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Prospective Study of Antioxidant Micronutrients in the Blood and the Risk of Developing Prostate Cancer

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Antioxidant micronutrients may have chemopreventive effects. The authors examined the associations between prediagnostic blood levels of micronutrients and prostate cancer risk in two nested case-control studies of 9,804 and 10,456 male residents of Washington County, Maryland, who donated blood in 1974 (CLUE I) and 1989 (CLUE II), respectively. Until 1996, 182 men for whom adequate serum remained for assays in the CLUE I cohort and 142 men in the CLUE II cohort developed prostate cancer. Each case was matched with two controls by age, gender, race, and date of blood donation. In both cohorts, cases and controls had similar concentrations of α-carotene, β-carotene, total carotene, β-cryptoxanthin, lutein, lycopene, retinol, and ascorbic acid; serum α-tocopherol was weakly associated with prostate cancer risk. Higher retinyl palmitate concentrations were associated with a lower risk in CLUE I but not CLUE II. In CLUE I, cases had lower concentrations of γ-tocopherol than did controls ($p = 0.02$), but no dose-response trend was observed. A strong inverse association between γ-tocopherol and prostate cancer risk was observed in CLUE II. Findings do not replicate previous reports of a protective association between lycopene and prostate cancer, but they suggest potential chemopreventive effects of γ-tocopherol on prostate cancer.

antioxidants; ascorbic acid; prostatic neoplasms; vitamin A; vitamin E

Abbreviation: PSA, prostate-specific antigen.

The etiology of prostate cancer remains unclear. The only well-documented risk factors are age, race, and family history. Other possible risk factors include fat intake, androgen metabolism, genetic susceptibility, and oxidative damage. A high intake of fat may lead to high levels of androgens (1, 2). Both fat and androgens are associated with higher levels of oxidative damage (3–5), which could be prevented or mitigated by antioxidants.

In vitro studies suggest that antioxidant micronutrients have cancer chemopreventive properties, such as antioxidant effects; regulation of cellular signaling; enhancement of immunity; and modulation of gene expression, cell differentiation, cell proliferation, and apoptosis (6, 7). To date, the antioxidant micronutrients that have been the target of considerable research interest include vitamin A (a subgroup of retinoids that exhibit qualitatively the biologic activity of all-*trans* retinol (8)), carotenoids, vitamin C (ascorbic acid), and vitamin E (tocopherols). Studies of dietary intake have produced equivocal results with respect to the associations between antioxidant micronutrients and prostate cancer (9). Methodological issues such as inaccuracy and imprecision of dietary recall data collected by questionnaires may have hindered the interpretations and implications.

Epidemiologic evidence is limited in regard to the associations between prediagnostic concentrations of antioxidant micronutrients in serum and subsequent risk of developing prostate cancer (10–17) (table 1). No previous studies have been known to examine the association between retinyl palmitate, a naturally occurring compound as well as a metabolite of retinol via esterification. Of the six studies that assessed serum concentrations of retinol, two (11, 14) reported statistically significant inverse associations, three (10, 12, 13) reported statistically nonsignificant inverse associations, and one (16) reported a positive association. No

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TABLE 1. Summary of prospective studies of prostate cancer and blood levels of retinol, selected carotenoids, α-tocopherol, γ-tocopherol, and ascorbic acid

Authors, year (reference no.), study design, sample size (cases/controls or cohort), study period, subjects' age (years), specimen storage temperature	Odds ratio estimate by 4th or 5th fifth (low to high levels unless otherwise indicated)									
	Retinol	α-Carotene	β-Carotene	β-Cryptoxanthin	Lycopene	Lutein	Zeaxanthin	α-Tocopherol	γ-Tocopherol	Ascorbic acid
Knekt et al., 1988 (10), nested case-control, n = 37/68, 1968–1972~1978, 15–99, –20°C	2.4% higher in cases than in controls		0.5% lower in cases than in controls					3.5% lower in cases than in controls		
Reichman et al., 1990 (11), cohort, n = 84/2,440, 1971–1975~1981–1984, 50–74, –20°C	1.0									
	1.7									
	1.6									
	2.4*,†									
Eichholzer et al., 1999 (12), cohort, n = 30 deaths/2,974, 1971–1973~1990, mean 64, –20°C	1.5*		0.92 *,‡					3.3 *,† in smokers, 0.76 in nonsmokers		0.93*
Nomura et al., 1997 (13), nested case-control, n = 142/142, 1971–1975~1993, 52–74, –70°C	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
	0.9	1.5	1.4	1.6	1.0	1.7	0.5	1.8	1.1	
	0.7	1.7	1.6	0.9	1.0	1.8	1.0	1.6	0.8	
	0.8	1.2	1.6	1.5	1.1	1.4	1.0	1.4	0.7	
Hsing et al., 1990 (14), nested case-control, n = 103/103, 1974~1986, 47–91, –70°C	1.0		1.0		1.0			1.00§		
	0.67		1.57		0.81			1.43		
	0.39		1.42		0.55			0.91		
	0.40¶		1.08		0.50			1.00		
Hartman et al., 1998 (15), ATBC trial at baseline, n = 317 incidences/29,133, 1985~1994, 50–69, –70°C								1.0# 1.0** 0.86 1.04 0.82 0.91 0.76 0.98		
Gann et al., 1999 (16), nested case-control, n = 578/1,294, 1982–1995, 40–84, –82°C	1.0	1.0		1.0	1.0	1.0	1.0	1.0	1.0	
	1.08	1.11		0.85	0.89	1.01	0.85	1.04	0.91	
	1.37	0.97		0.87	0.90	1.08	0.87	1.09	0.90	
	1.21	1.14		0.88	0.87	1.09	0.88	1.17	0.87	
	1.56†	0.77		0.80	0.75	1.10	0.80	1.06	0.98	
Helzlsouer et al., 2000 (17) nested case-control study, n=117/233, 1989–1996, 43–82, –70°C								1.0	1.0	
								0.91	1.3	
								0.67	1.0	
								0.93	0.68	
								0.65	0.19††	

* Odds ratio for low levels compared with high levels.
† p for trend < 0.05.
‡ 80% β-carotene and 20% α-carotene.
§ Total tocopherol.
¶ p for trend = 0.07.
# In the active vitamin E supplement group.
** In the placebo group.
†† p for trend = 0.002.

statistically significant association was observed between prostate cancer risk and serum β-carotene (10, 12–14), α-carotene, β-cryptoxanthin, lutein, or zeaxanthin (13, 16). Data on lycopene were equivocal; no association (13), a statistically nonsignificant inverse association (14), and a borderline significant inverse association (16) were reported. For α-tocopherol, inverse associations were found in some studies of smokers (12) or smokers who used vitamin E supplements (15), whereas no association was found in othe studies (10, 13, 14, 16). Evidence on γ-tocopherol (13, 16 17) is inconsistent. Only one small study examined the asso ciation of prediagnostic serum concentrations of vitamin (with subsequent prostate cancer risk (12).

We examined the associations between carotenoid: retinol, retinyl palmitate, tocopherols, and ascorbic acid i the blood and the subsequent risk of developing prostat

cancer among participants of two population-based cohorts established in Washington County, Maryland, in 1974 (CLUE I) and 1989 (CLUE II).

MATERIALS AND METHODS

Study population

In the autumn of 1974, a campaign named CLUE I (from the slogan, "Give us a CLUE to cancer") was conducted in Washington County to collect blood samples for a serum bank and a brief questionnaire that included demographic characteristics, smoking, medication use, and vitamin supplement use within 48 hours before blood sample collection. A total of 23,938 persons (9,804 men and 14,134 women), 30 percent of the Washington County residents, participated in this program. Participation was better among women, among those who had more years of education, and among those in the age group 45–64 years. Blood samples were drawn, were allowed to clot at room temperature for 30 minutes, and were then kept at 4°C for 3–4 hours until the serum was separated. Serum aliquots were stored at –70°C until assayed.

In the summer and autumn of 1989, a similar campaign against cancer and heart disease, named CLUE II, was conducted to collect blood samples and data on age, gender, height, weight, weight at age 21 years, cigarette and cigar smoking, medication use, and vitamin supplement use within 48 hours prior to a blood donation. Participants filled out a modified 62-item Block food frequency questionnaire that contained questions about use of vitamin supplements (multivitamins, vitamin A, vitamin C, and vitamin E) over the past year. A total of 25,081 persons (10,456 men and 14,625 women), 30 percent of the Washington County residents, took part in this program. Participation was slightly higher among women, among those who had more years of education, and among those in the age group 45–70 years. Blood samples were collected into Vacutainers containing heparin (Fisher Scientific, Pittsburgh, Pennsylvania). Samples were refrigerated at 4°C and were centrifuged within 2–6 hours. Plasma aliquots were stored at –70°C until assayed. For the ascorbic acid assays, an additional aliquot was collected and was mixed with an equal amount of 10 percent metaphosphoric acid solution.

In each cohort, incident cases of prostate cancer (*International Classification of Diseases*, Ninth Revision, code 185) were identified through linkage of the cohort participants with the Washington County Cancer Registry and, since 1992, with the Maryland State Cancer Registry. A comparison between the two registries for CLUE II cases diagnosed after 1992 showed that the Washington County Cancer Registry was more complete than the Maryland State Cancer Registry: 5 percent of the cases were identified solely from the Maryland State Cancer Registry, and 25 percent were identified solely from the Washington County Cancer Registry. Cases were confirmed histologically. The stage of prostate cancer was extracted from medical records at the Washington County Hospital and was determined by using the American Joint Committee on Cancer/Tumor, Node, and Metastasis system. Stage data from the Maryland State Cancer Registry, if available, were incorporated.

Completeness of case ascertainment was assessed by comparing the number of identified cases with the number based on the American Cancer Society estimates (101.4 percent of estimated cases). On the basis of a survey of 4 percent of the CLUE I households, the annual emigration rate was 1.4 percent from 1974 to 1985. Among the 8,396 persons who participated in both CLUE I and CLUE II, 750 were lost to follow-up from 1974 to 1998. Hence, the annual rate of loss to follow-up was less than 1 percent in this group. Until September 1996, 182 and 142 incident prostate cancer cases in the CLUE I cohort and CLUE II cohort, respectively, were included in the present studies. Serum specimens from the majority of the CLUE I cases diagnosed during the period 1975–1985 were used for other studies (14) and thus were not available for the present study.

Each case was matched with two controls on age, race, gender, and date of blood donation. Controls were selected from the cohort participants who were not known to have died or to have been diagnosed with cancer (except for basal or squamous cell skin cancer) at the time that a case was diagnosed. Henceforth in this paper, the terms "CLUE I" and "CLUE II" are used to refer to the nested case-control studies conducted among the cohorts established in 1974 and 1989, respectively.

We previously reported an interaction of selenium, α-tocopherol, and γ-tocopherol with respect to prostate cancer risk in a subset (117 matched sets) of the CLUE II participants who provided toenail samples along with blood samples (17). For completeness, we have included the results of blood concentrations of tocopherols for the total CLUE II study (142 matched sets).

Laboratory assay

Frozen samples were thawed in ice water under yellow, dim light. The aliquots for assays were refrozen and were shipped to the laboratory with dry ice in Styrofoam boxes (The Dow Chemical Company, Midland, Michigan). The samples were then thawed at room temperature and were protected from light (≅20 minutes), mixed by vortex (15 seconds), and centrifuged (at 2,500 *g*, 5 minutes, 4°C). The fibrin-free upper fraction was used to measure retinol, carotenoids, and tocopherols by reversed-phase high-pressure liquid chromatography with multiwavelength detection (18). Ascorbic acid assays were performed on additional CLUE II samples. These samples were thawed at room temperature (≅20 minutes), mixed by vortex (15 seconds), and centrifuged (10,000 *g*, 5 minutes, 4°C); the clear supernatant was used to measure ascorbic acid with 2,4-dinitrophenylhydrazine as chromogen (19). Repeated freezing to –70°C and thawing for up to six cycles had no appreciable effect on the plasma or serum concentrations of micronutrients (20). In CLUE I, serum samples were thawed a maximum of two times prior to the aliquots for prostate cancer study, for a total of three thaws. Only 15 percent of the samples were thawed three times. None of the CLUE II samples in this study had been thawed previously. Serum

TABLE 2. Characteristics of prostate cancer cases and matched controls in Washington County, Maryland

Characteristic	CLUE* I cohort (1974)				CLUE II cohort (1989)			
	Cases (n = 182)	Controls (n = 364)	Matched OR†	95% CI†	Cases (n = 142)	Controls (n = 284)	Matched OR	95% CI
Age (years) at blood draw (mean (SD†))	54 (9)	54 (9)			66 (8)	66 (8)		
Age (years) at diagnosis (mean (SD))	71 (8)				70 (7)			
Race (%)								
Caucasian	98.3	98.3			96.5	96.5		
African American	1.7	1.7			3.5	3.5		
Education (%)								
<12 years	34.1	41.3	1.00		26.1	38.7	1.00	
12 years	40.1	35.0	1.40	0.93, 2.12	42.2	37.0	1.73	1.05, 2.86
>12 years	25.8	23.7	1.33	0.84, 2.13	31.7	24.3	1.96	1.15, 3.34
Cigarette smoking (%)								
Never	33.5	34.3	1.00		38.7	41.2	1.00	
Former	40.1	41.2	1.00	0.66, 1.51	51.4	47.9	1.14	0.74, 1.77
Current	26.4	24.5	1.11	0.69, 1.77	9.9	10.9	0.96	0.44, 2.05
Hours since last meal (%)								
<1	10.3	9.2	1.00		11.3	11.6	1.00	
1–2	43.5	42.7	0.92	0.49, 1.72	29.6	28.2	1.03	0.51, 2.09
3–7	37.4	38.3	0.83	0.44, 1.55	44.4	48.6	0.93	0.48, 1.81
≥8	8.4	9.2	0.82	0.36, 1.90	14.8	11.4	1.32	0.60, 2.91
Unknown	0.5	0.6			0.0	0.7		
Supplement use (48 hours before blood draw) (%)								
Multivitamins	8.2	5.8	1.50	0.74, 3.05‡	14.1	9.5	1.54	0.84, 2.84‡
Vitamin A	0	0			0	0		
Vitamin C	1.6	1.6			1.4	2.1		
Vitamin E	2.2	1.6			1.4	2.5		
Supplement use (%)§								
Multivitamins	NA†				18.6	11.2	1.90	1.02, 3.53‡,¶

Table continues

concentrations of retinol, β-carotene, and α-tocopherol were stable for at least 15 years of storage at –70°C (21).

Laboratory technicians were instructed to analyze multiples of triplet sets (including one case and two controls) on the same day by using the same solvents and reagents. Reproducibility of the laboratory measurements was assessed in 20 sets of serum specimens for the CLUE I study and in 15 sets of plasma specimens for the CLUE II study. For the CLUE I study, the intra-assay coefficients of variation were 11.6 percent for α-carotene; 11 percent for β-cryptoxanthin; 18.8 percent for retinyl palmitate; and 2–6 percent for β-carotene, total carotene, lutein, lycopene, retinol, α-tocopherol, and γ-tocopherol. For CLUE II, the intra-assay coefficients of variation were 16.0 percent for α-carotene; 13.6 percent for retinyl palmitate; and 3–8 percent for β-carotene, total carotene, β-cryptoxanthin, lutein, lycopene, retinol, α-tocopherol, γ-tocopherol, and ascorbic acid.

Statistical analysis

Baseline characteristics of cases and controls were summarized and compared by using paired t tests for continuous variables and conditional logistic regression models for categorical variables. Mean micronutrient concentrations for the two controls in a matched set were calculated, and data from cases and the mean values of their respective controls were compared by using the Wilcoxon signed-rank test. Micronutrient levels were further categorized into fifths according to the distributions among controls. The odds ratios and 95 percent confidence intervals for developing prostate cancer for the upper fifths compared with the lowest fifth of each micronutrient were estimated by using conditional logistic regression models. Adjustments for total lipid levels (continuous variable) in the blood, hours since last meal (<3, 3–7, ≥8), and education (years of schooling) were performed. Body mass index at age 21 years (continuous variable) was also included in the adjustment analyses for CLUE II. Tests for trend were performed by entering into a conditional logistic regression model a categorical variable defined by the median in each fifth of micronutrient concentration. Stratification analyses were conducted according to age at diagnosis (by median), number of years since blood was drawn, disease stage at diagnosis, history of cigarette smoking, and body mass index at age 21 years. To retain adequate sample size, thirds of micronutrient concentrations in controls were used to define the categories for the odds ratio estimates in these stratification analyses.

TABLE 2. Continued

Characteristic	CLUE* I cohort (1974)				CLUE II cohort (1989)			
	Cases (n = 182)	Controls (n = 364)	Matched OR†	95% CI†	Cases (n = 142)	Controls (n = 284)	Matched OR	95% CI
BMI†,# at baseline								
17.23–24.10	NA				26.8	19.7	1.00	
24.11–25.39					16.9	20.1	0.63	0.34, 1.18
25.40–26.94					14.8	20.4	0.55	0.29, 1.04
26.94–28.92					18.3	20.8	0.66	0.35, 1.24
28.92–40.39					23.2	19.0	0.91	0.50, 1.67
BMI# at age 21 years								
14.78–19.75	NA				15.2	20.1	1.00	
19.75–21.15					24.1	20.1	1.87	0.95, 3.68
21.16–22.97					24.8	19.4	1.97	0.99, 3.90
22.98–24.64					16.3	20.8	1.09	0.55, 2.17
24.65–36.35					20.6	19.7	1.61	0.79, 3.24
Stage of disease (%)								
0	4.4				2.1			
1	14.8				10.6			
2	28.6				36.6			
3	12.1				17.6			
4	8.2				4.2			
Missing data	31.9				28.9			
Year of diagnosis (%)								
1975–1980	2.2							
1981–1985	7.7							
1986–1989	17.6							
1990–1991	19.8				23.9			
1992–1993	26.4				35.2			
1994–1996	26.4				40.8			

* Derived from the slogan, "Give us a CLUE to cancer."
† OR, odds ratio; CI, confidence interval; SD, standard deviation; NA, not applicable, data not collected; BMI, body mass index.
‡ Users compared with nonusers.
§ Regular supplement use during the past year (data obtained from food frequency questionnaire).
¶ $p = 0.04$.
# Weight/height^2 (kg/m^2), cutoff points were based on the quintiles of BMI in the control group.

All analyses were performed separately for each cohort because of the differences in storage (serum vs. plasma) and the time between blood donation and diagnosis.

RESULTS

In both the CLUE I and CLUE II cohorts, cases and controls were comparable in age, race, education, history of cigarette smoking, and hours since last meal (table 2). Compared with controls, cases tended to have more years of schooling, but the differences were not statistically significant. Neither cases nor controls used vitamin A supplements within 48 hours prior to blood donation. Only 1–2 percent of the participants used vitamin C or vitamin E supplements 48 hours prior to a blood donation. In CLUE II, 91 percent of the participants responded to the food frequency questionnaire. Cases were more likely to have used multivitamin supplements regularly. Ninety percent of the CLUE I cases were diagnosed 10 years after blood donation, whereas all of the CLUE II cases were diagnosed within 7 years of blood donation. The median follow-up period was 17 years in CLUE I and 3.5 years in CLUE II.

Table 3 presents the medians and interquartile ranges of micronutrient concentrations for cases and controls. In both studies, cases and controls had similar serum concentrations of α-carotene, β-carotene, total carotene, lutein, β-cryptoxanthin, lycopene, and retinol. Cases in the CLUE I study had significantly lower levels of retinyl palmitate ($p = 0.01$). Concentrations of α-tocopherol were slightly lower in cases, but the differences were not statistically significant ($p = 0.11$ and $p = 0.09$ in CLUE I and CLUE II, respectively). Gamma-tocopherol was significantly lower in cases than in controls ($p = 0.02$ and $p = 0.0001$ in CLUE I and CLUE II, respectively). Ascorbic acid was measured only in the CLUE II study; no appreciable difference between cases and controls was observed.

TABLE 3. Medians and interquartile ranges of micronutrient concentrations in the blood of cases and controls, by cohort participation, Washington County, Maryland

Micronutrient	CLUE* I cohort (1974–1996)					CLUE II cohort (1989–1996)				
	Cases (n = 182)		Controls (n = 364)		p value†	Cases (n = 142)		Controls (n = 284)		p value†
	Median	Interquartile range	Median	Interquartile range		Median	Interquartile range	Median	Interquartile range	
α-Carotene (μg/dl)	2.2	1.4, 3.5	2.3	1.4, 3.5	0.34	2.4	1.5, 4.6	2.4	1.4, 4.8	0.16
β-Carotene (μg/dl)	7.4	5.0, 13.4	8.2	5.0, 13.9	0.20	8.3	5.5, 14.9	8.4	4.8, 13.4	0.64
Total carotene (μg/dl)	56.0	43.0, 74.0	58.3	42.1, 74.9	0.81	52.0	40.0, 77.0	57.6	43.2, 75.6	0.43
β-Cryptoxanthin (μg/dl)	5.9	3.3, 10.8	5.6	2.9, 11.2	0.58	6.7	3.7, 10.4	7.2	3.7, 11.0	0.42
Lutein (μg/dl)	13.7	10.7, 18.2	13.4	9.8, 17.8	0.85	10.6	8.2, 14.1	11.7	8.8, 15.0	0.16
Lycopene (μg/dl)	34.2	22.9, 48.9	35.7	24.5, 51.5	0.55	38.4	25.2, 59.7	42.1	27.4, 59.0	0.18
Retinol (μg/dl)	67.5	57.8, 74.3	67.2	57.0, 76.9	0.99	67.6	59.5, 78.8	68.7	57.7, 78.5	0.81
Retinyl palmitate (μg/dl)	7.7	4.8, 12.5	8.0	5.6, 14.0	0.01	10.6	6.9, 16.2	10.1	7.2, 15.8	0.84
α-Tocopherol (mg/dl)	1.1	1.0, 1.3	1.2	1.1, 1.5	0.11	1.3	1.1, 1.6	1.3	1.1, 1.7	0.09
γ-Tocopherol (mg/dl)	0.20	0.17, 0.30	0.24	0.17, 0.30	0.02	0.25	0.19, 0.32	0.29	0.20, 0.38	0.0001
Ascorbic acid‡ (μg/dl)	NA§		NA		NA	1.19	0.90, 1.50	1.20	0.90, 1.50	0.93

* Derived from the slogan, "Give us a CLUE to cancer."
† Wilcoxon signed-rank test.
‡ Not measured in the CLUE I study.
§ NA, not applicable.

Table 4 presents the odds ratio estimates of prostate cancer risk by fifths of micronutrient levels in controls. No significant association was observed between prostate cancer risk and α-carotene, β-carotene, total carotene, β-cryptoxanthin, lycopene, and retinol. In CLUE II, a statistically nonsignificant lower risk was observed in men who had higher plasma lutein concentrations. Serum retinyl palmitate concentrations in the upper four fifths were associated with an overall 50 percent reduction in the risk of prostate cancer in CLUE I (odds ratio = 0.51, 95 percent confidence interval: 0.30, 0.85) but not in CLUE II. Compared with men in the lowest fifth, men in the highest fifth of α-tocopherol levels had a statistically nonsignificant lower risk of prostate cancer in both studies. Unlike the strong inverse trend noted in the previous report from CLUE II (17), no significant dose-response trend was observed regarding γ-tocopherol and prostate cancer risk in CLUE I (p = 0.30).

The results for carotenoids, retinol, retinyl palmitate, and γ-tocopherol remained similar after adjustment for serum concentrations of total lipids, body mass index at age 21 years (for CLUE II only), hours since last meal, and years of schooling. In CLUE I, the odds ratio estimates for α-tocopherol remained similar for the second–fourth fifths but were slightly attenuated for the highest fifth (odds ratio = 0.71, 95 percent confidence interval: 0.36, 1.42) after adjustment for serum total lipids; in the CLUE II study, results were unchanged after adjustment. The association between ascorbic acid and prostate cancer did not change after adjustment for hours since last meal and years of education in the CLUE II study. Results remained very similar after we excluded from the analyses cases diagnosed within 2 years of blood donation and their matched controls. Exclusion of men who used multivitamin supplements within 48 hours before blood draw had only a trivial effect on the observed associations, suggesting that the associations were not due to recent supplement use. In CLUE II, results also remained similar after exclusion of multivitamin, vitamin C, or vitamin E supplement users identified through food frequency questionnaires. Stratification analyses by age at diagnosis, disease stage at diagnosis, years since blood draw, cigarette smoking, or body mass index at age 21 years produced results similar to those for the total group with few exceptions that could be attributed to chance variation.

DISCUSSION

We examined the associations between prediagnostic blood concentrations of micronutrients and subsequent risk of developing prostate cancer among participants in two population-based cohorts, CLUE I and CLUE II, with a median follow-up period of 17 years and 3.5 years, respectively. With the exception of γ-tocopherol, none of the antioxidant micronutrient concentrations was consistently associated with a decreased risk of developing prostate cancer in the two cohorts. For γ-tocopherol, a strong dose-response trend was observed among CLUE II participants but not CLUE I participants.

In the CLUE I and CLUE II studies, information was available on vitamin supplement use within the 48 hours prior to blood draw. Regular users were likely to be captured by this approach, but duration of use was not determined. In CLUE II, additional information on supplement use in the past year was available from the food frequency questionnaire administered at baseline. Restriction of analyses to nonvitamin users identified by either one or both approaches did not alter the observed associations.

Our findings for α-carotene, β-carotene, total carotene, lutein, β-cryptoxanthin, lycopene, and ascorbic acid are

TABLE 4. Matched odds ratios and 95% confidence intervals for prostate cancer risk, by fifths of serum micronutrient concentrations, Washington County, Maryland

Micronutrient	Lowest fifth			2nd fifth				3rd fifth				4th fifth				Highest fifth				*p* for trend
	Cases	Controls	Matched OR*	Cases	Controls	Matched OR	95% CI*	Cases	Controls	Matched OR	95% CI	Cases	Controls	Matched OR	95% CI	Cases	Controls	Matched OR	95% CI	
CLUE I cohort (1974–1996)†																				
α-Carotene (μg/dl)	41	77	1.00	29	56	1.04	0.54, 1.98	30	51	1.20	0.63, 2.32	29	60	0.93	0.49, 1.77	30	63	0.93	0.49, 1.78	0.61
β-Carotene (μg/dl)	33	70	1.00	50	74	1.47	0.84, 2.55	23	73	0.66	0.34, 1.27	43	72	1.19	0.65, 2.19	33	74	0.94	0.50, 1.77	0.59
Total carotene (μg/dl)	36	72	1.00	31	73	0.84	0.47, 1.55	51	71	1.57	0.87, 2.84	33	74	0.92	0.50, 1.68	31	73	0.90	0.48, 1.68	0.57
β-Cryptoxanthin (μg/dl)	29	74	1.00	36	71	1.39	0.76, 2.57	46	74	1.71	0.93, 3.14	38	68	1.55	0.83, 2.90	33	72	1.25	0.65, 2.38	0.93
Lutein (μg/dl)	33	75	1.00	31	71	1.03	0.56, 1.89	32	68	1.10	0.58, 2.08	46	74	1.45	0.81, 2.60	40	75	1.26	0.70, 2.26	0.30
Lycopene (μg/dl)	41	71	1.00	36	73	0.86	0.51, 1.47	31	73	0.74	0.41, 1.33	39	71	0.96	0.55, 1.67	35	73	0.83	0.46, 1.48	0.72
Retinol (μg/dl)	5	73	1.00	39	71	1.15	0.66, 2.02	44	74	1.26	0.73, 2.17	34	74	0.95	0.53, 1.69	30	72	0.86	0.49, 1.53	0.48
Retinyl palmitate (μg/dl)	42	63	1.00	28	64	0.50	0.26, 0.96	33	64	0.63	0.32, 1.22	31	65	0.50	0.27, 0.94	25	65	0.42	0.21, 0.83	0.04
α-Tocopherol (mg/dl)	43	75	1.00	37	70	0.93	0.54, 1.62	34	73	0.80	0.45, 1.41	44	75	0.99	0.58, 1.70	24	71	0.58	0.31, 1.06	0.11
γ-Tocopherol (mg/dl)	41	78	1.00	35	68	0.99	0.57, 1.72	39	65	1.14	0.66, 1.97	39	86	0.86	0.51, 1.45	28	67	0.77	0.42, 1.43	0.30
CLUE II cohort (1989–1996)‡,§																				
α-Carotene (μg/dl)	20	47	1.00	29	51	1.32	0.64, 2.73	23	56	0.97	0.45, 2.09	34	58	1.27	0.63, 2.54	23	54	1.11	0.52, 2.36	0.98
β-Carotene (μg/dl)	22	57	1.00	29	59	1.32	0.65, 2.68	36	52	1.92	0.96, 3.85	24	59	1.05	0.51, 2.18	31	57	1.47	0.74, 2.92	0.60
Total carotene (μg/dl)	33	56	1.00	41	56	1.21	0.63, 2.32	14	59	0.41	0.19, 0.86	19	58	0.56	0.28, 1.12	35	55	1.06	0.56, 2.02	0.65
β-Cryptoxanthin (μg/dl)	34	59	1.00	22	55	0.69	0.36, 1.33	33	54	1.03	0.55, 1.90	21	58	0.62	0.32, 1.21	31	58	0.90	0.48, 1.70	0.82
Lutein (μg/dl)	33	54	1.00	44	60	1.19	0.65, 2.18	15	53	0.45	0.22, 0.94	24	57	0.67	0.34, 1.32	26	60	0.68	0.35, 1.32	0.07
Lycopene (μg/dl)	33	57	1.00	29	56	0.88	0.45, 1.70	26	58	0.77	0.40, 1.47	28	57	0.83	0.42, 1.62	26	56	0.79	0.41, 1.54	0.49
Retinol (μg/dl)	24	56	1.00	34	57	1.36	0.71, 2.58	36	58	1.40	0.75, 2.61	16	57	0.63	0.29, 1.36	32	56	1.29	0.65, 2.54	0.91
Retinyl palmitate (μg/dl)	29	56	1.00	21	52	0.89	0.44, 1.80	20	52	0.78	0.38, 1.59	29	52	1.11	0.56, 2.18	29	54	1.26	0.64, 2.47	0.27
α-Tocopherol (mg/dl)	32	58	1.00	31	54	1.03	0.56, 1.88	25	60	0.74	0.39, 1.43	29	55	0.92	0.49, 1.75	25	57	0.78	0.41, 1.50	0.46
γ-Tocopherol (mg/dl)	33	55	1.00	50	67	1.27	0.72, 2.24	25	50	0.77	0.40, 1.50	25	62	0.56	0.28, 1.10	9	50	0.21	0.08, 0.54	<0.001
Ascorbic acid (μg/dl)	30	55	1.00	31	57	0.98	0.54, 1.78	26	55	0.86	0.45,1.62	21	57	0.66	0.33, 1.32	31	55	1.02	0.54, 1.92	0.76

* OR, odds ratio; CI, confidence interval.

† CLUE I quintiles—α-carotene: 1.4, 1.9, 2.7, 3.9; β-carotene: 4.4, 7.0, 9.8, 15.6; total carotene: 39.7, 50.7, 65.3, 83.0; β-cryptoxanthin: 2.6, 4.1, 7.7, 12.6; lutein: 9.2, 11.9, 14.5, 19.1; lycopene: 21.7, 31.1, 41.1, 54.9; retinol: 55.1, 62.9, 70.9, 80.2; retinyl palmitate: 5.0, 6.9, 10.0, 16.2; α-tocopherol: 0.96, 1.11, 1.25, 1.55; γ-tocopherol: 0.16, 0.21, 0.27, 0.35.

‡ Results of α-tocopherol and γ-tocopherol in 117 matched sets, of which toenail samples were available for selenium analysis, have been reported previously (17).

§ CLUE II quintiles—α-carotene: 1.2, 2.0, 3.0, 5.5; β-carotene: 4.2, 6.7, 10.3, 15.8; total carotene: 40.0, 53.2, 63.6, 79.1; β-cryptoxanthin: 3.3, 5.6, 8.5, 12.1; lutein: 8.1, 10.7, 12.8, 16.0; lycopene: 24.3, 35.2, 48.8, 62.8; retinol: 55.7, 64.0, 72.2, 80.7; retinyl palmitate: 6.7, 8.9, 11.9, 17.0; α-tocopherol: 1.04, 1.20, 1.39, 1.75; γ-tocopherol: 0.18, 0.26, 0.32, 0.41; ascorbic acid: 0.867, 1.131, 1.295, 1.545.

consistent with previous evidence (10, 12–14, 16) that suggests no association with prostate cancer risk. Lycopene has recently received considerable attention regarding a possible protective association against prostate cancer. Of the three previous reports (table 1), only one found a statistically borderline significant decreased risk, which was limited to aggressive cases of prostate cancer (16). A previous study of the first 103 cases of prostate cancer in the CLUE I cohort (14) found a statistically nonsignificant lower risk of prostate cancer in men whose serum lycopene concentrations were in the highest fourth level. In the present study, the cutoff points for the fifths of lycopene concentrations in controls were similar to those in the study by Gann et al. (16), but we did not observe an association of circulating lycopene concentrations with prostate cancer risk. Lycopene is thought to be important primarily because of the higher rate constant, as compared with several other carotenoids, in quenching singlet oxygen in in vitro photosensitization reactions (22). It is unknown whether significant numbers of singlet oxygen molecules occur in biologic systems, particularly in prostate tissue. The fate and the impact of the lycopene molecules attached with singlet oxygen are also unknown. Whether and how the chemopreventive effects of lycopene would emerge only at late stages of prostate cancer (16) is particularly intriguing.

Retinyl esters (such as retinyl palmitate, retinyl stearate, and retinyl oleate) are the primary storage forms of vitamin A (23–25). Retinyl palmitate is formed and stored mostly in the liver (23), and it accounts for 80–90 percent of stored vitamin A (25). In serum or plasma, retinyl palmitate was found to be the predominant retinyl ester, followed by retinyl stearate (18, 26). Approximately 4 hours after using vitamin A supplements, circulating levels of retinyl palmitate and retinyl stearate increase transiently (26). On the other hand, long-term use of vitamin A supplements (>5,000 IU/day) leads to a consistent elevation in circulating retinyl palmitate (27, 28). Retinyl esters have been linked to functionality in the vision cycle and may protect the retina from night blindness (23, 29). Functional dependence on vitamin A also occurs in the tracheobronchial tract (30). It is unknown whether retinyl palmitate alone or following conversion to retinol or retinol metabolites (retinoic acid or others) has functional importance in the prostate tissue.

Previous studies on retinol and prostate cancer have produced inconsistent results (10, 14, 16), which may be in part due to small sample sizes in some studies. The study based on the first 103 cases in the CLUE I cohort suggested a protective association between circulating retinol concentrations and prostate cancer (14). However, we did not replicate the finding in this larger study with cases diagnosed 10 or more years following blood donation (CLUE I) or cases diagnosed within 7 years of blood donation (CLUE II).

We did not observe an overall protective association against prostate cancer with higher α-tocopherol concentrations, a result consistent with some previous studies (10, 13, 14, 16). Unlike some other studies suggesting that serum α-tocopherol level is particularly important for smokers (12) and for cases with aggressive prostate cancer (16), we did not observe such associations in the subgroups. These findings may be due to the generally low intake of α-tocopherol in the US diet (typically 7–9 mg/day in adults); α-tocopherol is the major or sole ingredient in vitamin E supplements, and levels are high in certain types of vegetable oil, for example, wheat germ oil, sunflower oil, and cottonseed oil (31). Previous studies have suggested that supplementation with vitamin E in doses higher than those consumed from food may confer benefits on reducing the risk of prostate cancer. In the Alpha-Tocopherol Beta-Carotene (ATBC) trial of Finnish heavy smokers, a 32 percent reduction in prostate cancer incidence and a 41 percent reduction in prostate cancer mortality were observed in the group that used α-tocopherol supplements of 50 mg/day for 5–8 years compared with the placebo group (32); a statistically nonsignificant protective association was observed between serum α-tocopherol levels at baseline and subsequent prostate cancer risk, but only in the group that received α-tocopherol supplementation (15). Vitamin E supplementation was also associated with a lower prostate cancer risk in a case-control study (33) and a prospective study in which the association for metastatic or fatal prostate cancer was limited to current smokers or recent quitters (34). We previously reported possible interactive associations of α-tocopherol, γ-tocopherol, and selenium with respect to prostate cancer risk (17). It appears that benefits, yet to be confirmed, of α-tocopherol on prostate cancer may be acquired from supplements of doses higher than those from diet or from simultaneously adequate levels of other nutrients such as γ-tocopherol and/or selenium.

This expanded analysis of the role of γ-tocopherol in prostate cancer showed differences between the CLUE I and CLUE II cohorts, with stronger associations observed in CLUE II. In 1989, 4 percent of the cases and 8 percent of the controls ever used vitamin E supplements regularly. Although α-tocopherol supplement use decreases circulating levels of α-tocopherol, because use was less common in cases than in controls, it is unlikely that the lower α-tocopherol concentration in cases was a consequence of α-tocopherol supplement use. We know of only two previous prospective studies that have examined the association of serum γ-tocopherol with prostate cancer risk. A study of 142 sets of Japanese-American men (13), during a study period similar to CLUE I, reported a nonsignificant inverse association, a result similar to our findings from CLUE I. In contrast, a study among US physicians observed no association (16). The potential role of γ-tocopherol in prostate cancer prevention was supported by several in vitro studies showing γ-tocopherol to be more effective than α-tocopherol in inhibiting prostate cancer cells (35), reducing oxidative DNA damage as measured by sister chromatid exchange (36), and scavenging electrophilic mutagens such as peroxinitrite, a potent oxidizing nitrating compound (37).

Tests of prostate-specific antigen (PSA) have been increasingly used in prostate cancer screening and diagnosis since the early 1990s. Frequent use of screening may result in detection of disease in the early stages. In the present study, we found no evidence of overdiagnoses of early-stage prostate cancer by using PSA tests and digital rectal examinations. In CLUE I, we observed no important changes in the association between micronutrients and prostate cancer after excluding cases diagnosed before 1990. In CLUE II (1990–

1996), 92 percent of the cases and 64 percent of the controls who responded to a survey questionnaire in 1996 reported ever having a PSA test; 94 percent of the cases and 82 percent of the controls reported ever having a digital rectal examination. Among the prostate cancer cases who ever underwent a PSA test, 14 percent, 54 percent, and 31 percent were diagnosed to have stage 0 or 1, stage 2, and stage 3 or 4 disease, respectively—the same frequency distribution applied to digital rectal examination. No statistically significant difference was found in disease stage by PSA testing ($p = 0.77$).

An interesting aspect of this study is the difference in time from blood draw to clinical diagnosis for CLUE I and CLUE II participants. Duration of preclinical asymptomatic prostate cancer in Caucasians was estimated to be at least 11–12 years (38). Whether inconsistencies in the results of γ-tocopherol and retinyl palmitate between the two cohorts can be attributed to chance or other factors such as secular changes in diet over time need to be determined by additional studies. With respect to potential changes in diet over time, changes in fat intake from the 1970s to the late 1980s among the study participants could have accounted for inconsistencies in the γ-tocopherol results. For example, polyunsaturated fat, including corn oil and soybean oil, both of which contain the highest amount of γ-tocopherol compared with other types of oil, has been increasing in the US diet since 1970 (39). In the present study, circulating levels of γ-tocopherol were indeed higher in 1989 than in 1974, particularly among controls. Replication of these findings in other studies with prolonged follow-up is needed to improve our understanding of potentially modifiable factors in the prevention of prostate cancer.

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