^c
No	45 (87)	96 (81)	
Yes	7 (13)	22 (19)	

^a: Abbreviations are as follows: SD, standard deviation; BMI, body mass index.

^b: P value derived from Student's *t*-test.

^c: P value derived from standard χ^2 test.

Table 2. Median Plasma Carotenoid Levels Among Prostate Cancer Cases and Controls and Adjusted Estimates for Risk of Prostate Cancer Associated With High Plasma Carotenoid Levels^a

Plasma carotenoids (μmol/l)	Median (25–75%)		Age-adjusted OR (95% CI)	Age, smoking, and height-adjusted OR (95% CI)
	Controls, n = 52	Cases, n = 118		
Total carotenoids	1.14 (0.88–1.63)	1.18 (0.82–1.45)	0.98 (0.50–1.90)	1.01 (0.48–2.13)
β-Cryptoxanthin	0.08 (0.05–0.11)	0.04 (0.01–0.09)	0.45 (0.23–0.89)	0.48 (0.23–1.02)
α-Carotene	0.10 (0.07–0.12)	0.08 (0.06–0.10)	0.31 (0.15–0.62)	0.33 (0.16–0.72)
trans-β-Carotene	0.34 (0.17–0.46)	0.25 (0.16–0.40)	0.44 (0.22–0.87)	0.46 (0.21–0.98)
Lutein and zeaxanthin	0.22 (0.15–0.26)	0.16 (0.09–0.23)	0.39 (0.19–0.79)	0.32 (0.15–0.69)
Total lycopenes	0.47 (0.27–1.62)	0.51 (0.38–0.66)	1.21 (0.62–2.35)	1.30 (0.63–2.71)
trans-Lycopene	0.16 (0.10–0.22)	0.16 (0.12–0.23)	1.16 (0.60–2.25)	1.19 (0.57–2.48)
Total cis-lycopenes	0.32 (0.19–0.42)	0.33 (0.24–0.43)	1.21 (0.62–2.35)	1.16 (0.56–2.42)
cis-Lycopene-1	0.04 (0.01–0.05)	0.02 (0.00–0.03)	0.21 (0.10–0.44)	0.28 (0.13–0.62)
cis-Lycopene-2	0.08 (0.05–0.11)	0.09 (0.06–0.11)	1.26 (0.65–2.43)	1.27 (0.61–2.62)
cis-Lycopene-3	0.06 (0.02–0.07)	0.06 (0.04–0.07)	0.96 (0.50–1.86)	1.04 (0.51–2.15)
cis-Lycopene-5	0.14 (0.09–0.20)	0.16 (0.12–0.23)	1.75 (0.89–3.43)	1.83 (0.86–3.88)

a: Abbreviations are as follows: OR, odds ratio; CI, confidence interval. Low plasma carotenoid levels were defined as values lower than the median level among controls. Men with plasma levels higher than the median were used as the reference group.

tate cancer (Gleason score of <7) were leaner (BMI_{aggressive PC} = 27.3 kg/m² vs. BMI_{less aggressive PC} = 28.7 kg/m², *P* = 0.05) and older (64.8 yr for men with aggressive PC vs. 61.1 yr for men with less aggressive PC, *P* = 0.01).

Plasma Carotenoids Among Prostate Cancer Cases and Healthy Controls

Median plasma levels of total carotenoids were similar for prostate cancer cases and healthy controls (Table 2), mainly because the concentrations of the largest contributors to total carotenoids, *trans*-β-carotene and total lycopenes, were similar between cases and controls. However, healthy men had significantly higher median plasma levels of β-cryptoxanthin, α-carotene, and lutein and zeaxanthin than prostate cancer cases had. These patterns remained consistent whether we compared healthy men with all prostate cancer cases or with only aggressive cases because plasma carotenoid levels did not differ by disease aggressiveness (data not shown). Except for *cis*-lycopene-1, for which levels were lower in cases than controls, there were no notable case-control differences in levels of lycopene isomers.

Multivariate analysis revealed that men with high plasma levels of β-cryptoxanthin, α-carotene, *trans*-β-carotene, lutein and zeaxanthin, and *cis*-lycopene-1 had less than half the risk of prostate cancer diagnosis than men with lower levels. The concentrations of other carotenoids were not significantly associated with prostate cancer risk, and adjustment for the potentially confounding effects of BMI and family history did not alter our findings materially. For models estimating risk of aggressive disease in a subset analysis among prostate cancer cases, no associations for any individual plasma carotenoids were statistically significant (data not shown).

Discussion

Research regarding the role of lycopene in prostate carcinogenesis has been inconsistent. Epidemiological studies assessing dietary intake, although firmly based on biologically plausible hypotheses, have provided support for the association between risk and lycopene intake in most, but not all, studies published to date. At the same time, the hope has been, although not yet realized, that studies measuring biological samples—putatively more accurate than dietary assessment—would advance the understanding of this relationship. Among the studies based on blood samples published to date, four of nine studies, including ours presented here (2,3,8), reported no statistically significant associations between lycopene levels and prostate cancer risk (Table 3).

To provide further insight into the hypothesized protective mechanism of lycopene, we also evaluated lycopenes by structural class group, as members of the same class may act differently from each other and from the class considered all together. No remarkable patterns for risk were revealed for either total *cis*-lycopenes, the subclass of lycopenes found in highest concentrations in prostate tissue and serum (9), or all-*trans*-lycopene, the principal lycopene isomers in tomato products. We did, however, observe a significant association with the *cis*-lycopene-1 peak, the peak that corresponds mainly with 15-*cis*-lycopene, although the biological significance of this finding is not clear. Readers should note that this peak is consistently the smallest peak measured and can be difficult to quantify in chromatographic analysis. However, before forming definitive conclusions, the need exists to confirm these findings as other investigations have not evaluated subclasses of lycopenes.

In weighing the evidence from plasma studies that supports a protective role for lycopenes in prostate

Table 3. Plasma Carotenoid Levels ($\mu\text{mol/l}$) Among Cases and Controls in Epidemiological Studies of Prostate Cancer

	Sample Collection Years																					
	Wu et al. (2004)		Huang et al. (2003)				Vogt et al. (2002)				Lu et al. (2001)		Gann et al. (1999)		Rao et al. (1999)		Nomura et al. (1997)		Hsing et al. (1990)			
	Median		Median		Median		Median		Median		Mean		Median		Mean		Median		Median			
	1996–1998		1993–1998		1974–1996		1989–1996		1986–1989		8/1993–7/1995		9/1982–11/1982		—		1971–1975		8/1974–11/1974			
Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Black	Black	White	White	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	
<i>n</i> = 118	<i>n</i> = 52	<i>n</i> = 460	<i>n</i> = 460	<i>n</i> = 182	<i>n</i> = 364	<i>n</i> = 142	<i>n</i> = 284	<i>n</i> = 99	<i>n</i> = 108	<i>n</i> = 110	<i>n</i> = 120	<i>n</i> = 65	<i>n</i> = 130	<i>n</i> = 578	<i>n</i> = 578	<i>n</i> = 12	<i>n</i> = 12	<i>n</i> = 142	<i>n</i> = 142	<i>n</i> = 103	<i>n</i> = 103	
Total Carotenoids ^a	62.03	63.89	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	95.5	88.9	108	104	
β -Cryptoxanthin	0.042 ^b	0.079 ^b	0.114	0.116	0.11	0.13	0.12	0.13	0.14	0.136	0.123	0.12	0.081	0.107	0.11	0.118	0.121	0.104	0.266	0.221	—	—
α -Carotene	0.077 ^b	0.099 ^b	0.105	0.107	0.04	0.04	0.04	0.04	0.06	0.058	0.067	0.065	0.097 ^b	0.086 ^b	0.104	0.107	—	—	0.078	0.076	0.069	0.071
β -Carotene	0.25 ^b	0.344 ^b	0.344	0.351	0.13	0.15	0.15	0.16	0.328	0.308	0.291	0.258	0.361	0.267	—	—	0.152	0.199	0.287	0.265	0.447	0.447
Lutein	0.16 ^{b,c}	0.217 ^{b,d}	0.21	0.218	0.24	0.24	0.19	0.21	0.445 ^c	0.373 ^c	0.333 ^c	0.322 ^c	0.131	0.158	0.180	0.181	0.235	0.27	0.225	0.207	—	—
Zeaxanthin	—	—	—	—	—	—	—	—	—	—	—	—	0.048 ^b	0.065 ^b	—	—	—	—	0.04	0.042	—	—
Total lycopenes	0.51	0.47	0.698 ^b	0.726 ^b	0.64	0.67	0.72	0.79	0.271 ^e	0.287 ^e	0.315 ^e	0.349 ^e	0.223 ^b	0.307 ^b	0.688 ^{b,f}	0.723 ^{b,f}	0.244 ^b	0.434 ^b	0.25	0.25	0.559	0.597

a: Values are presented in original units, $\mu\text{g/dl}$.

b: A statistically significant association was reported (95% confidence interval).

c: Lutein and zeaxanthin were combined in the original article.

d: Reported *P* values of <0.05 .

e: A statistically significant difference was reported between aggressive cases and controls.

f: The statistically significant finding was restricted to comparisons by aggressive disease among only men with prostate cancer who were not taking β -carotene supplements.

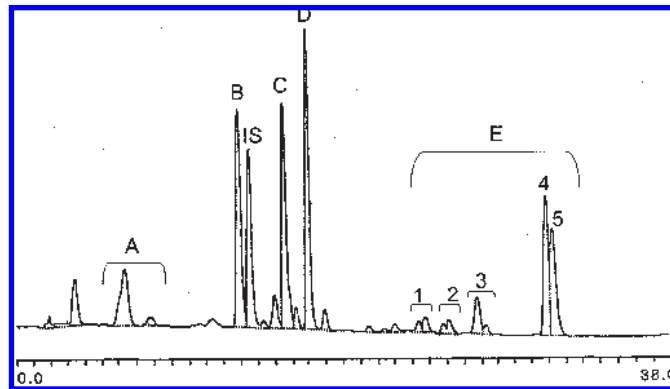


Figure 1. High-pressure liquid chromatography separation of human serum. The carotenoids were monitored at 450 nm. Peak numbers correspond to (A) lutein plus zeaxanthin, (B) β -cryptoxanthin, (C) all-*trans*- α carotene, (D) all-*trans*- β carotene, and (E) lycopenes. 1E, putative 15-*cis*-lycopene; 2E, putative 13-*cis*-lycopene; 3E, putative 9-*cis*-lycopene; 4 E, all-*trans*-lycopene; and 5E, 5-*cis*-lycopene (16). IS, internal standard (that is, echinenone).

carcinogenesis, several factors must be considered. First, the temporal relationship between blood sample collection and prostate cancer diagnosis in our study, as well as in the two other hospital-based, case-control studies (4,5), differs from that for the three case-control studies nested in established cohort studies (2,3,6); in the three latter studies, sample collection preceded cancer diagnosis. In our analysis, samples from the majority of cases (93%) were collected no sooner than 6 wk postoperatively. This may have been a sufficient length of time for carotenoid concentrations to have stabilized from the effects of hospitalization and surgery, although we cannot rule out the possible influence of patient-initiated dietary changes on blood carotenoid levels following surgery. Also, the three hospital-based studies measured blood samples collected from men in the 1990s and therefore may differ from the other studies that used samples collected earlier, from 1971 to 1982 (2,3,6), as carotenoid intake and other relevant risk factors are subject to secular trends. Another aspect of the time frame for blood collection is that the diet of subjects enrolled into studies over a short interval may be biased by foods consumed more frequently during the season of enrollment. Within our sample, carotenoid concentrations did not differ significantly by month of blood draw (data not shown) except for two carotenoids (that is, α -carotene and *cis*-lycopene-1). That we observed significant associations with both of these carotenoids underscores the need to replicate analyses such as ours, even though some evidence suggests that single plasma measurements can generally represent micronutrient exposures, even over extended periods of time (18). Whether such approaches capture the exposure adequately—both accurately and at the relevant time during carcinogenesis—needs to be evaluated further.

Other factors may also affect both carotenoid levels and comparison of studies, albeit indirectly, such as the composition of study subjects and their geographic location. For example, except for the studies among Japanese Americans (2) and Finnish men (19), most study samples were predominantly composed of Caucasian men recruited from a single center: New York, New York (4); Toronto, Canada (5); Washington County, Maryland (3); and Houston, Texas (our

study). In the two largest studies, led by Gann et al. (6) and Wu et al. (13), both of which were not geographically based in a single location, plasma levels of several carotenoids were somewhat higher than those from the other studies, which may reflect the higher education level of the subjects, physicians from the Physician's Health Study and men from the Health Professionals Follow-up Study. A third multicenter study in the United States reported differences by racial group, in which African-American controls had 18% lower median serum lycopene levels than Caucasian controls (7). Special attributes of study subjects, such as cultural practices and education, may reflect differing consumption patterns of certain ethnic foods or healthy lifestyles that may result in higher levels of micronutrients such as carotenoids. In light of these issues, the associations for each carotenoid need to be interpreted within the context of both its distribution (that is, absolute values) and the distributions in other studies as their ranges may vary meaningfully.

It follows that, if low levels of carotenoids are associated with increased prostate cancer risk but the carotenoid levels observed in studies, particularly among cases, are higher than the low levels associated with risk, then detecting significant associations with carotenoids in those studies may be impossible if not irrelevant for those particular prostate cancer cases. For lycopene levels, which were higher in our study than in the other studies, we did not observe a significant association with prostate cancer risk. However, plasma levels for all other carotenoids evaluated in our study— β -cryptoxanthin, α - and *trans*- β -carotene, and lutein and zeaxanthin—were both comparable with or lower than national levels for men (20,21) and significantly associated with prostate cancer risk. Moreover, in our study and that of Lu et al. (4), the only two to report significant associations with risk for lutein and zeaxanthin, plasma levels of these two carotenoids were lower than those in studies that assessed but did not report significant associations for lutein and zeaxanthin (2,5,6). This suggests that detection of a significant association with prostate cancer risk may be most probable below a maximum level of lutein and zeaxanthin, although it is also plausible that studies whose carotenoid

levels vary little between cases and controls will be unable to detect significant associations without the statistical power that comes from large sample sizes.

Indeed, small sample size limited the statistical power to detect modestly sized associations of several studies, including ours. However, the strongly protective associations reported in our study warrant further evaluation in larger studies. Although it is possible that bias from the patient and screening groups that was used to recruit study subjects may have affected our results, the majority of cases and all of the controls were recruited from the same institution (that is, M. D. Anderson Cancer Center). As in several other studies (2,3,4,5,8), we did not adjust for cholesterol levels in our analysis due to limited plasma volumes, which may have introduced some error into our findings, although others who adjusted for cholesterol reported little effect on their findings (6,7).

Despite limitations, we report interesting results in our analysis for β -cryptoxanthin, lutein and zeaxanthin, and α - and *trans*- β -carotene in addition to our findings for lycopene. For β -cryptoxanthin and lutein and zeaxanthin, our findings are similar to those from the hospital-based, case-control study by Lu et al. (4) but not other studies (3–5,11). For β -carotene, not all studies (2–5,22,23) found an effect as we did, although Knekt et al. (19) also reported a decreased risk of prostate cancer strongly associated with high β -carotene levels. In a subset analysis among only men with prostate cancer, we also found no significant association for any carotenoids with greater risk of diagnosis with aggressive disease (that is, a Gleason score of ≥ 7) compared with risk of less aggressive disease. The lack of associations with Gleason stage, in the context of our report that lower prostate cancer risk was associated with higher levels of some types of carotenoids, suggests that some carotenoids may influence the risk of developing disease but not progression to a more aggressive tumor (that is, from a low to a high Gleason score).

The observed inverse associations between plasma levels of several carotenoids and prostate cancer are biologically plausible, although it is impossible to determine whether these nutrients play direct or indirect roles in prostate cancer based on our results. Oxidative damage to DNA or other cellular macromolecules is thought to contribute to the development of prostate and other cancers. A direct effect of increased levels of circulating carotenoids, therefore, could involve increased antioxidant capacity, resulting in reduced levels of reactive oxygen species. Although lycopene has been shown to be the most efficient of the biological carotenoids at quenching singlet oxygen (9,24), several serum carotenoids, including α -carotene, *trans*- β -carotene, and the xanthophyll carotenoids lutein, zeaxanthin, and β -cryptoxanthin, have also demonstrated potent antioxidant properties against an array of reactive oxygen species in a variety of settings (25–28). Alternatively, plasma carotenoids could play an indirect role as markers of vegetable and fruit intake. For instance, the xanthophyll carotenoids may be an indicator of cruciferous vegetable intake (29), and it is possi-

ble that other protective compounds in these vegetables, independent of or in combination with the carotenoids, exert the protective effects against prostate carcinogenesis.

Because so much attention has focused on the hypothesized protective effects of lycopene and tomato products against prostate and other cancers, the potential benefits of other carotenoids have received less attention possibly because studies evaluating dietary intake, which has not been well correlated to biological measures of carotenoids (7,30), have not yielded strong associations with the wide number of foods that contain these carotenoids. It is also possible that the specific diet consumed by the men in our sample allowed us to capture different nuances in dietary components that have not been as prominent in other studies. For example, the carotenoids α -carotene, β -cryptoxanthin, lutein, and zeaxanthin are present in high quantities in dark, leafy green vegetables and corn and corn products, which are consumed in high quantities by people in southeast Texas relative to many other parts of the United States. High consumption of corn and corn products by Mexican Americans is thought to contribute to their lower risk of several cancers relative to non-Hispanic whites and African Americans in the United States (24). Currently, our analyses of dietary intake of carotenoids are under way, permitting a fuller understanding of the relationships between plasma and dietary levels of carotenoids with prostate cancer.

Although carotenoids measured in serum have been reported to be significantly and highly correlated (7) and serum patterns of lycopene isoforms have shown “limited between-person variability” in the same study sample group (31), whether these patterns hold constant for all groups is unknown. Until such observations have been validated in other groups and among larger numbers, we suggest that future investigations continue to evaluate individual carotenoids, including the various lycopene isoforms, for their independent associations with prostate cancer risk. That we reported significant associations with several, but not all, carotenoids and with one, but not all, lycopene isoforms evaluated—even though levels of lycopene isomers were strongly correlated (data not shown)—indicates that associations with risk may differ importantly. The link between carotenoids and prostate cancer would also be strengthened by further mechanistic research on specific carotenoids, including individual lycopene isoforms. In summary, we found that, in a group of Texas men, higher circulating levels of specific carotenoids, especially β -cryptoxanthin, α -carotene, *trans*- β -carotene, and lutein and zeaxanthin, may contribute to lower risk of developing prostate cancer. In contrast, carotenoids did not appear to be linked with prostate cancer aggressiveness at diagnosis.

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References

- Etminan M, Takkouche B, and Caamano-Isorna F: The role of tomato products and lycopene in the prevention of prostate cancer: a meta-analysis of observational studies. *Cancer Epidemiol Biomarkers Prev* **13**, 340–345, 2004.
- Nomura AMY, Stemmermann GN, Lee J, and Craft NE: Serum micronutrients and prostate cancer in Japanese Americans in Hawaii. *Cancer Epidemiol Biomarkers Prev* **6**, 487–491, 1997.
- Hsing AW, Comstock GW, Abbey H, and Polk BF: Serologic precursors of cancer. Retinol, carotenoids, and tocopherol, and the risk of prostate cancer. *JNCI* **82**, 941–946, 1990.
- Lu QY, Hung JC, Heber D, Go VLW, Reuter VE, et al.: Inverse associations between plasma lycopene and other carotenoids and prostate cancer. *Cancer Epidemiol Biomarkers Prev* **10**, 749–756, 2001.
- Rao AV, Fleshner N, and Agarwal S: Serum and tissue lycopene and biomarkers of oxidation in prostate cancer patients: a case-control study. *Nutr Cancer* **33**, 159–164, 1999.
- Gann PH, Ma J, Giovannucci E, Willett WC, Sacks FM, et al.: Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. *Cancer Res* **59**, 1225–1230, 1999.
- Vogt TM, Mayne ST, Graubard BI, Swanson CA, Sowell AL, et al.: Serum lycopene, other serum carotenoids, and risk of prostate cancer in US blacks and whites. *Am J Epidemiol* **155**, 1023–1032, 2002.
- Huang HY, Alberg AJ, Norkus EP, Hoffman SC, Comstock GW, et al.: Prospective study of antioxidant micronutrients in the blood and the risk of developing prostate cancer. *Am J Epidemiol* **157**, 335–344, 2003.
- Clinton SK, Emehiser C, Schwartz SJ, Bostwick DG, Williams AW, et al.: *cis-trans* Lycopene isomers, carotenoids, and retinol in the human prostate. *Cancer Epidemiol Biomarkers Prev* **5**, 823–833, 1996.
- Khachik F, Carvalho L, Bernstein PS, Muir GJ, Zhao DY, et al.: Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. *Exp Biol Med* **227**, 845–851, 2002.
- Gann PH and Khachik F: Tomatoes or lycopene versus prostate cancer: is evolution anti-reductionist? *JNCI* **95**, 1563–1565, 2003.
- Nguyen ML and Schwartz SJ: Lycopene stability during food processing. *Proc Soc Exp Biol Med* **218**, 101–105, 1998.
- Wu K, Erdman JW Jr, Schwartz SJ, Platz EA, Leitzmann M, et al.: Plasma and dietary carotenoids and the risk of prostate cancer: a nested case-control study. *Cancer Epidemiol Biomarkers Prev* **13**, 260–269, 2004.
- Spitz MR, Strom SS, Yamamura Y, Troncoco P, Babaian RJ, et al.: Epidemiologic determinants of clinically relevant prostate cancer. *Int J Cancer* **89**, 259–264, 2000.
- Babaian RJ, Troncoco P, Steelhammer LC, Lloreta-Trull J, and Ramirez EI: Tumor volume and prostate specific antigen: implications for early detection and defining a window of curability. *J Urol* **154**, 1808–1812, 1995.
- Yeum KJ, Booth SL, Sadowski JA, Liu C, Tang G, et al.: Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. *Am J Clin Nutr* **64**, 594–602, 1996.
- SAS Institute: Cary, NC: Statistical Analysis System, 1996.
- Comstock GW, Burke AE, Hoffman SC, Norkus EP, Gross M, et al.: The repeatability of serum carotenoid, retinoid, and tocopherol concentrations in specimens of blood collected 15 years apart. *Cancer Epidemiol Biomarkers Prev* **10**, 65–68, 2001.
- Knekt P, Aromaa A, Maatela J, Aaran RK, Nikkari T, et al.: Serum vitamin A and subsequent risk of cancer: cancer incidence follow-up of the Finnish Mobile Clinic Health Examination Study. *Am J Epidemiol* **132**, 857–870, 1990.
- Ford ES: Variations in serum carotenoid concentrations among United States adults by ethnicity and sex. *Ethn Dis* **10**, 208–217, 2000.
- Dixon LB, Winkleby MA, and Radimer KL: Dietary intakes and serum nutrients differ between adults from food-insufficient and food-sufficient families: Third National Health and Nutrition Examination Survey, 1988–1994. *J Nutr* **131**, 1232–1246, 2001.
- Hayes RB, Bogdanovicz JFA, Schroeder FH, DeBrujin A, Raatgever JW, et al.: Serum retinol and prostate cancer. *Cancer* **62**, 2021–2026, 1988.
- Eichholzer M, Stahelin HB, Ludin E, and Bernasconi F: Smoking, plasma vitamins C, E, retinol, and carotene, and fatal prostate cancer: seventeen-year follow-up of the prospective Basel study. *Prostate* **38**, 189–198, 1999.
- DiMascio P, Kaiser S, and Sies H: Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys* **274**, 532–538, 1989.
- Williams AW, Boileau TW, Clinton SK, and Erdman JW Jr: Beta-carotene stability and uptake by prostate cancer cells are dependent on delivery vehicle. *Nutr Cancer* **36**, 185–190, 2000.
- Williams AW, Boileau TW, Zhou JR, Clinton SK, and Erdman JW Jr: Beta-carotene modulates human prostate cancer cell growth and may undergo intracellular metabolism to retinol. *J Nutr* **130**, 728–732, 2000.
- Haegele AD, Gillette C, O'Neill C, Wolfe P, Heimendinger J, et al.: Plasma xanthophyll carotenoids correlate inversely with indices of oxidative DNA damage and lipid peroxidation. *Cancer Epidemiol Biomarkers Prev* **9**, 421–425, 2000.
- Rock CL: Carotenoids: biology and treatment. *Pharmacol Ther* **75**, 185–197, 1997.
- Campbell DR, Gross MD, Martini MC, Grandits GA, Slavin JL, et al.: Plasma carotenoids as biomarkers of vegetable and fruit intake. *Cancer Epidemiol Biomarkers Prev* **3**, 493–500, 1994.
- Freeman VL, Meydani M, Yong S, Pyle J, Wan Y, et al.: Prostatic levels of tocopherols, carotenoids, and retinol in relation to plasma levels and self-reported usual dietary intake. *Am J Epidemiol* **151**, 109–118, 2000.
- Wu K, Schwartz SJ, Platz EA, Clinton SK, Erdman JW Jr, et al: Variations in plasma lycopene and specific isomers over time in a cohort of U.S. men. *J Nutr* **133**, 1930–1936, 2003.