Plasma and Dietary Carotenoids, and the Risk of Prostate Cancer: A Nested Case-Control Study

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Abstract

The association between plasma carotenoids and prostate cancer risk was investigated in a case-control study nested within the prospective Health Professionals Follow-up Study. We matched 450 incident prostate cancer cases diagnosed from 1993–1998 to 450 controls by age, time, month, and year of blood donation.

Modest inverse, but not statistically significant, associations were observed among plasma α-carotene, β-carotene, and lycopene concentrations, and overall risk of prostate cancer diagnosis {odds ratio (highest versus lowest quintile; OR), α-carotene: OR, 0.67 [95% confidence interval (CI), −0.40–1.09]; β-carotene: OR, 0.78 (95% CI, 0.48–1.25); lycopene: OR, 0.66 (95% CI, 0.38–1.13)}. The inverse association between plasma lycopene concentrations and prostate cancer risk was limited to participants who were 65 years or older (OR, 0.47; 95% CI, 0.23–0.98) and without a family history of prostate cancer (OR, 0.48; 95% CI, 0.26–0.89). Combining, older age and a negative family history provided similar results (OR, 0.43; 95% CI, 0.18–1.02). Inverse associations between β-carotene and prostate cancer risk were also found among younger participants (<65 years of age; OR, 0.36; 95% CI, 0.14–0.91; P_{trend} = 0.03). Combining dietary intake and plasma data confirmed our results.

We found a statistically significant inverse association between higher plasma lycopene concentrations and lower risk of prostate cancer, which was restricted to older participants and those without a family history of prostate cancer. This observation suggests that tomato products may exhibit more potent protection against sporadic prostate cancer rather than those with a stronger familial or hereditary component. In addition, our findings also suggest that among younger men, diets rich in β-carotene may also play a protective role in prostate carcinogenesis.

Introduction

Dietary factors are strongly implicated as critical risk factors for prostate cancer (1, 2). Epidemiological studies, clinical trials, and experimental studies suggest that oxidative stress may be related to the initiation and progression of prostate cancer (3–6). Thus, dietary carotenoids, which possess antioxidant properties, are hypothesized to be one component in the host defense against reactive oxygen that may potentially reduce the risk of prostate cancer (7). However, results from both observational studies and clinical trials are far from conclusive (4, 8–11). Most of the observational studies that have examined the relationship between carotenoids and risk of prostate cancer have been based on estimated dietary intake (reviewed in Ref. 8) rather than plasma or serum concentrations of carotenoids (12–18).

Earlier studies have measured lycopene and/or β-carotene concentrations (15–17). More recent studies have also included measurements of other carotenoids such as lutein, zeaxanthin, and β-cryptoxanthin (12–14). Findings from the blood-based studies have been inconsistent, possibly because of low statistical power of many small studies and reliance on a single blood measurement of carotenoids as a biomarker of long-term exposure (12, 14–18).

We investigated the associations between plasma levels of the carotenoids lutein/zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, and lycopene, and risk of prostate cancer in the Health Professionals Follow-up Study (HPFS). Two studies published recently (19, 20) have found inverse associations between tomato sauce intake (19) and intake of cooked tomatoes (20) to be stronger among older participants, suggesting that some carotenoids may influence primarily sporadic cases that are largely attributed primarily to environmental influences. Men developing prostate cancer at a younger age may have a stronger contribution from familial and hereditary factors, and may be less sensitive to certain dietary factors (21, 22). Therefore, we also investigated associations between carotenoid intake and prostate cancer risk after stratification by age.

Because dietary intake data on carotenoids were available for this cohort, dietary intake data and plasma levels were also combined to better assess carotenoid exposure (23–25).

Materials and Methods

HPFS. The HPFS was started in 1986 when 51,529 male health professionals, between 40 and 75 years of age responded

to a questionnaire on medical history and lifestyle habits (26), and a 131-item semiquantitative food frequency questionnaire. Participants who were diagnosed with cancer before 1986, those without a completed food frequency questionnaire at baseline, those reporting very high (>4200 calories) or very low (<800 calories) energy intake, or those who had left >70 items blank on the food frequency questionnaire were excluded from the baseline cohort. Follow-up questionnaires were mailed every 2 years (*i.e.,* in 1988, 1990, 1992, 1994, 1996, and 1998), and every 4 years a food frequency questionnaire was also included in the mailings (*i.e.,* in 1990 and 1994). This study was approved by the Harvard School of Public Health Human Subjects Committee.

Between 1993 and 1995 surviving participants of the baseline cohort were asked to provide a blood sample; a blood collection kit was then mailed to those participants who had agreed. A total of 18,259 participants returned EDTA preserved blood samples, chilled in ice, using a prepaid overnight courier. Upon arrival in our laboratory, samples were centrifuged into white cell-enriched buffy coats, plasma, and RBCs, and subsequently kept frozen in liquid nitrogen at temperatures ranging from $-196°C$ to $-130°C$.

Selection of Cases and Controls. Participants were asked on each follow-up questionnaire whether they had been diagnosed with prostate cancer in the prior 2 years. Whenever a diagnosis of prostate cancer was reported either on the follow-up questionnaire or on the death certificate (if the participant had died during the follow-up), permission to review medical records was sought from the participant or the surviving next of kin. Medical records were reviewed by physicians blinded to the participant questionnaire information. For this study we used nonstage A1 incident prostate cancer as our main end point. This analysis included two separate nested case-control sets nested within the HPFS blood cohort. The first set included 203 incident prostate cancer cases diagnosed after initiation of blood collection between 1993 and 1997, and the second set included 257 incident prostate cancer diagnoses subsequently identified through January 31, 1998. All of the cases and controls were selected from the existing HPFS blood cohort. Each case was only considered a case for this study if the diagnosis of prostate cancer occurred after the date of blood donation. One case was matched to one control by age (± 1 year of age), time of blood donation (*i.e.,* midnight to before 9 a.m., 9 a.m. before noon, noon to before 4 p.m., and 4 p.m. before midnight), same season of the year of blood donation (*i.e.,* winter: January-March, spring: April-June, summer: July-September, and autumn: October-December), and same year of blood donation. In addition to this, cases and controls were also matched to whether or not they had a PSA (prostate specific antigen) test before blood draw. Controls had to be alive and free of cancer at the time the matched case was diagnosed with prostate cancer. All of the controls had a negative PSA test after blood donation (because the majority of prostate cancer cases in this time period were diagnosed through the PSA test). Four controls set who became cases after completion of the assays within the first set were included as cases in the second follow-up. Because of missing carotenoid measurements, 10 case-control sets from the second follow-up set were excluded from the final analysis resulting in 247 case-control pairs for the second follow-up set.

Assessment of Nutrient Intake. On the food frequency questionnaire, participants were asked to report the average frequency of eating specific foods and beverages during the past year (27). Nine possible answers were offered ranging from never or less than one serving per month to six or more servings per day. Nutrient intakes were then calculated by multiplying the frequency of consumption of a certain food or beverage item by the nutrient content of that serving, and then contributions from all food and beverage items were summed. Energy adjustment of nutrient intakes was performed using the residuals method (28). Estimated carotenoid intake corresponding to the time of blood sampling *i.e.,* between 1993 and 1995 was evaluated based on the 1994 food frequency questionnaire (nonupdated nutrient intake). Long-term carotenoid intake was evaluated by averaging nutrient intakes from the 1986, 1990, and 1994 food frequency questionnaires (cumulative updated intake; Ref. 29). Results from a study on the correlation between plasma and dietary carotenoids on a subsample in the HPFS have been published by our group earlier (30). Adjusted correlations between estimated dietary carotenoid intake and plasma carotenoids in 110 nonsmoking men were 0.40 for lutein, 0.43 for β-cryptoxanthin, 0.47 for α-carotene, 0.35 for β-carotene, and 0.47 for lycopene.

Assessment of Plasma Carotenoid Concentrations. All of the carotenoid analyses were performed at the laboratory of J. W. E. Analyses were conducted under yellow lights. Plasma was stored at $-80°C$, thawed on ice, allowed to stand at controlled room temperature for 30 min, sonicated for 5 min at controlled room temperature, returned to ice bath, and maintained on ice whenever possible throughout the procedure. Butylated hydroxytoluene (Sigma Chemical Company, St. Louis, MO) and echinenone internal standard (a gift from F. Hoffmann-La Roche Ltd., Basel, Switzerland) were added to duplicate portions. Carotenoids were extracted with hexane (Fisher Scientific, Fair Lawn, NJ) and preparations dried under vacuum. Extracts were reconstituted in methyl-tert-butyl ether (Fisher Scientific) and analyzed by reverse-phase high performance liquid chromatography using the method of Yeum *et al.* (31). The chromatographic conditions were described previously (32). The laboratory participates quarterly in the National Institutes of Standards and Technology micronutrient measurement proficiency testing program.

Mean intrapair coefficients of variation were calculated based on 6 paired quality control samples from pooled plasma for the first set and on 8 paired quality control samples from pooled plasma for the second set. In the first set, the mean intrapair coefficients of variation were 8.1% for lutein, 4.6% for β-cryptoxanthin, 1.3% for α-carotene, 4.7% for β-carotene, and 5.2% for lycopene; in the second set, the mean intrapair coefficients of variation were 10.1% for lutein, 7.2% for β-cryptoxanthin, 10.9% for α-carotene, 4.7% for β-carotene, and 11.9% for lycopene.

Cholesterol measurements were performed at the laboratory of S. K. C. using an Infinity Total Cholesterol enzymatic assay kit (Sigma Diagnostics, St. Louis, MO) following the manufacturer's recommendations. Control plasma samples were included in each assay to verify that interassay variations were <5%.

Statistical Analysis. Because plasma carotenoid concentrations were right skewed, we tested for differences in carotenoid concentrations between cases and controls by using the nonparametric Wilcoxon signed rank test. Conditional logistic regression models were run to assess the association between quintiles of carotenoid concentrations and risk of total prostate cancer. For the multivariate analysis we included known and suspected risk factors for prostate cancer in the models [family history of prostate cancer, history of vasectomy, body mass index (in quintiles), height (in quintiles), vigorous exercise (in

quintiles), current smoking (yes *versus* no), vitamin E supplementation (yes *versus* no), and selenium supplementation (yes *versus* no)]. We also included plasma cholesterol concentrations (in quintiles) in all of the multivariate models to control for the variation in plasma carotenoids, because they are transported in the plasma via lipoproteins (33, 34). Possible confounding with multivitamin and β-carotene supplementation, alcohol intake, intakes of fruits and vegetables, red meat, α-linoleic acid, calcium, and vitamin D were assessed by adding those nutrients separately to the multivariate models. Because results were similar after adding those nutrients individually, they were not included in the final multivariate models. Trend tests were performed by using the median of each quintile of carotenoid concentrations as exposure scores.

When two response variables are obtainable to assess exposure, combining exposure information from those two sources may reduce misclassification of exposure (23–25). Therefore, we also analyzed data after combining dietary and plasma data. We calculated dietary/plasma scores by summing deciles of dietary intake of carotenoids and deciles of plasma levels of carotenoids. For example, a participant in decile 1 of dietary carotenoid intake and decile 1 of plasma carotenoid level would get a score of 2, and a participant in decile 5 of dietary carotenoid intake and decile 10 of plasma level would get a score of 15. Then quintiles were calculated based on the sum and the association between quintiles of dietary/plasma scores, and risk of prostate cancer was assessed. We calculated two different types of dietary/plasma scores based on the dietary carotenoid data used (*i.e.,* nonupdated dietary intake and cumulative updated dietary intake). Trend tests were performed by including the dietary/plasma score (*i.e.,* 2–20) as a continuous variable in the conditional regression models.

Two studies published recently (19, 20) have found inverse associations between tomato sauce intake (19) and intake of cooked tomatoes (20) to be stronger among older participants. Therefore, associations between carotenoid intake and prostate cancer risk were also analyzed after stratification by age. The stronger inverse associations in older men suggests that diets rich in certain carotenoids may have greater benefits for sporadic cases in contrast with those with a strong genetic component typically observed in younger men (21, 22). Thus, we conducted additional analyses excluding men with a positive family history of prostate cancer (first-degree relative). Plasma carotenoid concentrations tended to be lower in older men; therefore, strataspecific quintiles (*i.e.,* quintiles calculated according to the distribution in each specific strata) were used to investigate the association between the carotenoids and prostate cancer by age and family history. We did not investigate associations within the subgroup of men with a positive family history of prostate cancer because of limited sample size (*n* = 117). *P*s for interactions between carotenoid concentrations and family history were calculated for older participants. A cross-product consisting of carotenoid levels (as a continuous variable) and family history (yes/no) was included in the multivariate models. *P*s were calculated by assessing the differences in log-likelihood statistics between the multivariate model containing the interaction term and a model without the interaction term.

Results

With the exception of 2 case-control sets, which had donated blood 1 year apart, all of the case-control sets were matched to the same year of blood donation. Of case-control sets, 95% were matched within the same season of blood donation, and

87% were matched within the same category of time of blood donation. Of case-control sets, 95% were matched within 1 year of age (*i.e.,* ≤1 year), and the remaining 5% were matched within 3 years of age (*i.e.,* 1.1–2.8 years). The baseline characteristics for the combined study population by case-control status are shown in Table 1. There were no appreciable differences between cases and controls with regard to body mass index, smoking status, or the matching factors month of blood

Table 1   Baseline characteristics[a] of combined study population by case-control status, Health Professionals Follow-up Study 1993–1994

	Cases (*n* = 450)	Controls (*n* = 450)
Age group		
≥55 yrs	42 (9.3%)	47 (10.4%)
56–65 yrs	123 (27.3%)	119 (26.4%)
>=66 yrs	285 (63.3%)	284 (63.1%)
Family history of prostate cancer		
No	384 (85.5%)	398 (88.4%)
Yes	65 (14.5%)	52 (11.6%)
Body mass index (kg/m^2)		
≥25	173 (38.4%)	172 (38.2%)
>25	277 (61.6%)	278 (61.2%)
Smoking status		
Nonsmoker	424 (94.6%)	439 (97.8%)
Current smoker	24 (5.4%)	10 (2.2%)
Race		
Caucasian	424 (99.1%)	421 (99.5%)
African-American	2 (0.5%)	1 (0.2%)
Asian	1 (0.2%)	1 (0.2%)
Month of donation		
January-March	66 (14.7%)	69 (15.3%)
April-June	112 (24.9%)	108 (24.0%)
July-September	115 (33.6%)	153 (34.0%)
October-December	121 (26.9%)	120 (26.7%)
Hours since last meal		
1–2 h	51 (12.3%)	61 (14.8%)
3–4 h	51 (12.3%)	41 (9.9%)
5–6 h	23 (5.5%)	16 (3.9%)
>=7 h	290 (69.9%)	295 (71.43%)
Time of donation		
12 a.m.–8 a.m.	194 (43.1%)	188 (41.2%)
9 a.m.–11 a.m.	199 (44.2%)	198 (44.0%)
12 p.m.–3 p.m.	41 (9.1%)	49 (10.9%)
4 p.m.–11 p.m.	16 (3.6%)	15 (3.3%)
Fruit intake		
0–2 servings/day	154 (34.2%)	151 (33.6%)
2.1–4 servings/day	215 (47.8%)	214 (47.6%)
>4 servings/day	67 (14.9%)	68 (15.1%)
Vegetable intake		
0–2 servings/day	70 (15.6%)	60 (13.3%)
2.1–4 servings/day	193 (42.9%)	177 (39.3%)
>4 servings/day	173 (38.4%)	196 (43.6%)
Multivitamin supplementation		
No	189 (42.0%)	214 (44.6%)
Yes	237 (52.7%)	205 (45.6%)
Vitamin E supplementation		
No	244 (54.2%)	253 (56.2%)
Yes	166 (36.9%)	145 (32.2%)
Selenium supplementation		
No	352 (78.2%)	352 (78.2%)
Yes	33 (7.3%)	35 (7.8%)
β-Carotene supplementation		
No	315 (70.0%)	321 (71.3%)
Yes	108 (24.0%)	94 (20.9%)

[a] Information on baseline characteristics was obtained from 1994 questionnaires; not all numbers add up to the total or 450 cases and 450 controls because there are missing values for some variables from the 1994 questionnaire.

Table 2 Median plasma carotenoid concentrations and Wilcoxon signed rank test by time of study and in combined study population, Health Professionals Follow-up Study

Carotenoids (nmol/l)	1996 Set			1998 Set			Combined study population		
	Cases	Controls	P^a	Cases	Controls	P^a	Cases	Controls	P^a
Total carotene	1553.2	1556.0	0.95	1598.3	1719.1	0.17	1582.7	1625.5	0.28
Lutein/zeaxanthin	228.7	232.8	0.64	193.7	199.8	0.17	209.9	217.7	0.20
β-Cryptoxanthin	94.3	89.4	0.91	127.8	131.7	0.44	114.1	116.3	0.51
α-Carotene	114.2	110.6	0.67	97.6	100.1	0.10	105.3	107.0	0.29
β-Carotene	339.0	354.2	0.99	350.2	343.8	0.36	343.7	351.2	0.47
Lycopene	658.4	696.0	0.60	739.5	757.2	0.38	698.3	726.0	0.31

[a] Ps were calculated using Wilcoxon signed rank test.

donation and hours since last meal. Fruit intake and supplementation with selenium were also similar between cases and controls. Cases tended to have lower vegetable intake and were more likely to take multivitamin, vitamin E, and β-carotene supplements when compared with controls.

Table 2 shows the median plasma carotenoid concentrations by time of study and in the combined study population.

Cases tended to have lower concentrations for the measured carotenoids, but none of the differences was statistically significant.

Table 3 shows odds ratios (ORs) and 95% confidence intervals (CIs) of prostate cancer by quintiles of carotenoid plasma concentrations in the total study population and by age at time of blood donation using conditional logistic regression

Table 3 Odds ratios (OR) and 95% confidence interval (CI) of prostate cancer by quintiles of carotenoid plasma concentrations in the total study population and by age at time of donation using conditional logistic regression, Health Professionals Follow-up Study

	Total study population			Age at blood donation <65 years			Age at blood donation ≥65 years		
	Cases/controls	OR^a (95% CI)	OR^b (95% CI)	Cases/controls	OR^a (95% CI)	OR^b (95% CI)	Cases/controls	OR^a (95% CI)^a	OR^b (95% CI)
Lutein/zeaxanthin									
Q1	86/94	1.00	1.00	30/36	1.00	1.00	58/56	1.00	1.00
Q2	96/84	1.36	1.37 (0.82–2.27)	34/32	1.39	1.20 (0.46–3.12)	62/52	1.15	1.26 (0.64–2.47)
Q3	91/89	1.16	1.16 (0.70–1.93)	33/33	1.26	1.08 (0.45–2.62)	56/58	0.88	0.89 (0.44–1.80)
Q4	103/77	1.58	1.73 (1.02–2.95)	39/27	1.90	2.10 (0.83–5.31)	63/51	1.13	1.24 (0.61–2.54)
Q5	74/106	0.81	0.83 (0.49–1.40)	29/37	1.00	1.09 (0.43–2.73)	46/68	0.62	0.59 (0.29–1.20)
		$P_{trend} = 0.16$	$P_{trend} = 0.27$		$P_{trend} = 0.89$	$P_{trend} = 0.71$		$P_{trend} = 0.07$	$P_{trend} = 0.07$
β-Cryptoxanthin									
Q1	98/82	1.00	1.00	35/31	1.00	1.00	62/52	1.00	1.00
Q2	79/101	0.67	0.71 (0.46–1.10)	30/36	0.79	0.97 (0.43–2.18)	48/66	0.62	0.68 (0.39–1.20)
Q3	94/86	0.92	0.85 (0.53–1.37)	36/30	1.13	1.18 (0.47–2.95)	60/54	0.92	0.85 (0.47–1.54)
Q4	86/94	0.80	0.81 (0.51–1.29)	28/38	0.66	0.56 (0.22–1.43)	59/55	0.92	0.92 (0.52–1.63)
Q5	93/87	0.92	0.94 (0.56–1.58)	36/30	1.16	1.67 (0.58–4.76)	56/58	0.83	0.85 (0.45–1.61)
		$P_{trend} = 0.96$	$P_{trend} = 0.94$		$P_{trend} = 0.86$	$P_{trend} = 0.63$		$P_{trend} = 0.89$	$P_{trend} = 0.99$
α-Carotene									
Q1	99/81	1.00	1.00	38/28	1.00	1.00	60/54	1.00	1.00
Q2	83/97	0.68	0.62 (0.38–0.99)	26/40	0.46	0.52 (0.22–1.24)	58/56	0.92	0.82 (0.46–1.48)
Q3	87/93	0.74	0.69 (0.43–1.10)	34/31	0.73	0.62 (0.25–1.54)	53/61	0.79	0.78 (0.44–1.39)
Q4	96/84	0.91	0.89 (0.54–1.47)	36/31	0.81	0.76 (0.31–1.85)	58/56	0.92	0.95 (0.50–1.78)
Q5	85/95	0.71	0.67 (0.40–1.09)	31/35	0.62	0.61 (0.25–1.47)	56/58	0.86	0.80 (0.41–1.54)
		$P_{trend} = 0.38$	$P_{trend} = 0.39$		$P_{trend} = 0.56$	$P_{trend} = 0.52$		$P_{trend} = 0.67$	$P_{trend} = 0.65$
β-Carotene									
Q1	96/84	1.00	1.00	38/28	1.00	1.00	58/56	1.00	1.00
Q2	91/89	0.89	0.86 (0.55–1.35)	37/29	0.89	0.70 (0.30–1.63)	54/60	0.88	0.93 (0.52–1.65)
Q3	81/99	0.72	0.69 (0.45–1.08)	30/36	0.57	0.53 (0.21–1.33)	52/62	0.83	0.88 (0.50–1.54)
Q4	92/88	0.91	0.92 (0.57–1.47)	31/35	0.62	0.56 (0.23–1.36)	62/52	1.20	1.39 (0.73–2.62)
Q5	90/90	0.87	0.78 (0.48–1.25)	29/37	0.54	0.36 (0.14–0.91)	59/55	1.07	0.99 (0.53–1.85)
		$P_{trend} = 0.71$	$P_{trend} = 0.49$		$P_{trend} = 0.08$	$P_{trend} = 0.03$		$P_{trend} = 0.49$	$P_{trend} = 0.69$
Lycopene									
Q1	101/79	1.00	1.00	32/34	1.00	1.00	64/50	1.00	1.00
Q2	88/92	0.70	0.72 (0.45–1.15)	37/29	1.31	1.08 (0.46–2.55)	57/57	0.72	0.67 (0.36–1.25)
Q3	82/98	0.59	0.56 (0.34–0.93)	28/38	0.81	0.60 (0.24–1.54)	57/57	0.69	0.64 (0.33–1.27)
Q4	91/89	0.74	0.73 (0.44–1.19)	35/31	1.23	0.93 (0.36–2.39)	54/60	0.63	0.64 (0.34–1.23)
Q5	88/92	0.66	0.66 (0.38–1.13)	33/33	1.07	1.17 (0.47–2.88)	53/61	0.57	0.47 (0.23–0.98)
		$P_{trend} = 0.26$	$P_{trend} = 0.33$		$P_{trend} = 0.95$	$P_{trend} = 0.59$		$P_{trend} = 0.09$	$P_{trend} = 0.07$

[a] Adjusted for matching variables only using conditional logistic regression.
[b] Adjusted for matching variables plus cholesterol levels (in quintiles), selenium supplementation, vitamin E supplementation, family history of prostate cancer, body mass index, height, vigorous exercise, history of vasectomy and current smoking using conditional logistic regression; 3 case-controls sets were excluded from the multivariate analysis because of missing information on at least one of the covariates. For each strata strataspecific quintiles were used.

models. ORs adjusted for the matching variables only, and those adjusted for other known and suspected risk factors for prostate cancer and cholesterol levels were similar. In the total population, no evidence for a protective association between plasma concentrations of lutein/zeaxanthin and β-cryptoxanthin, and overall risk of prostate cancer was found (highest *versus* lowest quintile: lutein/zeaxanthin: OR, 0.83; 95% CI, 0.49–1.40; β-cryptoxanthin: OR, 0.94; 95% CI, 0.56–1.58). Modest inverse, but not statistically significant, associations were observed among plasma α-carotene, β-carotene, and lycopene concentrations, and overall risk of prostate cancer diagnosis (highest *versus* lowest quintile: α-carotene: OR, 0.67; 95% CI-0.40–1.09; β-carotene: OR, 0.78; 95% CI, 0.48–1.25; lycopene: OR, 0.66; 95% CI, 0.38–1.13). Among participants who donated blood at a younger age, *i.e.,* <65 years of age, an inverse association between higher concentrations of β-carotene and lower risk of prostate cancer was observed (OR, 0.36; 95% CI, 0.14–0.91). For the other carotenoids no evidence for an inverse association between higher concentrations of carotenoids and risk of prostate cancer were found among younger participants. Statistically significant inverse associations between higher concentrations of lycopene and prostate cancer risk were found among participants who were 65 years or older at time of blood donation (highest *versus* lowest quintile: lycopene: OR, 0.47; 95% CI, 0.23–0.98). Among older participants, results were also suggestive of an inverse association between higher lutein/zeaxanthin levels and prostate cancer risk (OR, 0.59; 95% CI, 0.29–1.20), but the confidence interval included one. Adding intakes of calcium, vitamin D, α-linoleic acid, red meat, fruits, and vegetables, as well as multivitamin supplementation separately into the multivariate models did not change the overall results, although in some instances confidence intervals became wider (data not shown). When analysis was stratified by age at time of diagnosis of prostate cancer (rather than age at blood donation), results were similar to those presented in Table 3.

The stronger inverse associations in older men supports a hypothesis that certain carotenoid-rich foods may act to prevent sporadic prostate cancer (21, 22). Thus, we conducted additional analyses excluding men with a positive family history of prostate cancer among first-degree relatives. Table 4 shows ORs and 95% CI of prostate cancer by quintiles of plasma carotenoid concentrations in the total study population and by age at time of donation after excluding participants with a positive family history of prostate cancer. After excluding participants with a family history of prostate cancer, inverse associations between higher lycopene concentrations and risk of prostate cancer were also limited to older participants (highest *versus* lowest quintile: OR, 0.43; 95% CI, 0.18–1.02). Among participants who donated blood at a younger age, *i.e.,* <65 years of age, and did not have a family history of prostate cancer, findings were suggestive of an inverse association between higher levels of β-carotene and risk of prostate cancer, but the CIs included one. *P*s for interaction between carotenoid concentrations and family history were calculated for older participants. Among older participants no statistically significant interaction between the measured carotenoids and family history was found (lycopene: $P = 0.15$; all other carotenoids, $P > 0.70$).

To address the possibility of residual confounding due to smoking [current smoking (yes/no) was included in all of the final multivariate models], analyses were also performed after excluding all of the current smokers. Among older participants without a family history of prostate cancer and who had never

smoked, observed associations between carotenoids and risk of prostate cancer were similar to those observed in Table 4.

Estimated dietary carotenoid consumption and plasma concentrations were only moderately correlated among controls (Spearman correlation coefficient for 1994 dietary intake *versus* plasma levels: α-carotene, $r = 0.27$; β-carotene, $r = 0.26$; β-crytoxanthin, $r = 0.30$; lycopene, $r = 0.26$; lutein/zeaxanthin, $r = 0.16$; all *P*s <0.0001; correlations were similar for the average 1986, 1990, and 1994 carotenoid intake). Table 5 shows the risk of prostate cancer according to combinations of 1994 dietary intake (which is the dietary assessment closest to the time of blood donation) and plasma concentrations of carotenoids using quintiles of the combined dietary and plasma scores by age at donation among participants without a family history of prostate cancer. Younger participants in the highest quintile of dietary/plasma scores of β-carotene appeared to have a 77% lower risk of prostate cancer when compared with those in the lowest quintile, but the confidence interval was wide due to limited sample size (OR, 0.27; 95% CI, 0.08–0.97; $P_{trend} = 0.15$). Among older participants in the highest quintile of dietary/plasma scores of lycopene, a 60% decreased risk of prostate cancer was found when compared with those in the lowest quintile (OR, 0.40; 95% CI, 0.19–0.88; $P_{trend} = 0.05$). When associations were investigated using dietary/plasma scores based on 1986, 1990, and 1994 average dietary intake, results for lycopene were similar (for age $\geq$65 years: highest quintile *versus* lowest quintile OR, 0.46; 95% CI, 0.20–1.03; $P_{trend} = 0.05$). However, it appeared that the inverse associations observed between higher lycopene dietary/plasma scores and risk of prostate cancer were strongest among participants who were both $\geq$65 years of age and had no family history of prostate cancer. Substantially weaker and statistically nonsignificant inverse associations between lycopene dietary/plasma scores and prostate cancer were found among all of the older participants (highest quintile *versus* lowest quintile OR , 0.61; 95% CI, 0.32–1.19; $P_{trend} = 0.21$) and all of the participants without a family history of prostate cancer (highest quintile *versus* lowest quintile OR, 0.73; 95% CI, 0.42–1.28; $P_{trend} = 0.25$).

Discussion

The majority of epidemiological studies investigating the association among carotenoids, carotenoid-rich foods, and risk of prostate cancer have focused on the relationship between estimated dietary intake of carotenoids and risk of prostate cancer (reviewed in Ref. 8). Only a limited number of studies, of various statistical and analytic power, have investigated the association between carotenoid serum concentrations and risk of prostate cancer (12–18, 35). The strengths of this present study include its prospective design, its large sample size, and its ability to combine dietary and plasma data, which may have decreased the possibility of misclassification of exposure, and consequently strengthens our confidence in these results. Our findings suggest an inverse association between higher plasma lycopene concentrations and risk of prostate cancer, particularly among participants who were 65 years or older at time of blood donation and those without a family history of prostate cancer. Combining dietary and plasma data confirmed these results. Our study also identified an inverse association between higher β-carotene concentrations and risk of prostate cancer risk among younger participants.

β-Carotene. Several case-control studies found evidence for a statistically significant inverse association between higher intakes of β-carotene and prostate cancer risk (36–39). However,

Table 4 Odds ratios (OR) and 95% confidence interval (CI) of prostate cancer by quintiles of carotenoid plasma concentrations in the total study population and by age at time of donation after exclusion of participants with family history of prostate cancer using conditional logistic regression, Health Professionals Follow-up Study

	Total study population			Age at blood donation <65 years			Age at blood donation ≥65 years		
	Cases/controls	OR[a] (95% CI)	OR[b] (95% CI)	Cases/controls	OR[a] (95% CI)	OR[b] (95% CI)	Cases/controls	OR[a] (95% CI)	OR[b] (95% CI)
Lutein/zeaxanthin									
Q1	67/69	1.00	1.00	25/25	1.00	1.00	44/42	1.00	1.00
Q2	74/63	1.28	1.31 (0.74–2.34)	24/26	0.97	1.17 (0.37–3.67)	47/39	1.15	1.26 (0.59–2.72)
Q3	65/72	0.93	0.97 (0.55–1.74)	24/27	0.91	0.95 (0.35–2.57)	42/45	0.80	0.79 (0.34–1.85)
Q4	77/60	1.37	1.57 (0.86–2.86)	30/20	1.59	2.09 (0.68–6.45)	47/39	1.09	1.32 (0.58–3.01)
Q5	59/78	0.79	0.86 (0.48–1.53)	23/28	0.87	1.07 (0.37–3.08)	36/51	0.61	0.61 (0.27–1.36)
		$P_{trend} = 0.27$	$P_{trend} = 0.54$		$P_{trend} = 0.89$	$P_{trend} = 0.84$		$P_{trend} = 0.13$	$P_{trend} = 0.20$
β-Cryptoxanthin									
Q1	79/57	1.00	1.00	29/21	1.00	1.00	47/39	1.00	1.00
Q2	58/79	0.53	0.55 (0.33–0.93)	22/28	0.57	0.86 (0.31–2.36)	39/47	0.71	0.69 (0.37–1.31)
Q3	70/67	0.72	0.66 (0.38–1.16)	25/26	0.66	0.64 (0.21–1.91)	44/43	0.83	0.71 (0.36–1.41)
Q4	68/69	0.71	0.72 (0.42–1.25)	23/27	0.62	0.68 (0.22–2.05)	45/41	0.90	0.88 (0.45–1.73)
Q5	67/70	0.69	0.68 (0.38–1.20)	27/24	0.80	1.08 (0.34–3.46)	41/46	0.74	0.74 (0.36–1.52)
		$P_{trend} = 0.39$	$P_{trend} = 0.44$		$P_{trend} = 0.82$	$P_{trend} = 0.86$		$P_{trend} = 0.55$	$P_{trend} = 0.70$
α-Carotene									
Q1	75/61	1.00	1.00	28/22	1.00	1.00	45/41	1.00	1.00
Q2	68/69	0.78	0.71 (0.41–1.23)	23/27	0.64	0.76 (0.27–2.15)	46/40	1.05	0.92 (0.45–1.87)
Q3	63/74	0.68	0.65 (0.38–1.12)	24/27	0.66	0.50 (0.16–1.54)	41/46	0.82	0.81 (0.42–1.58)
Q4	73/64	0.90	0.86 (0.48–1.54)	28/22	1.02	1.11 (0.39–3.16)	43/43	0.88	0.87 (0.41–1.83)
Q5	63/74	0.68	0.63 (0.36–1.12)	23/28	0.64	0.52 (0.18–1.52)	41/46	0.79	0.74 (0.34–1.59)
		$P_{trend} = 0.23$	$P_{trend} = 0.23$		$P_{trend} = 0.51$	$P_{trend} = 0.38$		$P_{trend} = 0.40$	$P_{trend} = 0.44$
β-Carotene									
Q1	70/66	1.00	1.00	26/24	1.00	1.00	45/41	1.00	1.00
Q2	76/61	1.18	1.23 (0.72–2.11)	31/19	1.50	2.58 (0.77–8.63)	41/45	0.84	0.87 (0.45–1.70)
Q3	61/76	0.77	0.76 (0.45–1.27)	25/26	0.82	1.13 (0.34–3.77)	41/46	0.84	0.82 (0.44–1.56)
Q4	70/67	0.98	0.97 (0.57–1.66)	25/25	0.89	1.38 (0.44–4.32)	44/42	0.97	1.05 (0.52–2.13)
Q5	65/72	0.84	0.77 (0.43–1.36)	19/32	0.51	0.42 (0.13–1.32)	45/42	1.00	0.97 (0.46–2.02)
		$P_{trend} = 0.35$	$P_{trend} = 0.20$		$P_{trend} = 0.04$	$P_{trend} = 0.03$		$P_{trend} = 0.78$	$P_{trend} = 0.85$
Lycopene									
Q1	80/56	1.00	1.00	28/22	1.00	1.00	49/37	1.00	1.00
Q2	67/70	0.63	0.63 (0.37–1.07)	24/26	0.70	0.50 (0.17–1.44)	47/39	0.83	0.75 (0.37–1.54)
Q3	64/73	0.54	0.51 (0.29–0.92)	23/28	0.63	0.40 (0.13–1.24)	43/44	0.62	0.58 (0.27–1.27)
Q4	70/67	0.67	0.66 (0.37–1.15)	28/22	0.95	0.73 (0.24–2.21)	38/48	0.51	0.48 (0.22–1.02)
Q5	61/76	0.47	0.48 (0.26–0.89)	23/28	0.65	0.66 (0.23–1.94)	39/48	0.46	0.43 (0.18–1.02)
		$P_{trend} = 0.03$	$P_{trend} = 0.06$		$P_{trend} = 0.50$	$P_{trend} = 1.00$		$P_{trend} = 0.02$	$P_{trend} = 0.03$

[a] Adjusted for matching variables only using conditional logistic regression.
[b] Adjusted for matching variables plus cholesterol levels (in quintiles), selenium supplementation, vitamin E supplementation, family history of prostate cancer, body mass index, height, vigorous exercise, history of vasectomy and current smoking using conditional logistic regression; 3 case-controls sets were excluded from the multivariate analysis because of missing information on at least one of the covariates. For each strata strataspecific quintiles were used.

in other case-control studies β-carotene intake was not associated with prostate cancer risk (40–46). Results from two prospective studies, one in the Western Electric Study cohort (47) and another from a previous published report on this cohort (HPFS; Ref. 48) also observed no association between β-carotene intake and subsequent risk of prostate cancer. Randomized clinical trials evaluating β-carotene supplementation and risk of various disease outcomes have not consistently reported a change in prostate cancer risk (4, 10). In fact, in the α-Tocopherol, β-Carotene Cancer Prevention Study, men who were supplemented with 20 mg of β-carotene seemed to be at slightly higher risk of developing prostate cancer, although those findings were not statistically significant (4). In the Physicians' Health Study (10) supplementation with β-carotene seemed to be associated with lower risk of prostate cancer but only in men in the lowest quartile of baseline plasma β-carotene levels. Serum-based studies also do not support an inverse association between β-carotene concentrations and risk of prostate cancer. In a small hospital based case-control study by Hayes *et al.* (16), no association between β-carotene levels and

risk of prostate cancer was found. A case-control study nested within the Washington County cohort (17) also did not support an association between serum levels of β-carotene and risk of prostate cancer. In a later study from the Washington County cohort blood concentrations of total carotene, β-carotene, and α-carotene were not associated with risk of prostate cancer (18). In the prospective Basel Study no association between carotene levels and prostate cancer mortality ($n = 30$) was observed after 17 years of follow-up (15). Results from a recently published multicenter case-control study on 209 cases and 228 controls even suggest an increased risk of prostate cancer with higher concentrations of β-carotene, but results were not statistically significant (highest *versus* lowest quartile: OR, 1.64; $P = 0.22$; Ref. 35).

Overall, most studies do not detect an inverse association between β-carotene and risk of prostate cancer. In our study higher β-carotene plasma levels were associated with decreased risk of prostate cancer only among younger men. However, only a few observational studies have investigated associations

Table 5 Multivariate risk of prostate cancer according to combinations of dietary intake in 1994 and plasma levels (using quintiles of summary scores) of carotenoids by age at donation among participants without family history of prostate cancer

	Lutein/zeaxanthin		β-Cryptoxanthin		α-Carotene		β-Carotene		Lycopene	
	Cases/controls	OR[a] (95% CI)	Cases/controls	OR[a] (95% CI)	Cases/controls	OR[a] (95% CI)	Cases/controls	OR[a] (95% CI)	Cases/controls	OR[a] (95% CI)
Age <65 years										
Quintiles of dietary/plasma scores[b]										
Q1	25/20	1.00	26/25	1.00	22/21	1.00	24/17	1.00	21/20	1.00
Q2	26/23	0.63 (0.22–1.77)	31/26	1.32 (0.46–3.82)	25/26	1.01 (0.35–2.94)	25/28	0.29 (0.09–0.95)	29/22	1.33 (0.46–3.87)
Q3	24/30	0.48 (0.18–1.26)	17/22	0.82 (0.25–2.67)	30/26	1.28 (0.48–3.42)	25/23	0.55 (0.17–1.77)	23/31	1.21 (0.42–3.49)
Q4	14/20	0.55 (0.18–1.68)	22/25	1.21 (0.38–3.80)	24/23	1.11 (0.34–3.61)	29/26	0.56 (0.19–1.63)	21/26	0.45 (0.14–1.49)
Q5	31/27	0.89 (0.31–2.54)	24/22	1.63 (0.41–6.45)	19/24	0.54 (0.20–1.47)	17/26	0.27 (0.08–0.97)	26/21	1.96 (0.67–5.72)
		$P_{trend} = 0.47$		$P_{trend} = 0.78$		$P_{trend} = 0.47$		$P_{trend} = 0.15$		$P_{trend} = 0.83$
Age ≥65 years										
Quintiles of dietary/plasma scores[b]										
Q1	42/32	1.00	40/38	1.00	44/34	1.00	43/35	1.00	44/31	1.00
Q2	46/46	0.71 (0.33–1.51)	39/34	0.96 (0.46–2.01)	42/43	0.68 (0.33–1.39)	42/42	0.77 (0.38–1.60)	35/37	0.70 (0.31–1.56)
Q3	40/42	0.77 (0.35–1.69)	45/44	0.88 (0.43–1.81)	43/42	0.86 (0.43–1.72)	40/51	0.74 (0.37–1.45)	52/49	0.65 (0.32–1.31)
Q4	37/47	0.44 (0.20–1.00)	37/50	0.63 (0.31–1.28)	32/37	0.54 (0.25–1.20)	31/45	0.57 (0.27–1.21)	40/42	0.57 (0.28–1.17)
Q5	37/35	0.68 (0.30–1.59)	41/36	1.11 (0.52–2.38)	41/46	0.61 (0.29–1.30)	46/29	1.41 (0.59–3.37)	31/43	0.40 (0.19–0.88)
		$P_{trend} = 0.14$		$P_{trend} = 0.66$		$P_{trend} = 0.22$		$P_{trend} = 0.83$		$P_{trend} = 0.05$

[a] OR, odds ratio; CI, confidence interval; Adjusted for matching variables plus cholesterol levels (in quintiles), selenium supplementation, vitamin E supplementation, family history of prostate cancer, body mass index, height, vigorous exercise, history of vasectomy and current smoking using conditional logistic regression. P_{trend} is calculated using the dietary/plasma scores as continuous variable in the models.
[b] Dietary/plasma scores are calculated by summing deciles of dietary intake and plasma concentrations, *i.e.* a participant in decile 1 of dietary intake and decile 1 of plasma level would get a score of $1 + 1 = 2$.

between β-carotene blood levels and prostate cancer by age (see below).

Lutein/Zeaxanthin. In our study no association between lutein/zeaxanthin levels and risk of prostate cancer was found. Studies evaluating the relationship between lutein/zeaxanthin and prostate cancer risk have been inconclusive. A case-control study (49) reported a significant inverse association between diets rich in lutein/zeaxanthin and risk of prostate cancer (for ≥2000 μg/day *versus* <800 μg/day; OR, 0.68; 95% CI, 0.45–1.00), whereas no association between estimated lutein intake and risk of prostate cancer was found in the previously published study from our cohort (48). Similarly, no evidence for a protective association between plasma levels of lutein and zeaxanthin, and risk of prostate cancer was observed in a cohort of Japanese Americans residing in Hawaii followed for a period of up to 20 years ($n = 142$ cases and 142 controls; Ref. 14). However, another recently published case-control study based on 65 prostate cancer patients and 132 controls found higher zeaxanthin and lutein levels (highest versus lowest quartile: zeaxanthin: OR, 0.22; 95% CI, 0.06–0.83; lutein: OR, 0.30; 95% CI, 0.09–1.03) to be inversely related to the risk of prostate cancer (12). On the contrary, findings from a recently published multicenter case-control study are suggestive of an increased risk of prostate cancer with higher concentrations of lutein/zeaxanthin, but results were not statistically significant (highest *versus* lowest quartile: OR, 1.51; $P = 0.17$; Ref. 35).

β-Cryptoxanthin. Consistent with the findings from this study, results from other epidemiological studies do not support a protective role for foods rich in β-cryptoxanthin on the risk of prostate cancer. In our report published previously from this cohort (48) no evidence for an inverse association between higher intakes of β-cryptoxanthin and prostate cancer risk was found. On the other hand, one case-control study by Jain *et al.* (46) reported a positive association between higher intake of β-cryptoxanthin and risk of prostate cancer (highest *versus* lowest quartile: OR, 1.44; 95% CI, 1.09–1.89). Two nested case-control studies, a cohort of Japanese Americans residing in

Hawaii and the Physicians' Health Study cohort, did not observe an association between plasma levels of β-cryptoxanthin and risk of prostate cancer (13, 14).

Lycopene. Several studies have investigated recently a possible protective effect of lycopene, a carotenoid primarily found in tomatoes and tomato based products (50), on prostate cancer. Of the more recent case-control studies, most found no appreciable association between lycopene intake and risk of prostate cancer (46, 49, 51), but results from one case-control study (44) were suggestive of a small inverse association between higher intake of lycopene and risk of prostate cancer, but results were not statistically significant (highest versus lowest quartile: OR, 0.76; 95% CI, 0.50–1.17). However, in our report published previously from the HPFS cohort (48) higher consumption of tomato products and estimated lycopene intake were associated with a significantly lower risk of prostate cancer (highest quartile *versus* lowest quartile: lycopene: relative risk, 0.79; 95% CI, 0.64–0.99); tomato products: relative risk, 0.74; 95% CI, 0.58–0.93). These results were confirmed in a later publication with longer follow-up and multiple dietary assessments (19).

Several prospective and retrospective studies evaluating the relationship between plasma or serum lycopene concentrations and risk of prostate cancer also support an inverse association. In a case-control study nested within the Washington County cohort (17) participants in the highest quartile of blood lycopene concentrations had a 50% lower risk of prostate cancer when compared with those in the lowest quartile, but results were not statistically significant (OR, 0.50; 95% CI, 0.20–1.29). In the later study from the Washington County cohort, blood concentrations of lycopene were not appreciably associated with risk of prostate cancer (18). No evidence for a protective association between plasma levels of lycopene and risk of prostate cancer was found in a cohort of Japanese Americans residing in Hawaii (14). However, average lycopene concentrations in that study were ~3-fold lower than observed in other United States studies. On the other hand, a nested case-control study using data from the Physicians' Health

Study, with a follow-up period of up to 13 years, demonstrated a strong and statistically significant inverse association between plasma lycopene levels and risk of aggressive prostate cancer (highest *versus* lowest quintile: OR, 0.56; 95% CI, 0.34–0.91; Ref. 13). Findings from blood-based studies using retrospectively collected blood samples were similar to those from the prospective studies. A recent multicenter case-control study demonstrated an inverse association between higher blood lycopene concentrations and risk of prostate cancer, a relationship that was strongest for aggressive prostate cancer (highest *versus* lowest quartile: OR, 0.37; P_{trend} = 0.04; Ref. 35). Another recently published case-control study also found higher lycopene levels to be inversely related to the risk of prostate cancer (highest *versus* lowest quartile: lycopene: OR, 0.17; 95% CI, 0.04–0.78; Ref. 12).

Combining Dietary and Plasma Data. One factor contributing to inconsistencies among blood-based studies is the lack of statistical power; with the exception of the Physicians' Health Study, all of the above cited studies were based on <250 cases and reliance on one plasma measurement, which may lead to misclassification of long-term exposure. When two response variables are obtainable to assess exposure, combining exposure information from those two sources may result in increased validity of the study by reducing misclassification of exposure (23–25). Combining dietary intake data and plasma data in this study reinforced our observations based on plasma concentrations or estimated dietary intake when examined independently.

Associations by Age and Family History. The inverse associations between higher plasma lycopene concentrations and risk of prostate cancer were restricted to older participants and those without a family history. Our findings suggest an inverse association between higher β-carotene concentrations and risk of prostate cancer among participants <65 years of age. A few observational studies have examined associations between carotenoid intake or plasma concentrations and risk of prostate cancer stratified by age (12, 13, 17, 35–37). No evidence for an interaction between age (<70 years of age *versus* ≥70 years of age) and serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, and lutein/zeaxanthin was observed in one case-control study (35). In contrast, statistically significant inverse associations between β-carotene intake and risk of prostate cancer were restricted to men ≤68 years of age in one study (37). In another case-control study by Hsing *et al.* (36) inverse associations between β-carotene intake and risk of prostate cancer were only seen for older participants (≥75 years of age). In the Physicians' Health Study (13) plasma lycopene levels appeared to be decreased among older men, but stratification by age groups yielded similar results. In the Washington County cohort (17), inverse associations between lycopene and risk of prostate cancer appeared to be slightly more pronounced among participants <70 years at diagnosis (highest *versus* lowest quartile OR, 0.35; P_{trend} = 0.14) than in participants ≥70 years at diagnosis (highest *versus* lowest quartile: OR, 0.65; P_{trend} = 0.69), although in both age groups the results were not statistically significant. On the other hand, in the recent hospital-based case-control study by Lu *et al.* (12), inverse relations between higher lycopene, zeaxanthin, and lutein levels, and risk of prostate cancer were restricted to participants <60 years of age. However, cases and controls were not matched by age, and the majority of controls (89% of all controls) belonged to the younger age group.

The recently published prospective study from our cohort (HPFS) also found that the inverse association between higher tomato sauce intake, which is the strongest predictor of plasma lycopene in our cohort, and risk of prostate cancer was more pronounced among participants who were ≥65 years of age at diagnosis (19). Similarly, in the case-control study based on 320 prostate cancer cases and 246 controls by Lagiou *et al.* (20), the inverse association between intake of cooked tomatoes and risk of prostate cancer was restricted to older participants (i.e., ≥70 years of age). Why the inverse association between lycopene level and prostate cancer risk may differ by age is unresolved, but one possible reason may be that some carotenoids may influence primarily sporadic cases that are largely attributed primarily to environmental influences. Men developing prostate cancer at a younger age may have a stronger contribution from familial and hereditary factors and may be less sensitive to certain dietary factors (21, 22). When we conducted additional analyses excluding men with a positive family history of prostate cancer, our results for plasma lycopene and dietary/plasma lycopene generally became stronger. These results suggest that lycopene may play a more important role for the development of sporadic prostate cancer than for prostate cancer associated with familial and hereditary prostate cancer. To our knowledge no other study has investigated the association between lycopene intake or serum concentrations and risk of prostate cancer in groups with or without a positive history of prostate cancer. Thus, these findings need to be confirmed by other studies.

There are several methodologic issues in the present study that should be discussed. First, although our study was based on fairly many cases we did not have enough power to investigate associations based on stage of the disease at the time of diagnosis, *i.e.,* advanced prostate cancer *versus* nonadvanced prostate cancer. Results from previous blood studies have suggested inverse associations between lycopene levels and risk of prostate cancer to be stronger for aggressive prostate cancer (13, 35). Another limitation of this study is that the vast majority of men included in this study were Caucasians. However, in a recent case-control study (35), the only blood-based study that examined associations between racial groups separately, inverse associations between lycopene levels, and prostate cancer risk were similar in blacks and whites. Considering that risk factors for prostate cancer may differ by race (52, 53), future studies investigating this association should also incorporate men with different ethnicities in their study design. Thirdly, our study was based on two nested case-control sets with laboratory measurements performed at two different time points. However, assays for each case-control pair were run in the same batch, measured plasma carotenoid concentrations were similar between these two nested case-control sets, and matched analysis was performed. Furthermore, findings from a reliability study on the HPFS cohort indicated good reproducibility of lycopene measurements drawn up to 4 years apart (Spearman correlation coefficient = 0.63, based on 144 paired samples; Ref. 54). In addition, results from the Washington County cohort also suggest that in blood specimens collected 15 years apart plasma measurements of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene were reliable with regard to their rankings after having been stored at −70°C (55). Finally, our results are based on a relatively short follow-up period of up to 5 years. However, most cases were asymptomatic, organ-confined, and detected primarily through PSA screening. When analysis was limited to cases who were diagnosed at least 2 years after blood donation, results were comparable, although due to the lower number of cases all of the CIs included 1.

In conclusion, we found a statistically significant inverse association between higher lycopene levels and risk of prostate

cancer, which was restricted to participants over the age of 65 and those without a family history of prostate cancer. This group is enriched for sporadic, as opposed to familial or hereditary prostate cancer, suggesting that lycopene may play a more important role for the development of sporadic prostate cancer than for familial and hereditary prostate cancer. Our present results support our recent findings from the HPFS cohort based on tomato based products. However, we cannot exclude the possibility that lycopene intake and plasma levels may be a marker for intake of tomato products or other foods that contain lycopene, and that other components, (*e.g.,* other carotenoids such as neurosporene or γ-carotene) found in tomato products (50) may have led to the observed associations.

In addition, our findings also suggest that among younger men, β-carotene may also play a role in prostate carcinogenesis. Mechanisms whereby β-carotene may influence prostate cancer incidence or progression remain obscure, but may include the conversion of β-carotene to retinol within the prostate, leading to activation of retinoid receptors (56). Because prospective data on the association between plasma lycopene and risk of prostate cancer are sparse, our results need to be confirmed by future studies, which should also focus on possible differences by age, family history of prostate cancer, and stage or severity of disease.

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