The Association between Lung and Prostate Cancer Risk, and Serum Micronutrients: Results and Lessons Learned from β-Carotene and Retinol Efficacy Trial[1]

Gary E. Goodman,[2] Steven Schaffer, Gilbert S. Omenn, Chu Chen, and Irena King

The Fred Hutchinson Cancer Research Center, Seattle, Washington 98109 [G. E. G., S. S., C. C., I. K.]; Swedish Medical Center Cancer Institute, Seattle, Washington [G. E. G.]; and University of Michigan, Ann Arbor, Michigan [G. S. O.]

Abstract

β-Carotene and Retinol Efficacy Trial is a nationwide chemoprevention trial that recruited 18,314 high-risk individuals to test the effect of supplemental β-carotene and retinol on lung cancer incidence. In this report, we conducted a prospective nested case-control study of the association between serum carotenoids, retinoids, and tocopherols on both lung and prostate cancer incidence. Prerandomization serum samples were selected from 278 lung cancer cases and 205 prostate cancer cases, and 483 controls matched by high-risk population, study center location, age, sex (lung cancer only), smoking status, and year of randomization. Carotenoids, retinoids, and tocopherols were analyzed by high-performance liquid chromatography. Endpoints were confirmed by pathology review (lung cancer) or review of the pathology report (prostate cancer). In the control-only population, there was a significant association between tobacco use and serum micronutrient concentration. Current smokers compared with former smokers had lower mean levels of all of the micronutrients tested with zeaxanthin, β-cryptoxanthin, α-carotene, α-tocopherol, retinol, and retinyl palmitate reaching statistical significance at $P = 0.05$. In the overall population, the mean serum concentrations of all of the micronutrients except γ-tocopherol were lower for lung cancer cases than controls. Statistically significant trends across quartiles were observed in lutein ($P = 0.02$), zeaxanthin ($P = 0.02$), and α-tocopherol ($P = 0.03$). The carotenoid findings in the overall population were because of the strong inverse association between serum micronutrients and lung cancer in females. Statistically significant odds ratios (ORs) comparing 4th to 1st quartiles in the female population were seen in lutein [OR, 0.31; confidence interval (CI), 0.13–0.75], zeaxanthin (OR, 0.31; CI, 0.12–0.77), and β-cryptoxanthin (OR, 0.34; CI, 0.14–0.81). For prostate cancer, mean serum concentrations were lower in cases for all of the nutrients except α-carotene. Only for α-tocopherol ($P_{trend} = 0.04$) were the findings statistically significant. There was no statistically significant association between serum carotenoids and prostate cancer. Our findings provide additional support for the association between physiological levels of dietary micronutrients and cancer incidence.

Introduction

Individuals whose diets are high in fresh fruits and vegetables have a lower incidence of many cancers than those with a diet low in these food groups. It has been hypothesized that this association is because of the presence of naturally occurring micronutrients or trace compounds, which act as inhibitors of carcinogenesis. If the food constituents responsible for this "prevention" activity can be identified, modification of the diet to include foods rich in these compounds or supplementation with the specific agent(s) may be an effective method of cancer prevention. The challenge has been to identify the relevant dietary constituents.

One method used to identify candidate prevention nutrients is the analysis and comparison of serum obtained from individuals who later developed cancer with those from matched healthy controls. Using this methodology, inverse correlations have been described between the serum concentration of many dietary micronutrients and cancer incidence. In the 1970s and 1980s these types of analyses pointed to β-carotene as the micronutrient with the most consistent evidence of playing a role in the cancer prevention activity attributed to diet (1–3). Individuals with a high intake of β-carotene-rich foods and those who had high serum concentrations of β-carotene had a 40–60% lower incidence of cancer than those with a low dietary intake or serum concentrations (4–7). The consistency of these studies was part of the rationale to initiate clinical trials testing the effect of moderate-dose supplemental β-carotene on the incidence of lung, skin, colon, and total cancer. However, the findings of these trials were uniformly discouraging (8–10). Two lung cancer prevention trials, the ATBC[3] (11), testing α-tocopherol (50 mg/day) and β-carotene (20 mg/day) and the CARET, testing β-carotene (30 mg/day) plus vitamin A (25,000 IU/day; Ref. 12), found the incidence of lung cancer to

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[1] This work was supported by Grant 2 U01 CA63673 from the National Cancer Institute.
[2] To whom requests for reprints should be addressed, at Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, MP-859, Seattle, WA 98109. Phone: (206) 667-5722; Fax: (206) 667-5964; E-mail: gary.goodman@swedish.org.

[3] The abbreviations used are: ATBC, Alpha Tocopherol Beta Carotene; CARET, β-Carotene and Retinol Efficacy Trial; HPLC, high-performance liquid chromatography; OR, odds ratio; CI, confidence interval; PHS, Physicians' Health Study.

be higher (18% and 28%, respectively) in the groups receiving β-carotene than in those who received a placebo.

An important legacy of these large clinical intervention trials has been the establishment of blood and tissue specimen banks. These remain as valuable resources to additionally explore the association between micronutrients and cancer. As part of the CARET lung cancer prevention trial, we collected and stored serum on all of the participants before randomization. In the analysis reported here we re-examine the serum concentrations of micronutrients in 278 individuals who developed lung cancer, 205 who developed prostate cancer, and 483 matched controls. This analysis includes all of the major carotenoids, α- and γ-tocopherol, retinol, and retinyl palmitate. We have already reported our findings with selenium (13), and the results for folic acid and B12 will be reported separately. We have also studied the variables of sex, age, smoking history, and the temporal relationship between the time of serum sampling to the diagnosis of lung cancer. Our findings in conjunction with results of the β-carotene intervention trials have caused us to re-evaluate the interpretation of this type of serum based epidemiological investigation.

Materials and Methods

CARET is a randomized, double-blind, placebo-controlled, lung cancer chemoprevention trial of β-carotene 30 mg and retinol 25,000 IU/day. CARET randomized members of two high-risk groups: asbestos-exposed workers, who were men 45–69 years of age with occupational exposure to asbestos at least 15 years before randomization, with either a chest radiograph positive for asbestos-related disease or a 5-year work history in specific high-risk trades, and who were either currently smoking or had smoked within the previous 15 years; and heavy smokers, who were men and women 50–69 years of age with at least 20 pack-years of cigarette smoking and either currently smoking or had quit within the previous 6 years. All of the participants were cancer-free for 5 years or more and were not taking any β-carotene supplement, and vitamin A supplements were ≤5,500 IU/day. At the first visit, height, weight, and blood pressure were measured, and participants self-reported whether they were currently taking supplemental vitamins (yes or no), their smoking status (current or former) and intensity (pack-years), ethanol use, and their race. A food frequency questionnaire was also completed. Enrollment to two pilot trials began in 1983. After showing effective recruitment strategies and no detectable toxicity, the study was expanded to include five additional study centers. Enrollment ended in April 1994. A total of 4,060 male asbestos workers and 14,254 heavy smokers (44% of whom were women) were randomized at six study centers around the United States. The design of CARET projected active intervention until the end of 1997. At an interim analysis in late 1995, an increased risk of both lung cancer and cardiovascular disease was observed in the active intervention arm. In conjunction with the National Cancer Institute, and the Safety and Data Monitoring Committee, the CARET Steering Committee made the decision to end the active intervention 21 months early in January 1996. Participants continue to be followed for cancer and death outcomes postintervention.

CARET has a well-established end point assessment protocol (14). During the intervention phase of CARET, when a cancer end point was reported by a participant, the medical records and pathology reports were obtained. Central CARET pathology review was conducted on all of the lung cancer cases (the CARET primary end point). For prostate cancer, surgical pathology reports from the diagnosing institution were reviewed. End point materials were reviewed independently by three physician adjudicators. A consensus was required on the site of the primary cancer, histology, and date of diagnosis to consider the end point confirmed. As of December 31, 1995, 423 CARET participants (291 men and 132 women) had developed a confirmed histological diagnosis of lung cancer, and 332 male participants had developed prostate cancer.

At the time of the first CARET study center visit, all of the participants had nonfasting blood collected in foil-covered vacutainers, which were centrifuged to separate serum. Serum aliquots of 2.0 and 0.5 ml were stored in amber glass vials at −25°C degrees for a maximum of 48 h before being placed in long-term storage at −70°C. At the time of this analysis, constraints imposed upon the CARET serum bank allowed only samples with a minimum of 3 aliquots of never-thawed serum to be chosen. Prerandomization blood draw samples were available for 278 lung cancer cases and 205 prostate cancer cases. The cases were independently matched with 278 and 205 controls, respectively, on high-risk population (asbestos or heavy smoker), study center location at randomization, age at randomization (within 5-year age intervals *i.e.*, 45–49, 50–54, and so forth), sex, smoking status at randomization (former or current), and year of randomization. In the lung cancer analysis, 1 case and 1 control had invalid results for all of the analytes. These observations along with their respective matched control observation were removed from the analysis, leaving 276 matched pairs for the lung cancer analysis. The mean time from blood draw to lung and prostate cancer diagnosis is 2.9 and 3.0 years, respectively.

To assess the demographics of serum micronutrients in cancer-free CARET participants, we pooled the data from the participants selected as controls for the lung and prostate cancer case-control studies. However, at the time of statistical analysis, we found that 65 participants had been selected independently as a control for each study. The serum samples had also been analyzed independently. In these participants there was no statistically significant difference in micronutrient values between the two sets of laboratory analyses. To not count these individuals twice, only one set of serum results, randomly selected, was included in the control participant analyses. One participant with no history of smoking and the control with invalid results for all of the analytes were excluded leaving a total of 416 participants for the control-only analysis.

Micronutrient/Vitamin Assay

HPLC Analysis. The extraction of analytes from serum, the quality control parameters, and the HPLC methods for the serum micronutrients have been published previously (15). The HPLC method was modified to include a complete profile of carotenoids, retinoids, and tocopherols in a single HPLC run. Briefly, a hexane extract of 0.4 ml of serum was injected onto a 3-μm C-18 Spherisorb ODS-2 HPLC column and eluted with an isocratic solvent consisting of 73% acetonitrile, 12% tetrahydrofuran, 8% methanol, 7% water, 0.025% ammonium acetate, and 0.05% diethylamine (v/v) at 1.2 ml/min. Lutein, zeaxanthin, β-cryptoxanthin, and lycopene were detected at 476 nm, α-carotene and β-carotene at 452 nm, retinol and retinyl palmitate at 325 nm, and α-tocopherol and γ-tocopherol were detected at 292 nm. The HPLC was a fully automated Hewlett-Packard (Avondale, PA) 1050 HPLC system equipped with quaternary pumps, electronic degasser, insulated column housing, automatic sampler diode array detector, and software to run the system and perform data management. The coefficient of

variation for the pooled quality control samples for all of the analytes was ≤10%.

Statistical Methods. Exploratory analysis indicated that serum concentration values were highly skewed and were log-transformed to comply with normality assumptions. In the control-only analysis, arithmetic means of the transformed values, back-transformed to the original units, (and, hence, equal to the geometric mean of the untransformed values) are given for these measures. Tests for heterogeneity were performed using ANOVA. For the matched analysis, univariate statistics (proportions) were used to describe the distributions of key factors among subgroups of the population. Differences in log-transformed means were assessed using a paired *t* test. Cases and controls were placed into quartiles based on the respective overall control serum micronutrient levels. Sex-specific control quartiles were also examined but did not significantly change the ORs or results of the statistical tests in the sex-specific models. Conditional logistic regression stratified on the matched set was used to obtain ORs of cancer and to test for linear trends. For lung cancer, to control for the duration and intensity of smoking we included covariates for pack-years and years since quitting smoking in the conditional logistic regression models. Covariates to control for intervention arm, BMI, alcohol use, supplemental vitamins, asbestos exposure and the seasonal effects on carotenoid intake were tested but were not statistically significant, and were not included in the model. Interactions between these and other covariates in the model were not statistically significant. For prostate cancer, none of these covariates nor any interaction was statistically significant. An ordinal score variable was created to test for a linear trend in ORs across serum micronutrient quartiles assuming equal increments in the log odds from one quartile to the next. Likelihood ratio tests were used in testing for the statistical significance of an interaction between the micronutrients and sex. All of the analyses were performed using SAS Version 6.12 (SAS Institute 1989).

Results

The demographics for our lung and prostate cancer populations, and their controls are seen in Table 1. For the lung cancer matched case and control population, participants were predominantly white (93%), male (63%), and in the heavy smoker exposure population (78%). Fifty-eight percent of cases and 51% of controls were in the active intervention arm, and 70% were current smokers at blood draw. The mean pack-years of smoking was 57.7 for cases and 48.5 for controls. Thirty-seven percent of controls had <40 pack-years of smoking compared with 18% of cases; 40% of cases had >59 pack-years of smoking compared with 24% of controls. For the prostate cancer-matched case and control population, participants were primarily white (case 89% and control 92%) and from the heavy smoker exposure population (66%). Forty-eight percent of cases and 46% of controls were in the active intervention arm. The mean pack-years of smoking for cases was 51.9 and 50.9 for controls, with a similar distribution of pack-years; 56% of cases and controls were current smokers at blood draw.

In our first set of analyses we studied the determinants of serum micronutrients in the control populations. The combined lung and prostate control-only participants were predominantly white (93%), male (75%), and from the heavy smoker population (74%). At the time of blood draw, 65% were current smokers, and 39% reported using supplemental vitamins. The association between the smoking history of the control participants and micronutrient serum concentrations is seen in Table

Table 1 Characteristics of the lung and prostate cancer case-control study participants

Variable	Lung cancer		Prostate cancer	
	Case (%)	Control (%)	Case (%)	Control (%)
Sex				
Male	174 (63)	174 (63)	205 (100)	205 (100)
Female	102 (37)	102 (37)	—	—
Race				
White	256 (93)	257 (93)	183 (89)	189 (92)
Black	11 (4)	10 (4)	16 (8)	9 (4)
Other/unknown	9 (3)	9 (3)	6 (3)	7 (4)
Exposure population				
Asbestos	60 (22)	60 (22)	69 (34)	69 (34)
Heavy smoker	216 (78)	216 (78)	136 (66)	136 (66)
Intervention arm				
Combination	161 (58)	140 (51)	99 (48)	94 (46)
Placebo	115 (42)	136 (49)	106 (52)	111 (54)
Age group				
45–49	2 (1)	2 (1)	1 (<1)	1 (<1)
50–54	48 (17)	48 (17)	18 (9)	18 (9)
55–59	62 (22)	62 (22)	38 (19)	38 (19)
60–64	93 (34)	93 (34)	72 (35)	72 (35)
65–69	70 (25)	70 (25)	69 (34)	69 (34)
70–74	1 (<1)	1 (<1)	7 (3)	7 (3)
Body mass index[a]				
Normal	170 (62)	146 (53)	100 (49)	84 (42)
Overweight	63 (23)	87 (32)	59 (29)	73 (36)
Obese	42 (15)	42 (15)	46 (22)	45 (22)
Alcohol use[b]				
Nondrinker	112 (40)	94 (37)	52 (27)	78 (42)
≤1 drink/day	88 (34)	95 (37)	75 (39)	60 (32)
>1 drink/day	68 (26)	68 (26)	64 (34)	48 (26)
Study center				
Baltimore	24 (9)	24 (9)	21 (10)	21 (10)
Irvine	43 (16)	43 (16)	23 (11)	23 (11)
New Haven	9 (3)	9 (3)	10 (5)	10 (5)
Portland	83 (30)	83 (30)	67 (33)	67 (33)
San Francisco	10 (4)	10 (4)	8 (4)	8 (4)
Seattle	107 (39)	107 (39)	76 (37)	76 (37)
Baseline smoking status				
Current	192 (70)	192 (70)	115 (56)	115 (56)
Former	84 (30)	84 (30)	89 (43)	89 (43)
Never	—	—	1 (<1)	1 (<1)
Pack-years[c]				
<40	51 (18)	101 (37)	69 (34)	69 (34)
40–59	116 (42)	109 (39)	71 (35)	74 (36)
>59	109 (40)	66 (24)	64 (31)	61 (30)
Years quit smoking (former smokers)				
<1	5 (6)	6 (7)	5 (6)	4 (4)
1	20 (24)	5 (6)	9 (10)	7 (8)
2	13 (15)	9 (11)	8 (9)	8 (9)
3	7 (8)	16 (19)	14 (16)	8 (9)
4	10 (12)	10 (12)	3 (3)	12 (13)
5	10 (12)	11 (13)	13 (15)	9 (10)
6	6 (7)	6 (7)	8 (9)	10 (11)
7+	13 (15)	21 (25)	29 (33)	31 (35)
Take supplemental vitamins[d]				
Yes	104 (39)	108 (41)	72 (38)	66 (35)
No	160 (61)	156 (59)	115 (62)	123 (65)

[a] Participants were grouped as normal (<27.8), overweight (27.8–31.1), and obese (>31.1). Two lung cancer and 3 prostate cancer participants are missing BMI data.
[b] Thirty-seven lung cancer and 38 prostate cancer participants are missing alcohol use data.
[c] Two prostate cancer participants missing pack-year data.
[d] Twenty-four lung cancer participants and 34 prostate cancer participants are missing supplemental vitamin data.

Table 2 Association between control participants' smoking history and micronutrient serum concentrations (ng/ml) geometric means

Analytes	Overall	Smoking Status[a]			Pack-years[b]			P^c	$P_{trend}{}^d$
	(1st and 3rd Quartile)	Former	Current	P^c	<40	40–60	>60		
Lutein	137 (104, 178)	144	133	0.07	146	136	126	0.02	0.004
Male	136 (105, 173)	142	132	0.09	150	133	126	0.01	0.002
Female	140 (103, 194)	152	137	0.35	139	146	132	0.77	0.91
Zeaxanthin	27.2 (21.0, 36.0)	29.6	26.0	0.004	28.6	26.6	26.3	0.23	0.12
Male	27.8 (21.0, 36.0)	29.6	26.7	0.04	30.7	26.6	26.4	0.02	0.01
Female	25.5 (20.0, 33.0)	29.4	24.3	0.08	24.9	26.3	25.4	0.84	0.71
β-Cryptoxanthin	58.0 (38.0, 88.0)	69.8	52.5	<0.001	65.5	57.5	50.4	0.004	<0.001
Male	55.3 (37.0, 84.0)	68.3	48.6	<0.001	64.8	54.8	48.0	0.003	0.001
Female	67.1 (49.0, 97.0)	77.7	64.0	0.14	66.1	66.5	72.7	0.86	0.66
Lycopene	305 (219, 437)	325	296	0.09	319	297	299	0.45	0.29
Male	303 (220, 431)	325	290	0.07	314	291	306	0.56	0.76
Female	313 (213, 464)	324	309	0.73	330	318	245	0.22	0.14
α-Carotene	31.1 (19.0, 49.5)	34.7	29.4	0.02	32.9	32.5	27.3	0.06	0.04
Male	29.0 (18.0, 45.0)	31.9	27.4	0.05	31.2	29.6	26.3	0.19	0.07
Female	38.6 (24.0, 61.0)	51.5	35.2	0.01	36.2	42.9	36.2	0.46	0.61
β-Carotene	148 (91, 236)	160	142	0.13	162	154	125	0.01	0.01
Male	136 (87, 213)	147	130	0.15	156	138	118	0.03	0.01
Female	194 (134, 287)	245	180	0.07	176	217	200	0.43	0.33
α-Tocopherol	13800 (10700, 16900)	14800	13200	0.003	13600	13900	13900	0.88	0.68
Male	13600 (10600, 16600)	14600	12900	0.005	13300	13700	13700	0.89	0.67
Female	14500 (11200, 17500)	15900	14000	0.14	14100	14600	15400	0.73	0.43
γ-Tocopherol	2300 (1700, 3500)	2470	2250	0.17	2230	2320	2460	0.47	0.22
Male	2500 (1800, 3600)	2602	2408	0.26	2470	2340	2670	0.27	0.35
Female	1900 (1400, 3000)	1929	1908	0.95	1840	2270	1340	0.07	0.60
Retinol	666 (572, 772)	695	651	0.01	662	673	661	0.79	1.00
Male	675 (580, 782)	699	660	0.04	681	675	667	0.84	0.55
Female	641 (545, 756)	678	629	0.18	628	665	616	0.46	0.79
Retinyl-palmitate	45.6 (27.0, 69.0)	53.1	42.0	0.01	42.4	48.8	45.6	0.37	0.45
Male	44.8 (26.0, 69.0)	52.6	40.6	0.01	40.4	47.6	46.0	0.41	0.33
Female	48.1 (29.0, 69.0)	55.3	45.6	0.27	46.3	52.5	42.5	0.59	0.96

[a] There are 144 (119 male, 25 female) former smokers and 272 (195 male, 77 female) current smokers.
[b] There are 146 (96 male, 50 female) participants with <40 pack-years, 157 (118 male, 39 female) participants with 40–60 pack-years, and 113 (100 male, 13 female) with >60 pack-years.
[c] P from test for heterogeneity.
[d] P from test for trend.

2. Serum concentrations of all of the micronutrients measured were numerically lower in current smokers *versus* former smokers overall, and achieved statistical significance for zeaxanthin ($P = 0.004$), β-cryptoxanthin ($P < 0.001$), α-carotene ($P = 0.02$), α-tocopherol ($P = 0.003$), retinol ($P = 0.01$), and retinyl-palmitate ($P = 0.01$). Male former smokers had lower serum concentrations than female former smokers for all of the micronutrients except zeaxanthin. In current smokers, males were lower for all except zeaxanthin, γ-tocopherol, and retinol. Male current smokers had significantly lower serum concentrations than former smokers for the same micronutrients as the overall population. However, in females the difference between current and former smokers was significant only for α-carotene ($P = 0.01$). In the overall population, there was a statistically significant inverse association between intensity of smoking (pack-years) and carotenoid serum concentrations for lutein ($P_{trend} = 0.004$), β-cryptoxanthin ($P_{trend} < 0.001$), α-carotene ($P_{trend} = 0.04$), and β-carotene ($P_{trend} = 0.01$). For the tocopherols and retinoids, serum levels are similar or higher in the higher pack-years of smoking. In males, similar associations were seen between current and former smokers with the addition that zeaxanthin ($P_{trend} = 0.01$) was also statistically significant and α-carotene ($P_{trend} = 0.07$) marginally statistically significant. Intensity of smoking and carotenoid serum concentrations were similar with zeaxanthin ($P = 0.01$) being statistically significant and α-carotene ($P = 0.07$) marginally statistically significant. No statistically significant associations or trends were seen for females.

Overall baseline geometric mean serum concentrations in the lung cancer cases were numerically lower than in controls for all of the micronutrients except γ-tocopherol (Table 3); among males, all of the geometric mean serum concentrations were lower, whereas among females γ-tocopherol and retinol were higher in cases than controls. Table 4 presents overall and sex-specific ORs of lung cancer by quartile of control micronutrient serum concentration. In the overall population, statistically significant downward trends across the quartiles in ORs were observed for lutein ($P = 0.02$), zeaxanthin ($P = 0.02$), and α-tocopherol ($P = 0.03$). In the point estimates, statistically significant 4th quartile to 1st quartile ORs were observed for lutein, zeaxanthin, and α-tocopherol. For males, a statistically significant downward trend was seen in retinol ($P = 0.046$). In the point estimates comparing 4th to 1st quartile, serum concentration of α-tocopherol (OR, 0.49; CI, 0.25–0.95) was significantly inversely associated with ORs of lung cancer. For females, statistically significant downward trends across quartiles in the ORs were observed in lutein ($P = 0.005$), zeaxanthin ($P = 0.007$), β-cryptoxanthin ($P = 0.005$), and marginal statistical significance in α-carotene ($P = 0.06$). In the point estimates, statistically significant 4th quartile ORs were seen for lutein, zeaxanthin, and β-cryptoxanthin. Only lutein had a statistically significant interaction with sex ($P = 0.04$). To assess

Table 3 Geometric means of micronutrient (ng/ml) distribution

	Lung cancer[a]			Prostate cancer[b]		
	Case	Control	P^c	Case	Control	P^c
Lutein						
Overall	121	137	0.0008			
Male	124	135	0.07	126	135	0.11
Female	115	141	0.002			
Zeaxanthin						
Overall	24.1	27.1	0.004			
Male	25.6	28.0	0.07	25.7	27.2	0.19
Female	21.8	25.5	0.02			
β-Cryptoxanthin						
Overall	51.6	59.1	0.01			
Male	51.6	54.8	0.39	53.5	54.8	0.69
Female	51.6	67.1	0.003			
Lycopene						
Overall	277	302	0.11			
Male	283	293	0.60	307	309	0.92
Female	267	320	0.04			
α-Carotene						
Overall	27.7	31.5	0.03			
Male	26.1	28.0	0.37	29.1	28.9	0.91
Female	30.5	38.6	0.001			
β-Carotene						
Overall	141	152	0.23			
Male	126	132	0.57	135	142	0.49
Female	169	194	0.20			
α-Tocopherol						
Overall	12900	13900	0.02			
Male	12500	13600	0.05	12600	13800	0.03
Female	13600	14500	0.22			
γ-Tocopherol						
Overall	2280	2220	0.60			
Male	2330	2420	0.56	2290	2430	0.35
Female	2200	1910	0.16			
Retinol						
Overall	651	669	0.18			
Male	650	687	0.03	670	674	0.80
Female	653	641	0.61			
Retinyl palmitate						
Overall	40.9	44.7	0.23			
Male	39.1	42.8	0.36	42.9	45.9	0.37
Female	44.2	48.1	0.40			

[a] Lung cancer distribution: overall $n = 552$ (case $n = 276$, control $n = 276$), male $n = 348$ (case $n = 174$, control $n = 174$), female $n = 204$ (case $n = 102$, control $n = 102$).
[b] Prostate cancer distribution: overall $n = 410$ (case $n = 205$, control $n = 205$).
[c] P from paired t test for difference between means.

the effects of preclinical disease on micronutrient levels, cases diagnosed with lung cancer <1 year after blood draw were identified ($n = 40$). Removing these cases and their matched controls from models showed negligible differences in OR.

For prostate cancer, Table 3 shows that geometric mean serum concentrations of all of the serum micronutrients except α-carotene were numerically lower for cases compared with controls; however, only the results for α-tocopherol ($P = 0.03$) were statistically significant. Table 5 presents ORs of prostate cancer by quartile of control micronutrient distribution. Only α-tocopherol exhibited a statistically significant downward linear trend across the quartiles ($P = 0.04$), with a protective OR of 0.59 (CI, 0.34–1.04) for the 4th quartile compared with the 1st quartile. Cases diagnosed with prostate cancer <1 year after blood draw were identified ($n = 22$). Removing these cases and their matched controls had a negligible effect on ORs. However, for α-tocopherol, the downward linear trend was no longer statistically significant ($P = 0.07$) with 4th quartile to 1st quartile OR of 0.60 (CI, 0.33–1.10).

Discussion

Literature from the 1970s and 1980s studying associations of diet and cancer incidence stressed the importance of carotenoids, primarily β-carotene (2, 3, 7, 16). More recent studies have suggested an inverse association between the incidence of both prostate and lung cancer, and the serum concentration of the carotenoid lycopene or a diet low in lycopene-containing food (17–22). Other investigators have reported an inverse association between both α- and γ-tocopherol, and prostate cancer (23–25). In our analysis we were able to measure all of the major serum carotenoids and tocopherols. We found the serum concentrations of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and α-tocopherol were all statistically significantly lower in the lung cancer population than in the age- and smoking-matched controls. Of these micronutrients, statistically significant inverse trends across quartiles were seen for lutein, zeaxanthin, and α-tocopherol. Notably, we did not see statistical evidence that lung cancer risk is inversely related to serum lycopene or β-carotene. Some of our findings are similar to that of Comstock *et al.* (26), who found an inverse association with lung cancer incidence and carotenoids but only "trivial differences" for lycopene, α-tocopherol, and selenium. Yuan *et al.* (22) reported recently their findings in 18,244 men, 45–64 years of age, living in Shanghai. Their results had some similarities to ours, although their population differed in many ways (37% were never-smokers and the population total serum carotenoids concentration was 510 *versus* 754 ng/ml in our population). As in our population, Yuan *et al.* (22) reported that smokers had a significantly lower concentration of all of the carotenoids than never-smokers (our population consisted of only current or former smokers). We both found the serum concentrations of many carotenoids were inversely associated with risk, but unlike our study, when Yuan *et al.* (22) corrected for smoking these findings were no longer significant. Yuan *et al.* (22) reported a significant inverse association between lung cancer and β-cryptoxanthin, but did not find any association between lung cancer risk and α-tocopherol serum concentration. Our study adds to the body of literature that suggests that the serum concentration of many carotenoids are inversely associated with lung cancer risk. This supports the view that a low dietary intake of foods rich in carotenoids as a whole is likely a risk factor for lung cancer. Whether this effect is because of a specific carotenoid or other co-consumed compounds remains unclear.

One of the new findings of this analysis is that in the 102 female lung cancer participants studied there was a strong inverse association between serum carotenoid concentration and lung cancer risk. The magnitude of the statistical significance of these results was much more dramatic than in the male population. In fact, the findings we report for the full sample size are because of the findings seen in the 102 females. The sex differences could not be explained by differences in diet, supplement use, high-risk population, or smoking history (data not shown). It has been reported previously that healthy females have higher serum β-carotene concentrations than matched male smokers, ex-smokers, or nonsmokers (27–30). When controlling for smoking we had similar findings in our control population. Females had significantly higher levels of all of the carotenoid analytes except for zeaxanthin. The mechanism of these sex differences is not known. It may be that the role of micronutrients in inhibiting tobacco-associated carcinogenesis

Table 4 Risks of lung cancer by quartile of control micronutrient (ng/ml) distribution[a]

	Quartile[b]				P_{trend}	$P_{interaction}$
	1st	2nd	3rd	4th		
Lutein						
Overall	1[c]	0.49 (0.30–0.80)	0.53 (0.32–0.87)	0.55 (0.33–0.93)	0.02	
Male	1	0.35 (0.19–0.66)	0.53 (0.28–0.99)	0.79 (0.40–1.57)	0.48	0.04
Female	1	1.01 (0.44–2.34)	0.55 (0.25–1.24)	0.31 (0.13–0.75)	0.005	
Zeaxanthin						
Overall	1	0.69 (0.43–1.11)	0.61 (0.37–1.02)	0.56 (0.33–0.95)	0.02	
Male	1	0.76 (0.40–1.44)	0.74 (0.39–1.41)	0.78 (0.40–1.51)	0.44	0.72
Female	1	0.62 (0.30–1.31)	0.46 (0.20–1.08)	0.31 (0.12–0.77)	0.007	
β-Cryptoxanthin						
Overall	1	0.98 (0.62–1.56)	0.49 (0.29–0.85)	0.76 (0.44–1.28)	0.07	
Male	1	1.25 (0.71–2.20)	0.70 (0.35–1.40)	1.21 (0.62–2.37)	0.97	0.32
Female	1	0.58 (0.25–1.35)	0.26 (0.10–0.64)	0.34 (0.14–0.81)	0.005	
Lycopene						
Overall	1	1.04 (0.64–1.69)	0.71 (0.43–1.17)	0.86 (0.52–1.43)	0.31	
Male	1	1.04 (0.58–1.88)	0.70 (0.37–1.34)	1.07 (0.56–2.05)	0.87	0.87
Female	1	1.09 (0.47–2.52)	0.72 (0.32–1.64)	0.61 (0.27–1.38)	0.16	
α-Carotene						
Overall	1	1.02 (0.62–1.69)	0.86 (0.52–1.42)	0.77 (0.45–1.32)	0.26	
Male	1	1.07 (0.58–1.98)	0.87 (0.47–1.63)	1.13 (0.58–2.22)	0.96	0.63
Female	1	0.88 (0.35–2.16)	0.74 (0.32–1.75)	0.43 (0.17–1.08)	0.06	
β-Carotene						
Overall	1	1.43 (0.88–2.32)	0.85 (0.49–1.45)	1.07 (0.63–1.83)	0.73	
Male	1	1.29 (0.72–2.32)	1.11 (0.57–2.15)	1.40 (0.71–2.74)	0.42	0.41
Female	1	1.71 (0.71–4.17)	0.53 (0.21–1.35)	0.72 (0.30–1.75)	0.11	
α-Tocopherol						
Overall	1	0.71 (0.42–1.18)	0.76 (0.46–1.26)	0.52 (0.31–0.88)	0.03	
Male	1	0.62 (0.33–1.18)	0.86 (0.47–1.59)	0.49 (0.25–0.95)	0.09	0.91
Female	1	0.83 (0.35–1.99)	0.63 (0.25–1.54)	0.57 (0.23–1.38)	0.17	
γ-Tocopherol						
Overall	1	0.94 (0.58–1.52)	1.00 (0.62–1.61)	0.95 (0.58–1.57)	0.92	
Male	1	0.87 (0.46–1.63)	0.80 (0.43–1.47)	0.73 (0.37–1.44)	0.34	0.84
Female	1	1.01 (0.48–2.14)	1.43 (0.65–3.16)	1.32 (0.62–2.82)	0.35	
Retinol						
Overall	1	0.62 (0.37–1.01)	0.59 (0.36–0.96)	0.69 (0.42–1.14)	0.11	
Male	1	0.53 (0.28–1.00)	0.49 (0.27–0.92)	0.54 (0.29–1.01)	0.046	0.88
Female	1	0.79 (0.35–1.78)	0.77 (0.34–1.75)	1.05 (0.46–2.37)	1.00	
Retinyl palmitate						
Overall	1	1.39 (0.84–2.30)	1.19 (0.71–1.98)	0.73 (0.42–1.28)	0.24	
Male	1	1.70 (0.90–3.23)	1.63 (0.86–3.09)	0.66 (0.33–1.33)	0.39	0.48
Female	1	0.98 (0.42–2.28)	0.69 (0.28–1.65)	0.77 (0.30–1.99)	0.41	

[a] OR from conditional logistic regression model stratified on exposure population, study center at randomization, age at randomization within 5-year intervals, sex, smoking status at randomization, and year of randomization, and controlling for pack-years of smoking and years quit smoking.
[b] Quartile cutpoints for lutein (106, 138, 175), zeaxanthin (21.0, 27.0, 36.0), β-cryptoxanthin (39.5.0, 60.0, 87.0), lycopene (213, 319, 437), α-carotene (19.0, 31.0, 51.5), β-carotene (87, 158, 255), polar carotenoids (184, 233, 289), nonpolar carotenoids (382, 533, 741), α-tocopherol (10760, 13016, 17038), γ-tocopherol (1687, 2416, 3417), retinol (577, 660, 777), and retinyl palmitate (25.0, 37.5, 69.5).
[c] Comparison quartile.

in females is more important than in males. This raises the hypothesis that dietary interventions may be more effective in females than males.

Smoking status is an important potential confounder of studies of serum micronutrients and cancer. We have reported previously in the CARET population that, at the time of enrollment, current cigarette use and years since quitting were major determinants of β-carotene serum concentration (28, 31). Other investigators have also reported an inverse association between current smoking and serum β-carotene (27, 31). In the analysis of the control participants reported here we found that current smoking was associated with numerically lower mean serum concentrations of all of the micronutrients we measured (Table 2). When analyzing by pack-years, lutein, β-cryptoxanthin, and α- and β-carotene were associated with statistically significant inverse relationships. Additional studies with a larger sample size will be needed to confirm these findings. It has been suggested that oxidants in cigarette smoke can act as an oxidative stress and may lower serum β-carotene concentrations (32–34). It is possible that this effect may also occur with the other serum carotenoids as well as other antioxidant micronutrients.

We also found the serum concentration of α-tocopherol ($P_{trend} = 0.03$) but not γ-tocopherol ($P_{trend} = 0.68$) to be lower in lung cancer patients than in matched controls. This was similar to an analysis of preintervention serum α-tocopherol concentration in ATBC participants (35). In that study of 1144 lung cancer cases the incidence of lung cancer was reported to be 19% less in the highest *versus* the lowest quartile of serum α-tocopherol ($P_{trend} = 0.09$). Other studies of the association between lung cancer and serum α-tocopherol have been mixed. Some have shown an inverse relationship (7, 26, 36), whereas others have shown no association (6, 22, 37, 38). It should be noted that all of our findings and those of ATBC were con-

Table 5 Risks of prostate cancer by quartile of control micronutrient (ng/ml) distribution[a]

	Quartile[b]				P_{trend}
	1st	2nd	3rd	4th	
Lutein	1[c]	0.94 (0.55–1.60)	0.77 (0.44–1.35)	0.86 (0.48–1.52)	0.44
Zeaxanthin	1	0.91 (0.53–1.55)	0.88 (0.52–1.49)	0.78 (0.43–1.41)	0.42
β-Cryptoxanthin	1	0.98 (0.58–1.66)	1.09 (0.62–1.91)	0.81 (0.46–1.43)	0.58
Lycopene	1	0.65 (0.36–1.15)	0.47 (0.26–0.87)	1.04 (0.61–1.77)	0.83
α-Carotene	1	0.98 (0.55–1.73)	0.83 (0.47–1.47)	1.18 (0.68–2.05)	0.72
β-Carotene	1	0.94 (0.55–1.62)	1.06 (0.61–1.83)	0.85 (0.49–1.49)	0.69
α-Tocopherol	1	0.72 (0.42–1.22)	0.61 (0.35–1.06)	0.59 (0.34–1.04)	0.04
γ-Tocopherol	1	0.91 (0.52–1.59)	0.87 (0.50–1.52)	0.86 (0.50–1.48)	0.57
Retinol	1	1.03 (0.59–1.80)	1.56 (0.88–2.74)	1.02 (0.56–1.87)	0.59
Retinyl palmitate	1	1.24 (0.69–2.22)	1.05 (0.60–1.82)	0.93 (0.52–1.64)	0.65

[a] OR from conditional logistic regression model stratified on exposure population, study center at randomization, age at randomization within 5-year intervals, sex, smoking status at randomization, and year of randomization.
[b] Quartile cutpoints for lutein (103, 135, 173), zeaxanthin (21.0, 27.0, 36.0), β-cryptoxanthin (36.0, 55.0, 90.0), lycopene (229, 321, 417), α-carotene (18.0, 30.0, 43.0), β-carotene (94, 139, 219), polar carotenoids (176, 225, 290), nonpolar carotenoids (374, 514, 686), α-tocopherol (10666, 12830, 16792), γ-tocopherol (1754, 2569, 3567), retinol (572, 657, 786), and retinyl palmitate (26.0, 38.0, 62.0).
[c] Comparison quartile.

ducted in a current and ex-cigarette smoking population. Neither trial had a never-smoker population. The associations we report may not apply to never-smokers.

Our study of the association between prostate cancer and α-tocopherol was more striking with a statistically significant inverse association ($P_{trend} = 0.04$) and a protective OR of 0.59 (95% CI , 0.34–1.04). Both Gann *et al.* (17) and Nomura *et al.* (39) also found significant inverse associations between α-tocopherol and prostate cancer. However, in similar analyses from ATBC, with 317 incident prostate cancer cases they found no significant association between baseline serum α-tocopherol or dietary vitamin E and prostate cancer (40). Helzlsouer *et al.* (23) have reported previously a nonstatistically significant inverse association for serum α-tocopherol ($P_{trend} = 0.65$) but a statistically significant inverse association ($P_{trend} = 0.02$) for γ-tocopherol (41). We found no association between γ-tocopherol and prostate cancer ($P_{trend} = 0.57$). The role of γ-tocopherol needs additional investigation, because Cooney *et al.* (42) reported that the *in vitro* detoxification of nitrogen dioxide by γ-tocopherol is superior to that of α-tocopherol, and it may be a more potent inhibitor of neoplastic transformation in some model systems.

Our analysis adds to the evidence suggesting that a low serum concentration of α-tocopherol is a risk factor for both lung and prostate cancer. On the basis of these and previous findings, the hypothesis has been proposed: can supplemental α-tocopherol act as a cancer prevention agent for individuals at high risk for lung or prostate cancer? The ATBC trial was able to directly address that question. In that trial, participants randomized to 50 mg of α-tocopherol daily had a 32% lower incidence of prostate cancer, and prostate cancer mortality was reduced by 41%. The effect on lung cancer incidence was not significant (43). It is important to note that the beneficial effect on prostate cancer incidence was observed with an α-tocopherol dose of 50 mg, which is only 1.66× the recommended minimum daily requirement. This dose increased serum α-tocopherol to a level that approximates the upper quartile seen in normal populations. This important finding from the ATBC intervention trial supported the hypothesis that α-tocopherol may be an important modifier of prostate cancer risk. That hypothesis is now being additionally tested in the Selenium and Vitamin E Cancer Prevention Trial (44). That trial is testing vitamin E (400 units) and selenium (200 μg) in a 2 × 2 design in 32,400 American men, and has the primary end point of prostate cancer incidence.

We found no association between prostate cancer incidence and the serum concentration of any carotenoid. This contradicts the many smaller and less rigorously controlled studies that show an inverse relationship between prostate cancer incidence and serum lycopene (18–20, 25). However, the PHS with 578 cases of prostate cancer showed a significant inverse association between prostate cancer incidence and lycopene (17). Additional epidemiological studies are necessary to further explore this relationship.

One of the shortcomings with our study as well as others is the fact that diets contain many potential cancer-inhibiting micronutrients (41) and likely cancer promoting agents as well. It is likely that synergistic, additive, and inhibitory interactions exist among these dietary components (45, 46). The cancer prevention effects of different food groups described in epidemiology dietary studies may be because of complex interactions of many nutrients, with the contribution of each individual micronutrient being small. Our finding that low serum concentrations of multiple carotenoids are potential risk factors for lung cancer is compatible with this hypothesis. An alternative explanation also exists that carotenoids themselves may be unimportant but act only as a marker for other coconsumed compounds of plant origin [such as flavonoids (47), phenolics (48), or other phytochemicals (49)] or possibly nondietary covariates.

The results of the study presented here, the large body of epidemiological studies, and the clinical intervention trials offer important insights when reviewing the clinical evaluation of β-carotene (50). In general, epidemiological studies of prospectively collected samples found that populations with the highest serum concentrations of β-carotene had a lung cancer relative risk of 0.6 compared with those in the lowest serum concentration groups (4). On the basis of these studies, ATBC and CARET were designed to test the hypothesis that daily β-carotene supplements (20 mg and 30 mg, respectively) would decrease lung cancer. They found, instead, unexpected 18% and 28% increases in incidence of lung cancer among those individuals taking β-carotene and β-carotene with vitamin A, respectively (11, 12). Both trials found that the most adverse effects were in current smokers. In contrast, the PHS (10), which administered 50 mg of β-carotene every other day to

male physicians (only 11% current smokers), detected no effect on cancer incidence, although the number of lung cancer end points was very modest. CARET, ATBC, and PHS all used doses of β-carotene, which raised the serum concentrations to 10 times the normal range.

The findings we report here in the CARET population, as well as other epidemiological studies cited, explore the association between cancer incidence and the serum concentrations of nutrients that are the result of variations in the dietary intake and physiology of an individual. These analyses show that small differences in many serum micronutrients are associated with significant differences in cancer incidence. Supplementation with individual micronutrients in supraphysiologic doses, as was used in CARET, ATBC, and PHS, result in dramatic increases in serum concentrations of those micronutrients. High-dose supplementation addressed a very different hypothesis than that suggested by epidemiological studies of individuals on normal diets with serum levels in a narrow range. Those studies suggest that modest doses of micronutrient supplementation may be an effective chemoprevention strategy. It is intriguing to consider the outcome of a trial studying the effect of low-dose β-carotene (or "carotenoid cocktail" 3–5 mg/day), a dose that would increase serum concentrations to levels well within the range found to be protective in epidemiological studies and achievable by prudent dietary choices. By contrast, to the doses of β-carotene used in ATBC, CARET, and PHS, the α-tocopherol arm of the ATBC trial tested relatively low-dose supplementation of α-tocopherol 50 units. This resulted in an increase in the serum concentrations of vitamin E to levels that were well within the range reported in the epidemiological studies. This modest elevation of serum vitamin E resulted in a significant decrease in prostate cancer incidence.

High-dose micronutrient supplementation must be considered like any pharmacologic drug intervention. The *in vivo* and *in vitro* physiology of β-carotene is only now being explored. Wang *et al.* (51) have shown that high doses of β-carotene can modify ferret bronchial epithelium. In conjunction with cigarette smoke, there were adverse interactions between β-carotene and retinoic acid receptor expression, as well as *C-Jun* and *C-Fos* oncogene activation. Interestingly, these authors extended their findings suggesting that low doses of β-carotene might possibly be beneficial (52). Other laboratories have reported similar adverse effects of β-carotene (53, 54). It has been shown that high concentrations of both β-carotene and lycopene can decrease their efficacy as a protector against oxidation damage (55). Although these studies are in animal models, they may explain the adverse outcomes seen in ATBC and CARET. Our lack of understanding of the biological activity of high doses of micronutrients should be considered when dosages are selected for clinical trials. High doses of micronutrients may result in adverse actions not anticipated.

Studying the associations between the serum concentrations of dietary micronutrients and cancer incidence is a classic method to explore the potential role of a nutrient in cancer prevention, and generate a hypothesis to test in randomized clinical trials. In the analysis reported here we studied the serum concentration of multiple micronutrients. Many carotenoids and α-tocopherol had a significant inverse association with lung cancer incidence, most much greater than β-carotene. Only α-tocopherol had a significant inverse association with prostate cancer. However, these analyses have many shortcomings. First, as discussed, these studies can only address serum concentrations within the physiological range, which occur as the result of *ad libitum* dietary variability. Whereas they do provide evidence to justify clinical trials testing dietary modification or physiological dose supplementation, they should not be used as the sole justification for trials administering supraphysiologic doses of a micronutrient where dose/toxicity relationships are not well described. Secondly, epidemiological studies studying micronutrients do not consider that foods contain many biologically active substances (known and unknown, and measured and unmeasured). They assume that a single nutrient independently may have a significant effect on cancer incidence and that other dietary factors associated or coconsumed with the nutrient or other factors (such as physical exercise) are less important modifiers of cancer incidence. Until the spectrum of biologically active compounds in food can be better identified and measured, it will be difficult to ascribe the inverse association seen between the consumption of fresh fruits and vegetables and cancer to a specific nutrient.

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