Plasma carotenoids, retinol, and tocopherols and the risk of prostate cancer in the European Prospective Investigation into Cancer and Nutrition study[1–3]

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ABSTRACT

Background: Previous studies suggest that high plasma concentrations of carotenoids, retinol, or tocopherols may reduce the risk of prostate cancer.

Objective: We aimed to examine the associations between plasma concentrations of 7 carotenoids, retinol, α-tocopherol, and γ-tocopherol and prostate cancer risk.

Design: A total of 137 001 men in 8 European countries participated. After a mean of 6 y, 966 incident cases of prostate cancer with plasma were available. A total of 1064 control subjects were selected and were matched for study center, age, and date of recruitment. The relative risk of prostate cancer was estimated by conditional logistic regression, which was adjusted for smoking status, alcohol intake, body mass index, marital status, physical activity, and education level.

Results: Overall, none of the micronutrients examined were significantly associated with prostate cancer risk. For lycopene and the sum of carotenoids, there was evidence of heterogeneity between the associations with risks of localized and advanced disease. These carotenoids were not associated with the risk of localized disease but were inversely associated with the risk of advanced disease. The risk of advanced disease for men in the highest fifth of plasma concentrations compared with men in the lowest fifth was 0.40 (95% CI: 0.19, 0.88) for lycopene and 0.35 (95% CI: 0.17, 0.78) for the sum of carotenoids.

Conclusions: We observed no associations between plasma concentrations of carotenoids, retinol, or tocopherols and overall prostate cancer risk. The inverse associations of lycopene and the sum of carotenoids with the risk of advanced disease may involve a protective effect, an association of dietary choice with delayed detection of prostate cancer, reverse causality, or other factors.

KEY WORDS Carotenoids, retinol, tocopherols, lycopene, prostate cancer, nested case-control study

INTRODUCTION

Previous studies indicated that dietary carotenoids, retinol, and tocopherols may affect the development of prostate cancer (1, 2). Overall, this research was inconclusive, but it suggested that any associations may be more marked for advanced than for localized prostate cancer, and there was particular interest in the possibility that high concentrations of lycopene may be associated with a reduction in risk (2). The present study examined

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these associations by using plasma measures of micronutrients within the European Prospective Investigation into Cancer and Nutrition (EPIC), a large cohort study in men in 8 European countries (3) who had a wide range in plasma carotenoid concentrations (4). We previously reported that the risk of prostate cancer in EPIC was not associated with the total consumption of fruit or vegetables (5).

The carotenoids studied were α- and β-carotene, lycopene, lutein, zeaxanthin, β-cryptoxanthin, and canthaxanthin and the sum of carotenoids and retinol. Some of these carotenoids can be converted in the body to retinol, whereas others cannot but may have biological effects independent of vitamin A activity (6). The α- and β-carotenes are carotenes that can be converted to retinol; in Europe, carrots are the major source of α-carotene, and both carrots and green vegetables are major sources of β-carotene (7). Lycopene is a carotene that cannot be converted to retinol; in European diets, tomatoes and tomato products are the major source of lycopene (7, 8). The other carotenoids studied are xanthophylls. Lutein and its stereoisomer zeaxanthin are not converted to retinol; major food sources are green vegetables and, to a lesser extent, eggs, because lutein is added to chicken feed to enhance the yellow color of egg yolks (7, 9). β-Cryptoxanthin can be converted to retinol; citrus fruit, especially oranges, is the major food source in Europe (7). Canthaxanthin may be converted to retinol; it occurs naturally in some plants and mushrooms, but most of the canthaxanthin in European diets is derived from its use as a feed additive to color foods such as eggs and farmed salmon. Canthaxanthin is also an ingredient in pills that are taken to make the skin look tanned (10).

The tocopherols studied were α- and γ-tocopherols, both of which have vitamin E activity. α-Tocopherol occurs in a wide range of foods, especially in vegetable oils such as sunflower seed oil, whereas the major sources of γ-tocopherol are specific vegetable oils such as soy and corn (maize) oil. α-Tocopherol is the predominant tocopherol in European diets, whereas γ-tocopherol is more predominant in the United States owing to the greater use of soy and corn oils. α-Tocopherol is generally present in the plasma at higher concentrations than is γ-tocopherol (11).

SUBJECTS AND METHODS

Study population

The EPIC recruitment procedures and collection of questionnaire data, anthropometric measurements, and blood samples were described in detail elsewhere (3). In brief, standardized questionnaire data on dietary and nondietary variables were collected between 1992 and 2000 from 519 978 individuals across Europe, including 153 457 men, of whom 137 001 provided a blood sample. The present study includes the cases of prostate cancer that occurred after blood collection and the matched control subjects from 8 of the 10 participating countries: Denmark, Germany, Greece, Italy, Netherlands, Spain, Sweden and the United Kingdom (UK). France and Norway were not included in the present study because these cohorts included only women.

A 30-mL (20 mL in the Umeå cohort) blood sample was collected according to a standardized protocol. Filled syringes were kept at 5–10 °C, protected from light, and transferred to a local laboratory for further processing and the preparation of aliquots. An exception to this procedure was blood collection from the subjects who were recruited through the Oxford center. For those subjects, blood samples were collected throughout the United Kingdom and were transported to a laboratory in Norfolk by mail at ambient temperature. Blood fraction aliquots (serum, plasma, red blood cells, and buffy coat) in 0.5-mL straws were heat-sealed and stored in liquid nitrogen tanks at −196 °C, except in Denmark, where samples were stored in 1-mL tubes in nitrogen vapor at −150 °C, and Umeå, Sweden, where samples were stored in 1.8-mL plastic tubes in −80 °C freezers.

Follow-up for cancer incidence and vital status

In Denmark, Italy, Netherlands, Spain, Sweden, and the United Kingdom, the incident cancer cases were identified through the linkage of records with regional or national cancer registries. In Germany and Greece, follow-up was based on a combination of methods, which included health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next of kin. Data on vital status in most EPIC study centers were collected from mortality registries at the regional or national level, in combination with data collected by active follow-up (Greece). For each EPIC study center, closure dates of the study period were defined as the latest dates of complete follow-up for both cancer incidence and vital status (dates varied between centers, from June 1999 to January 2003).

Selection of case and control subjects

In total, the 8 subcohorts contributing to the present study included 1111 men diagnosed with incident prostate cancer by the end of each center's follow-up period. The cases with no available blood sample and those subjects who had missing information on the date of the blood donation or who had a history of another cancer (except nonmelanoma skin cancer) at the time of the blood donation were excluded. After these exclusions, laboratory measurements for the current analysis were available for 966 cases: 292 cases in Denmark, 62 in Italy, 205 in Germany, 9 in Greece, 25 in Netherlands, 95 in Spain, 98 in Sweden, and

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180 in the United Kingdom. For each case, one male control subject (in Umeå, an EPIC-associated cohort, 2 male control subjects) was chosen at random from appropriate risk sets that consisted of all cohort members alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the index case. Matching criteria were the study center, the age at the time of enrollment (±6 mo), the time of day of the blood collection (±1 h), and the time between the blood drawing and the last consumption of food or drink (<3, 3–6, or >6 h).

All participants gave written informed consent to participate in the study. The research was approved by the local ethics committees in the participating countries and by the Internal Review Board of the International Agency for Research on Cancer (Lyon, France).

Data on stage and grade were collected from each center when possible. The sources of this information varied between individual cases and between centers and included both clinical and surgical staging and grade information from both biopsies and prostatectomies. When data were available from more than one source, the most advanced stage or highest grade was used to categorize individuals. Tumor stage was categorized as localized [tumor (T)-node (N)-metastasis (M) categories T0 or T1 or T2 and N0 or NX and M0, or stage-coded in the recruitment center as localized], advanced (T3 or T4, N1+, M1, or some combination of these, or stage-coded in the recruitment center as metastatic), or unknown. Histologic grade was categorized as Gleason sum <7 or equivalent (cases were coded as well differentiated or as moderately differentiated), Gleason sum ≥7 or equivalent (cases were coded as poorly differentiated or as undifferentiated), or unknown.

Laboratory assays

Plasma concentrations of carotenoids, retinol, and tocopherols were measured by using HPLC at the International Agency for Research on Cancer (12, 13). Briefly, plasma samples were analyzed for 7 carotenoids (α-carotene, β-carotene, β-cryptoxanthin, canthaxanthin, lutein, lycopene, and zeaxanthin) and for retinol, α-tocopherol, and γ-tocopherol by using a reverse-phase HPLC method on an HPLC-1100 system (Hewlett-Packard, Wilmington, IL) with a C18-Adsorbosphere column (Alltech, Deerfield, IL). There were 69 assay batches. Two samples of a quality-control pool (created by mixing plasma from 3 subjects) were run per analysis batch, and no significant between-day drift was observed. At mean concentrations similar to the mean concentrations in controls, overall CVs (between- and within-assay variations combined) for these quality-control samples ranged from 3% to 12% with a median of 4%. Cases were analyzed in the same batch as their matched controls (with the exception of 5 matched sets). All assays were performed by laboratory personnel who were blinded to the case-control status of the blood samples.

Statistical analysis

Differences in baseline characteristics between cases and controls were compared by using the 2-sample *t* test for continuous variables and the chi-square test for categorical variables. Odds ratios as estimates of relative risk (RR) of prostate cancer in relation to plasma concentrations of carotenoids, retinol, and tocopherols were calculated by using conditional logistic regression models. Micronutrient concentrations were categorized into fifths with cutoffs based on the quintiles in the control subjects. Trends in RRs were assessed by replacing the categorical variable with the logarithm of concentration. The 95% CIs were computed by using the SEs of the pertinent regression coefficients. The effects of potential confounders, other than the matching criteria that are controlled for by design, were examined by including additional terms in the logistic regression models. Potential confounders included smoking status (never, past, or current), alcohol intake (<8, 8–15, 16–39, or ≥40 g/d), body mass index (BMI; in kg/m^2; in fourths), physical activity (index of combined recreational, household, and occupational physical activity: inactive, moderately inactive, or active), marital status [married (or cohabiting) or unmarried (or not cohabiting)], and education level (primary school or equivalent, secondary school, and university degree or equivalent). For each of these variables, a small proportion of values were unknown; these values were included in the analyses as a separate category.

Likelihood ratio chi-square tests were used to examine the heterogeneity of the trends in prostate cancer risk with the logarithm of plasma micronutrient concentrations between categories of tumor stage (localized or advanced), histologic grade (Gleason sum <7, ≥7, or equivalent), lag time between blood collection and diagnosis (<2 or ≥2 y), age at blood collection (<60 or ≥60 y), smoking status at time of blood collection (no or yes), and country of recruitment (8 countries). All *P* values presented are 2-sided, and $P < 0.05$ was considered statistically significant. Analyses were performed with STATA software (version 9; Stata Corp, College Station, TX).

To put the results of the present study into the context of previous research, we conducted a meta-analysis of our results together with the results of previously published studies. Studies were identified by searching PubMed and the reference lists of relevant articles by using the search terms carotene, carotenoid, lycopene, retinol, tocopherol, and prostate cancer. Summary RRs were estimated as the weighted average of the study-specific RRs for men in the highest category of micronutrient concentrations compared with those in the lowest category, with weights determined by the inverse of the variance of each RR.

RESULTS

Baseline characteristics of cases and controls are shown in **Table 1**. There were no large differences between cases and controls, but there were some small differences that were statistically significant or of borderline statistical significance: when compared with controls, cases had a slightly lower weight and BMI, were less likely to smoke, and were less physically active. Similar analyses were conducted to compare the characteristics of men subsequently diagnosed with localized or advanced prostate cancer. The mean (±SD) BMI was 26.6 ± 3.2 and 27.1 ± 3.6 in cases with localized disease and advanced disease, respectively. For the other characteristics, the mean proportions in cases with localized and advanced disease, respectively, were 26.2% and 25.6% for current smokers, 20.2% and 22.8% for alcohol consumption of ≥40g/d, 42.6% and 44.6% for physically active, 88.7% and 86.2% for married or cohabiting, and 28.8% and 26.5% for education to degree level or equivalent. None of these differences between cases of localized and advanced disease were statistically significant.

In the cases, the median time between blood collection and diagnosis was ≈4 y, and the mean age at diagnosis was ≈65 y

TABLE 1
Baseline characteristics by case and control status

Characteristic	Cases (n = 966)	Controls (n = 1064)	P^1
All participants			
Age at blood collection (y)	60.4 ± 5.8[2]	60.1 ± 5.7	NA[3]
Weight (kg)[4]	80.1 ± 11.2	81.1 ± 11.9	0.05
Height (cm)[4]	173.6 ± 6.9	173.8 ± 6.9	0.51
BMI (kg/m^2)[4]	26.6 ± 3.3	26.8 ± 3.6	0.08
Smoking [n (%)][4]			
Never	307 (32.5)	317 (31.2)	
Former	408 (43.2)	407 (40.1)	
Current	230 (24.3)	291 (28.7)	0.09
Alcohol consumption (g/d)[4]			
<8 [n (%)]	326 (34.6)	381 (37.4)	
8–15 [n (%)]	187 (19.9)	211 (20.7)	
16–39 [n (%)]	251 (26.6)	235 (23.1)	
≥40 [n (%)]	178 (18.9)	191 (18.8)	0.29
Physical activity [n (%)][4]			
Inactive	196 (21.6)	179 (18.6)	
Moderately inactive	319 (35.1)	307 (31.9)	
Active	394 (43.3)	475 (49.4)	0.03
Marital status [n (%)][4]			
Married or cohabiting	501 (89.0)	572 (88.1)	
Unmarried or not cohabiting	62 (11.0)	77 (11.9)	0.64
Educational attainment [n (%)][4]			
Primary or equivalent	354 (38.1)	411 (40.6)	
Secondary	331 (35.6)	376 (37.1)	
Degree	245 (26.3)	226 (22.3)	0.11
Cases only			
Year of diagnosis	2000 (1994–2005)[5]	—	
Age at diagnosis (y)	64.9 (5.7)	—	
Time from blood collection to diagnosis (mo)	50 (0–181)[5]	—	
Stage [n (%)]			
Localized	484 (50.1)	—	
Advanced	205 (21.2)	—	
Unknown	277 (28.7)	—	
Grade [n (%)]			
Gleason sum <7 or well or moderately differentiated	445 (46.1)	—	
Gleason sum ≥7 or poorly differentiated or undifferentiated	286 (29.6)	—	
Unknown	235 (24.3)	—	

[1] Independent sample t tests of equality of the means, or chi-square tests of association, as appropriate.
[2] $\bar{x}$ ± SD (all such values).
[3] Significance test was not applicable because this was a matching criterion.
[4] Unknown for some subjects; the calculations of percentages exclude missing values.
[5] Median; range in parentheses.

(Table 1). The stage of disease at diagnosis was known for 71% of cases; of these, 70% were localized and 30% were advanced. The grade of disease at diagnosis was known for 76% of cases; of these, 61% were low grade (Gleason sum <7 or equivalent) and 39% were high grade (Gleason sum ≥7 or equivalent). The proportion of advanced-stage disease in cases with known high-grade disease was 65%.

Geometric mean plasma concentrations of carotenoids, retinol, and tocopherols in cases and controls are shown in **Table 2**. The only difference that was statistically significant was that for zeaxanthin, which was 5.5% higher in cases than in controls (P = 0.04). In the carotenoids, the highest mean plasma concentrations were for lycopene, followed by lutein and then β-carotene (geometric mean concentrations in controls of 27.0, 17.7 and 15.2 μg/dL, respectively); lycopene was the carotenoid with the highest plasma concentrations in the control men in all 8 countries (data not shown). All of the plasma concentrations of the individual carotenoids were positively correlated with one another (results not shown). Geometric mean concentrations of α-tocopherol were approximately tenfold those of γ-tocopherol.

The RR of prostate cancer associated with plasma concentrations of carotenoids, retinol, and tocopherols, both unadjusted and after adjustment for BMI, smoking status, alcohol intake, physical activity, marital status, and education level, is shown in **Table 3**. The only statistically significant association was observed in the unadjusted analysis for zeaxanthin: compared with men in the lowest fifth of zeaxanthin, the men in the highest fifth of zeaxanthin had a RR of disease of 1.41 (95% CI: 1.03, 1.83; P for trend = 0.07); this association was attenuated and was not statistically significant in the fully adjusted model.

Analyses were also conducted with the cases subdivided by stage (localized or advanced), grade (Gleason sum <7, ≥7, or equivalent), time between blood collection and diagnosis (<2 or ≥2 y), age at blood collection (<60 or ≥60 y), smoking at time

TABLE 2
Concentrations of carotenoids, retinol, and tocopherols in cases and controls

Micronutrient	Cases (n = 966)[1]	Controls (n = 1064)[1]	P for difference[2]
	$\mu g/dL$		
α-Carotene	5.45 (5.18, 5.74)[3]	5.36 (5.10, 5.63)	0.63
β-Carotene	14.9 (14.2, 15.6)	15.2 (14.5, 15.8)	0.53
Lycopene	27.1 (25.9, 28.3)	27.0 (25.9, 28.2)	0.95
Lutein	17.9 (17.4, 18.4)	17.7 (17.2, 18.2)	0.57
Zeaxanthin	3.99 (3.85, 4.14)	3.78 (3.65, 3.92)	0.04
β-Cryptoxanthin	6.49 (6.15, 6.85)	6.29 (5.97, 6.63)	0.42
Canthaxanthin	1.71 (1.64, 1.79)	1.63 (1.56, 1.70)	0.12
Sum of carotenoids	86.1 (83.6, 88.7)	86.4 (84.0, 88.9)	0.86
Retinol	54.9 (54.1, 55.6)	54.6 (53.9, 55.4)	0.67
α-Tocopherol	1351 (1330, 1373)	1381 (1360, 1402)	0.06
γ-Tocopherol	97.6 (94.0, 101.2)	100.5 (97.0, 104.1)	0.26

[1] Data were missing for 1 case and 1 control for lycopene and for the sum of carotenoids.
[2] Independent sample t tests of equality of the log-transformed mean concentrations.
[3] Geometric $\bar{x}$; 95% CI in parentheses (all such values).

of blood collection (no or yes; analyzed both in matched sets and in an unmatched analysis to increase power), and country (8 countries). Heterogeneity in the associations between prostate cancer risk and the 11 micronutrients in subgroups of these 6 variables was assessed: of the 66 significance tests conducted, 3 were statistically significant or were of borderline statistical significance. These included the associations subdivided by stage of disease for lycopene (P for heterogeneity = 0.01) and for the sum of carotenoids (P for heterogeneity = 0.06).

The estimated RRs associated with plasma concentrations of lycopene and the sum of carotenoids, subdivided by stage of disease, are shown in **Table 4**. The RRs were adjusted for BMI, smoking status, alcohol intake, physical activity, marital status, and education level; however, the unadjusted RRs were similar (data not shown). For these carotenoids, plasma concentrations were not significantly associated with risk of localized disease, but there was a significant inverse association with risk of advanced disease. The risk of advanced disease for men in the top fifth of plasma concentrations relative to those in the lowest fifth was 0.40 for lycopene (95% CI: 0.19, 0.88; P for trend = 0.05) and 0.35 for the sum of carotenoids (95% CI: 0.17, 0.78; P for trend = 0.04). Restriction of these analyses to the 161 advanced cases diagnosed at ≥2 y after blood collection slightly attenuated the RR; the risk for men in the top fifth of plasma concentration relative to those in the lowest fifth was 0.48 for lycopene (95% CI: 0.20, 1.11; P for trend = 0.10) and 0.52 for the sum of carotenoids (95% CI: 0.21, 1.28; P for trend = 0.22). To investigate whether the association of the sum of carotenoids with risk of advanced disease may have been largely explained by the association with lycopene, we calculated the RR of prostate cancer associated with the sum of carotenoids minus lycopene; the RRs in ascending fifths of this variable were 0.87 (95% CI: 0.48, 1.56), 0.80 (95% CI: 0.42, 1.55), 0.77 (95% CI: 0.37, 1.58), and 0.76 (95% CI: 0.37, 1.56) (P for linear trend = 0.15).

The only other test for heterogeneity that approached statistical significance was that for β-cryptoxanthin by grade: P for heterogeneity = 0.06, with a nonsignificant positive association of β-cryptoxanthin with the risk of low-grade disease (RR for top fifth: 1.34; 95% CI: 0.82, 2.19) and a nonsignificant inverse association with risk of high-grade disease (RR for top fifth: 0.57; 95% CI: 0.28, 1.17).

Previous prospective studies of plasma or serum micronutrients and prostate cancer risk, together with the overall results for the current study, are summarized in **Table 5** (14–23). Of the 65 RRs in Table 5, only 2 were statistically significant: inverse associations were found for retinol in the study by Reichman et al (15) and for γ-tocopherol in the study by Huang et al (21). Furthermore, for none of the micronutrients studied was there a consistent pattern of lower risks with higher plasma concentrations. Summary RRs are shown in the last row of Table 5. The summary RRs range from 0.84 to 1.20, and none of the risks were significantly different from 1.0, although those for lycopene and for α-tocopherol were of borderline statistical significance.

DISCUSSION

To our knowledge, this is the largest prospective study to date of plasma carotenoids, retinol, tocopherols, and prostate cancer risk. Overall, we observed no significant associations between plasma micronutrient concentrations and prostate cancer risk. However, we did observe significant heterogeneity between localized and advanced prostate cancer for the associations with lycopene and for the sum of carotenoids, both of which were significantly associated with a reduction in risk of advanced prostate cancer. Analysis of the association of risk of advanced prostate cancer with the sum of carotenoids minus lycopene suggested that the association of risk with the sum of carotenoids is partly but not completely driven by the association with lycopene.

The study sample size of ≈1000 cases was estimated to provide >80% power to detect RRs of <0.7 in the highest category of exposure. However, for the subgroup analyses, the numbers of cases in each subgroup were smaller, and the power to detect heterogeneity or associations within subgroups was modest. A priori, we were most interested in analyses subdivided by the stage of disease. We had data on stage for 71% of the cases, of which 30% (n = 205) were advanced; this is a higher proportion than that in recent North American studies, but it provided only a moderate sample size. Testing for prostate cancer by using the serum concentration of prostate-specific antigen (PSA) became more widely used during the follow-up period of the present study. Data on PSA use in the EPIC cohort are not available, but studies of annual rates of PSA testing in older middle-aged men in some of the participating countries suggest rates of 6% in England and Wales, 7% in Netherlands, 9% in Spain, and 16% in Italy, compared with ≈38% in US whites (24–28).

Our analyses were based on single measures of plasma micronutrient concentrations. The repeatability of serum carotenoids over 1 to 2 y is high, with intraclass correlations of ≈0.6 to 0.8 (29). An examination of the repeatability of carotenoids, retinol, and α-tocopherol over 15 y showed that repeatability was similar to that for blood pressure, with Spearman's correlation coefficients of ≈0.3 to 0.5, which suggested that these measures should be reliable enough to detect moderate or strong associations, should they exist (30).

TABLE 3
Relative risks (and 95% CIs) of prostate cancer in relation to plasma concentrations of carotenoids, retinol, and tocopherols[1]

Micronutrient quintile cutoffs	Cases/controls	Relative risk (95% CI)	
		Unadjusted	Adjusted[2]
	n		
α-Carotene (μg/dL)			
<2.59	156/213	1.0	1.0
2.59 to <4.43	211/213	1.41 (1.06, 1.88)	1.34 (1.00, 1.79)
4.43 to <6.70	214/213	1.45 (1.08, 1.93)	1.29 (0.96, 1.75)
6.70 to <10.51	193/213	1.34 (0.99, 1.81)	1.21 (0.88, 1.66)
≥10.51	192/212	1.41 (1.05, 1.89)	1.20 (0.87, 1.66)
P for trend	—	0.17	0.89
β-Carotene (μg/dL)			
<8.21	179/213	1.0	1.0
8.21 to <13.12	209/213	1.23 (0.92, 1.63)	1.17 (0.87, 1.56)
13.12 to <18.52	207/213	1.24 (0.93, 1.66)	1.14 (0.84, 1.54)
18.52 to <27.28	198/213	1.21 (0.89, 1.63)	1.06 (0.77, 1.45)
≥27.28	173/212	1.08 (0.80, 1.46)	0.92 (0.66, 1.28)
P for trend	—	0.99	0.24
Lycopene (μg/dL)			
<15.04	159/213	1.0	1.0
15.04 to <24.32	220/213	1.39 (1.04, 1.85)	1.36 (1.02, 1.83)
24.32 to <34.75	216/212	1.32 (0.99, 1.75)	1.25 (0.93, 1.68)
34.75 to <49.37	196/213	1.18 (0.89, 1.57)	1.11 (0.83, 1.49)
≥49.37	174/212	1.03 (0.76, 1.41)	0.97 (0.70, 1.34)
P for trend	—	0.77	0.41
Lutein (μg/dL)			
<12.22	201/213	1.0	1.0
12.22 to <15.82	174/213	0.89 (0.67, 1.18)	0.85 (0.64, 1.14)
15.82 to <19.84	171/213	0.88 (0.66, 1.17)	0.81 (0.61, 1.08)
19.84 to <26.41	226/213	1.19 (0.90, 1.57)	1.11 (0.83, 1.49)
≥26.41	194/212	1.01 (0.75, 1.37)	0.91 (0.67, 1.25)
P for trend	—	0.49	0.99
Zeaxanthin (μg/dL)			
<2.37	178/213	1.0	1.0
2.37 to <3.40	189/213	1.07 (0.80, 1.43)	0.99 (0.74, 1.34)
3.40 to <4.50	185/213	1.05 (0.78, 1.40)	0.96 (0.71, 1.30)
4.50 to <6.20	166/213	0.95 (0.70, 1.28)	0.88 (0.65, 1.21)
≥6.20	248/212	1.41 (1.03, 1.83)	1.30 (0.96, 1.74)
P for trend	—	0.07	0.22
β-Cryptoxanthin (μg/dL)			
<2.99	180/213	1.0	1.0
2.99 to <2.99	202/213	1.17 (0.88, 1.56)	1.09 (0.81, 1.47)
5.23 to <8.24	197/213	1.16 (0.86, 1.55)	1.04 (0.76, 1.42)
8.24 to <13.04	188/213	1.07 (0.79, 1.45)	0.95 (0.69, 1.31)
≥13.04	199/212	1.15 (0.84, 1.58)	1.02 (0.73, 1.44)
P for trend	—	0.31	0.79
Canthaxanthin (μg/dL)			
<0.90	182/213	1.0	1.0
0.90 to <1.27	171/213	0.94 (0.71, 1.26)	0.93 (0.69, 1.25)
1.27 to <1.81	194/213	1.01 (0.75, 1.36)	0.97 (0.72, 1.32)
1.81 to <2.90	202/213	1.05 (0.77, 1.45)	1.03 (0.74, 1.42)
≥2.90	217/212	1.15 (0.80, 1.67)	1.16 (0.80, 1.69)
P for trend	—	0.42	0.43
Sum of carotenoids (μg/dL)			
<59.82	186/213	1.0	1.0
59.82 to <79.56	214/213	1.15 (0.88, 1.50)	1.03 (0.77, 1.36)
79.56 to <99.48	190/212	1.05 (0.79, 1.40)	0.93 (0.69, 1.26)
99.48 to <127.31	189/213	1.02 (0.76, 1.37)	0.89 (0.65, 1.21)
≥127.31	186/212	1.00 (0.74, 1.35)	0.84 (0.61, 1.16)
P for trend	—	0.94	0.24

(*Continued*)

TABLE 3 (*Continued*)

Micronutrient quintile cutoffs	Cases/controls	Relative risk (95% CI)	
		Unadjusted	Adjusted[2]
	n		
Retinol (μg/dL)			
<46.22	204/213	1.0	1.0
46.22 to < 52.31	180/213	0.93 (0.70, 1.23)	0.92 (0.69, 1.23)
52.31 to <58.18	202/213	1.07 (0.81, 1.40)	1.07 (0.80, 1.42)
58.18 to <64.58	170/213	0.90 (0.68, 1.20)	0.88 (0.65, 1.18)
≥64.58	210/212	1.14 (0.86, 1.51)	1.12 (0.83, 1.44)
P for trend	—	0.23	0.27
α-Tocopherol (μg/dL)			
<1132	224/213	1.0	1.0
1132 to <1294	207/213	0.95 (0.72, 1.26)	0.96 (0.72, 1.27)
1294 to <1442	181/213	0.85 (0.64, 1.13)	0.86 (0.65, 1.15)
1442 to <1680	196/213	0.95 (0.72, 1.25)	0.95 (0.72, 1.26)
≥1680	158/212	0.81 (0.61, 1.08)	0.82 (0.61, 1.11)
P for trend	—	0.37	0.48
γ-Tocopherol (μg/dL)			
<62.52	199/213	1.0	1.0
62.52 to <94.06	238/213	1.26 (0.93, 1.72)	1.29 (0.94, 1.77)
94.06 to <121.80	168/213	0.94 (0.68, 1.30)	0.98 (0.70, 1.37)
121.80 to <161.11	165/213	0.96 (0.68, 1.36)	0.99 (0.70, 1.41)
≥161.11	196/212	1.24 (0.88, 1.76)	1.33 (0.93, 1.90)
P for trend	—	0.61	0.34

[1] *P* for trend is associated with the logarithm of micronutrient concentration.
[2] Adjusted for BMI, smoking status, alcohol intake, physical activity level, marital status, and educational level.

Meta-analysis of our results together with the results of previous prospective studies suggested that none of these micronutrients were likely to be strongly related to overall prostate cancer risk. Only 2 of the previous studies reported on these associations after subdivision of the cases according to whether the prostate cancer was localized or advanced at the time of diagnosis. Nomura et al (16) reported simply that excluding nonaggressive cases did not alter the results. Gann et al (19) reported analyses restricted to aggressive cases, defined as those diagnosed with extraprostatic disease or with a histologic grade of Gleason score ≥7 or equivalent, or both; in these cases, they observed a significant trend of decreasing risk for increasing plasma lycopene, with a RR in the top fifth of 0.56 (95% CI: 0.34, 0.92), which is similar to the results of the current study.

The associations of carotenoids and tocopherols with the risk of prostate cancer were also examined in observational studies of dietary intake (2, 31, 32). Some of these studies suggested that lycopene, other carotenoids, and tocopherols may be associated with a lower risk of prostate cancer, but the results have not been consistent (2).

TABLE 4
Relative risks (and 95% CIs) of prostate cancer in relation to plasma concentrations of lycopene and the sum of carotenoids, subdivided according to stage of disease[1]

Micronutrient quintile cutoffs	Localized disease		Advanced disease	
	Cases/controls	Relative risk (95% CI)	Cases/controls	Relative risk (95% CI)
	n		*n*	
Lycopene (μg/dL)				
<15.04	77/115	1.0	48/49	1.0
15.04 to <24.32	113/126	1.31 (0.87, 1.97)	45/38	1.35 (0.67, 2.74)
24.32 to <34.75	115/116	1.36 (0.90, 2.05)	44/38	1.19 (0.62, 2.29)
34.75 to <49.37	83/108	1.05 (0.69, 1.59)	43/42	0.93 (0.47, 1.85)
≥49.37	95/95	1.40 (0.89, 2.21)	25/51	0.40 (0.19, 0.88)
P for trend		0.50		0.05
Sum of carotenoids (μg/dL)				
<59.82	83/110	1.0	57/51	1.0
59.82 to <79.56	118/116	1.27 (0.87, 1.87)	46/47	0.66 (0.34, 1.28)
79.56 to <99.48	92/107	1.09 (0.70, 1.69)	37/35	0.74 (0.37, 1.49)
99.48 to <127.31	90/121	0.89 (0.59, 1.37)	37/37	0.62 (0.28, 1.35)
≥127.31	100/106	1.02 (0.65, 1.60)	28/48	0.35 (0.17, 0.78)
P for trend		0.56		0.04

[1] *P* for trend is associated with the logarithm of micronutrient concentration. *P* for heterogeneity by stage = 0.01 (lycopene) and 0.06 (sum of carotenoids).

TABLE 5
Prospective studies of plasma or serum micronutrients and prostate cancer: relative risks (and 95% CIs) for the highest compared with the lowest micronutrient concentrations in each study and the mean or median concentrations in the control subjects

Study	Cases	α-Carotene	β-Carotene	Lycopene	Lutein	Zeaxanthin	β-Cryptoxanthin	Retinol	α-Tocopherol	γ-Tocopherol
	n									
Hsing et al, 1990 (14)	103									
RR (95% CI)		—	1.08 (0.45, 2.62)	0.50 (0.20, 1.29)	—	—	—	0.40 (0.15, 1.07)	1.00 (0.37, 2.68)[1]	—
Concentration (μg/dL)		—	24	32	—	—	—	64	960	—
Reichman et al, 1990 (15)	84									
RR (95% CI)		—	—	—	—	—	—	0.5 (0.2, 0.9)[2]	—	—
Concentration (μg/dL)		—	—	—	—	—	—	65	—	—
Nomura et al, 1997 (16)	142									
RR (95% CI)		1.2 (0.5, 2.5)	1.6 (0.8, 3.5)	1.1 (0.5, 2.2)	1.0 (0.5, 2.0)	1.5 (0.7, 2.9)	1.8 (0.9, 3.9)	0.8 (0.4, 1.5)	1.4 (0.7, 2.9)	0.7 (0.3, 1.5)
Concentration (μg/dL)		4.1	14.2	13.4	11.8	2.4	12.2	61	1313	150
Hartman et al, 1998 (17)	190									
RR (95% CI)		—	—	—	—	—	—	—	0.98 (0.60, 1.60)	—
Concentration (μg/dL)		—	—	—	—	—	—	—	1190	—
Eichholzer et al, 1999 (18)	30									
RR (95% CI)		—	1.1 (0.5, 2.5)[2]	—	—	—	—	0.7 (0.3, 1.5)	—	—
Concentration (μg/dL)		—	23.6	—	—	—	—	82	—	—
Gann et al, 1999 *(19)*	578									
RR (95% CI)		0.77 (0.54, 1.10)	—	0.75 (0.54, 1.06)	1.10 (0.73, 1.65)	—	0.80 (0.57, 1.11)	1.56 (1.07, 2.27)	1.06 (0.76, 1.48)	0.98 (0.71, 1.35)
Concentration (μg/dL)		5.7	—	38.8	10.3	—	6.5	57	1107	170
Goodman et al, 2003 *(20)*	205									
RR (95% CI)		1.18 (0.68, 2.05)	0.85 (0.49, 1.49)	1.04 (0.61, 1.77)	0.86 (0.48, 1.52)	0.78 (0.43, 1.41)	0.81 (0.46, 1.43)	1.02 (0.56, 1.87)	0.59 (0.34, 1.04)	0.86 (0.50, 1.48)
Concentration (μg/dL)		2.9	14.2	30.9	13.5	2.7	5.5	67	1380	243
Huang et al, 2003 *(21)*										
Cohort 1	182									
RR (95% CI)		0.93 (0.49, 1.78)	0.94 (0.50, 1.77)	0.83 (0.46, 1.48)	1.26 (0.70, 2.26)	—	1.25 (0.65, 2.38)	0.86 (0.49, 1.53)	0.58 (0.31, 1.06)	0.77 (0.42, 1.43)
Concentration (μg/dL)		2.3	8.2	35.7	13.4	—	5.6	67	1200	240
Cohort 2	142									
RR (95% CI)		1.11 (0.52, 2.36)	1.47 (0.74, 2.92)	0.79 (0.41, 1.54)	0.68 (0.35, 1.32)	—	0.90 (0.48, 1.70)	1.29 (0.65, 2.54)	0.78 (0.41, 1.50)	0.21 (0.08, 0.54)
Concentration (μg/dL)		2.4	8.4	42.1	11.7	—	7.2	69	1300	290
Wu et al, 2004 *(22)*	450									
RR (95% CI)		0.67 (0.40, 1.09)	0.78 (0.48, 1.25)	0.66 (0.38, 1.13)	0.83 (0.49, 1.40)[3]	—	0.94 (0.56, 1.58)			
Concentration (μg/dL)		5.7	18.9	39.0	12.4	—	6.4			
Weinstein et al, 2005 *(23)*	100									
RR (95% CI)		—	—	—	—	—	—	—	0.49 (0.24, 1.01)	0.57 (0.31, 1.06)
Concentration (μg/dL)		—	—	—	—	—	—	—	1420	100
Current study	966									
RR (95% CI)		1.20 (0.87, 1.66)	0.92 (0.66, 1.28)	0.97 (0.70, 1.34)	0.91 (0.67, 1.25)	1.30 (0.94, 1.78)	1.02 (0.73, 1.44)	1.12 (0.83, 1.50)	0.82 (0.61, 1.11)	1.33 (0.93, 1.90)
Concentration (μg/dL)		5.4	15.2	27.0	17.7	3.8	6.3	55	1381	101
Summary										
RR (95% CI)		0.97 (0.81, 1.16)	0.98 (0.80, 1.19)	0.84 (0.71, 1.00)	0.94 (0.79, 1.13)	1.20 (0.92, 1.56)	0.96 (0.80, 1.14)	1.04 (0.88, 1.24)	0.85 (0.72, 1.00)	0.90 (0.75, 1.09)

[1] Total tocopherols.
[2] Estimated by current authors.
[3] Lutein combined with zeaxanthin.

There are also data on some of these micronutrients from large randomized controlled trials. In the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC), the RR of prostate cancer for men taking β-carotene (20 mg/d for a median of 6 y) compared with placebo was 1.26 (95% CI: 0.98, 1.62), which was attenuated in the posttrial period (33). In the Physicians' Health Study, treatment with 50 mg β-carotene on alternate days for an average of 12 y did not alter the risk of prostate cancer (RR: 0.99; 34), although there was some evidence for a protective effect in the men with the lowest plasma concentrations at the beginning of the trial (35). In the Carotene and Retinol Efficacy Trial, men in the intervention arm were given 30 mg β-carotene/d together with retinol for a mean of 4 y, and the RR of prostate cancer was 1.01 (95% CI: 0.80, 1.27), in comparison with the placebo group (36). For α-tocopherol, the ATBC trial reported that supplements of 50 mg/d reduced prostate cancer risk by 34% (95% CI: 14, 48), although this reduction in risk was no longer present after extended follow-up (33). However, the Heart Outcomes Prevention Evaluation trial showed no effect of 268 mg α-tocopherol/d for a median of 7 y (RR: 0.98; 95% CI: 0.76, 1.26; 37). The Heart Protection Study tested the effect of a combination of daily β-carotene (20 mg), α-tocopherol (600 mg), and vitamin C (250 mg) for 5 y and found no significant effect on prostate cancer (RR: 0.91; 38). Together, these trials provide strong evidence that supplementation with β-carotene for a few years does not reduce the risk of prostate cancer and moderately strong evidence that vitamin E does not reduce risk. The trials did not report on risk subdivided according to the stage of prostate cancer.

For lycopene and for the sum of carotenoids, there was weak evidence for a difference in association according to the stage of disease, which corresponded to an inverse association for advanced disease but no association with localized disease. One possible explanation for this observation is that these carotenoids, or associated dietary factors, actively reduce the risk of advanced prostate cancer; several potential mechanisms by which lycopene may reduce prostate cancer risk have been discussed (39). Given the known effect of the 5-α-reductase inhibitor finasteride in reducing risk (40), the possibility that lycopene may inhibit 5-α-reductase warrants attention (41). Alternatively, there may be little or no biological association between these carotenoids and the risk of disease, but low consumption of certain foods rich in lycopene or all carotenoids may be associated with lifestyles that lead to the detection of cancer at an advanced stage. Finally, the results may be related to reversed causality; the advanced cancers may have been present subclinically at the time of blood collection and may have influenced the plasma concentrations of some of the micronutrients. Our data do not enable us to choose between these interpretations. If lycopene or total carotenoids reduce the risk of advanced disease, it would be expected that, unless these carotenoids affect only disease progression, there would also be some reduction in risk of localized disease.

Further research on the effects of lycopene and other carotenoids on the prostate would be valuable. Although this is the largest prospective study of plasma carotenoids, retinol, tocopherols, and prostate cancer risk published so far, more data are needed to clearly differentiate the associations of lycopene and total carotenoids with localized and advanced disease, to examine whether these associations vary according to time between blood collection and diagnosis, and to examine whether these associations are confounded or modified by other potential risk factors for prostate cancer. Overall, the results from this and other studies have not established that these micronutrients have any clear effect on prostate cancer risk.

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