Association of Plasma Micronutrient Levels and Urinary Isoprostane with Risk of Lung Cancer: The Multiethnic Cohort Study

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Abstract

Although smoking is the primary risk factor for lung cancer, there is evidence to suggest that fruit and vegetable intake are important cofactors. The present case-control study, nested within the Multiethnic Cohort Study, examined the associations of biomarkers of fruit and vegetable intake (individual plasma micronutrient levels), serum selenium, and a urinary biomarker for total lipid peroxidation with lung cancer risk. Two hundred seven incident cases were matched to 414 controls on age, sex, ethnicity, study location (Hawaii or California), smoking status, date/time of collection, and hours of fasting. We measured prediagnositic circulating levels of individual tocopherols and carotenoids, retinol, and serum selenium, and urinary 15-isoprostane F_{2t}. Conditional logistic regression was used to compute odds ratios (OR) and 95% confidence intervals (CI). For men, strong reductions in risk were seen with increasing tertiles of each plasma carotenoid, with the ORs for the third tertile, compared with the first tertile, ranging from 0.24 to 0.45 (P_{trends}, 0.002-0.04). No associations were found among women for carotenoids or among either sex for tocopherols, selenium, and retinol. A doubling in risk was seen for men in the second and third tertiles, compared with the first tertile of urinary 15-isoprostane F_{2t} (OR, 2.31; 95% CI, 1.02-5.25; and OR, 2.16; 95% CI, 0.98-4.78). This study supports the previously observed association between circulating carotenoids and lung cancer risk in men, and adds to the limited literature regarding urinary 15-isoprostane F_{2t} as a marker of cancer risk. Future research examining the possible relationship between isoprostanes and lung cancer is warranted. (Cancer Epidemiol Biomarkers Prev 2009;18(7):1962–70)

Introduction

Lung cancer is the most commonly diagnosed cancer worldwide, and it is estimated that 215,000 new cases will arise in 2008 in the United States alone (1). Although smoking is the primary risk factor for lung cancer, accounting for over 80% of all lung cancer cases, there is evidence to suggest that diet is an important cofactor (2). Because experimental work showed a relationship between oxidation and carcinogenesis (3), antioxidants, such as carotenoids, tocopherols, and selenium, have been investigated for potential protective effects against lung cancer. According to the report produced by the World Cancer Research Fund in 2007, published evidence indicates that: fruit and foods containing carotenoids *probably* protect against lung cancer; and nonstarchy vegetables, foods containing selenium or quercetin, and selenium supplements *may* protect against lung cancer (4). A recent systematic review of carotenoids and lung cancer risk found that the pooled relative risk of lung cancer comparing the highest with the lowest category of total dietary carotenoid intake was 0.79 [95% confidence interval (95% CI), 0.71-0.87], whereas the pooled relative risk for circulating levels of carotenoids was even lower at 0.70, but not statistically significant (95% CI, 0.44-1.11; ref. 5). Pooled relative risks of several individual carotenoids in serum (α-carotene, β-carotene, β-cryptoxanthin, lutein-zeaxanthin, and lycopene) all suggested inverse associations, but only the association with lycopene was statistically significant (relative risk, 0.71, 95% CI, 0.51-0.98; ref. 5).

Although two large randomized supplementation trials have found that high dose of β-carotene increases risk of lung cancer among high-risk individuals (6, 7), higher baseline levels were seen to be protective, indicating that there is still a potential protective effect of carotenoids (8, 9). In fact, the Supplémentation en Vitamines et Minéraux Antioxydants study, a French randomized prevention trial, found that when antioxidant supplements are given in nutritional doses (which are significantly lower than those given in the two trials mentioned above) to the general population (not high-risk individuals specifically), there is a suggested decrease in risk of all cancers in men only (results of the associations with individual cancers have not yet been published; ref. 10).

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Table 1. Characteristics of lung cancer cases and controls, by sex

	Men		Women	
	Cases n = 136	Controls n = 272	Cases n = 71	Controls n = 142
General characteristics				
Age at blood draw (y), mean (SD)*	71.5 (6.5)	71.1 (6.8)	71.0 (7.1)	70.4 (7.2)
Fasting hours before blood draw, mean (SD)*	12.1 (4.5)	12.1 (4.4)	12.6 (3.9)	12.3 (3.7)
Smoking status, n (%)*				
Never	31 (23%)	62 (23%)	21 (30%)	42 (30%)
Former	51 (38%)	102 (38%)	23 (32%)	46 (32%)
Current	54 (40%)	108 (40%)	27 (38%)	54 (38%)
Pack-years, mean (SD)	37.7 (33.7)	19.7 (21.3)	26.9 (29.9)	11.6 (17.4)
Ethnicity, n (%)*				
African-American	45 (33%)	90 (33%)	20 (28%)	40 (28%)
Caucasian	22 (16%)	44 (16%)	19 (27%)	38 (27%)
Japanese-American	37 (27%)	74 (27%)	13 (18%)	26 (18%)
Latino	18 (13%)	36 (13%)	10 (14%)	20 (14%)
Native Hawaiian	14 (10%)	28 (10%)	9 (13%)	18 (13%)
Body mass index (kg/m^2), mean (SD)	26.4 (4.6)	25.8 (4.1)	25.5 (5.4)	26.8 (5.5)
High school education or less, n (%)	64 (47%)	100 (37%)	28 (40%)	67 (47%)
Physical activity (METS per day), mean (SD)	1.7 (0.3)	1.7 (0.3)	1.6 (0.2)	1.6 (0.2)
Family history of lung cancer, n (%)	18 (13%)	15 (6%)	3 (4%)	17 (12%)
Supplement use for > 1 year, n (%)	59 (44%)	131 (48%)	32 (45%)	73 (51%)

*Matching variable.

Markers of oxidation itself have rarely been examined in epidemiologic studies. Isoprostane is one measure of ''total lipid peroxidation,'' as it is a compound produced when free radicals peroxidate arachidonic acid (11). In particular, nitric oxide radical seems to be a significant mediator of isoprostane formation (12) and isoprostane levels may be an important indicator of inflammation-induced nitrosation damage.

In the present study, we examined the association between antioxidant plant constituents and risk of lung cancer using individual circulating antioxidant biomarkers (specifically for tocopherols, carotenoids, and selenium), plasma retinol levels, as well as a urinary biomarker for total lipid peroxidation (15-isoprostane F_{2t}). This nested case-control study was conducted within the larger Multiethnic Cohort Study.

Materials and Methods

Study Population. The Multiethnic Cohort Study, which recruited >215,000 individuals in Hawaii and Los Angeles, California from 1993 to 1996, has been described in detail previously (13). The study required that participants be ages 45 to 75 y in 1993, except for Native Hawaiians who were recruited at age 42 y and older, and targeted the five racial/ethnic groups of African Americans, Caucasians, Japanese Americans, Latinos, and Native Hawaiians. Participants completed a 26-page baseline questionnaire that included a quantitative food frequency questionnaire, and questions on demographics, medical history, and life-style. No biospecimens were collected at baseline.

The Biospecimen Subcohort. Cohort members were recruited by letter, and then by phone, to participate in a biospecimen subcohort. The biospecimen collection began in 1997, with the vast majority of biospecimens collected between 2001 and 2006. After agreeing to provide blood and urine, individuals were interviewed by phone and were given a short screening questionnaire and an update of a few items from the baseline questionnaire. Blood samples, 94% of which were fasting (8 h or more), were drawn at a clinical laboratory or in the subjects' homes and were then kept refrigerated and protected from light until processing, which took place on average 3 h after collection. After centrifugation and separation into serum, plasma, buffy coat, and red cells, blood components were stored in 0.5-cc cryovials in the vapor phase of liquid nitrogen. First-morning urine samples from Los Angeles and overnight urine samples from Hawaii were collected starting in 2001; spot urines were collected before that time, which were not included in the isoprostane analyses. The urine samples were aliquoted into 2-cc cryotubes, and stored in −80°C freezers. A total of 67,594 cohort members contributed to the biorepository, from which the cases and controls for this study were selected.

The baseline characteristics of individuals who provided specimens were compared with those who did not. Although the responders on average had more education (13.6 versus 12.7 y), were less likely to be current smokers (13% versus 17%), and were more likely to have a cancer family history (46% versus 42%), they were similar to the nonresponders on many other important exposures, including age, body mass index, percent energy from fat, consumption of vegetables, alcohol intake, and hours spent in moderate or vigorous activity per day. Thus, the participants in the biospecimen subcohort are broadly representative of all cohort members.

Selection of Cases and Controls. Linkage with the Hawaii and California tumor registries of the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute allowed for identification of incident lung cancer cases among the cohort. Cases for this nested case-control study were individuals who had contributed blood to the biorepository subcohort before their diagnosis of lung cancer, and whose diagnoses was reported in the 2006 Surveillance, Epidemiology, and End Results tumor linkage.

Two controls for each case were randomly chosen from a pool of potential controls of individuals who contributed blood to the biorepository and were alive and free of lung cancer at the age of the case's diagnosis, and who matched the case on sex, race/ethnicity, location (Hawaii or California), year of birth (± 1 y), date (± 6 mo) and time (± 2 h) of blood draw, hours of fasting before that blood draw (0-<6, 6-<8, 8-<10, and 10+ h), and smoking status (never, former, current).

Of the 219 eligible lung cancer cases and 438 eligible controls, 4 cases and 8 controls were missing information on pack-years, and thus, they, and individuals matched to them, were dropped from the analysis. Thus, the study population for the present study included 207 cases and 414 matched controls. The median time from blood draw to date of diagnosis for cases (or reference date for controls) was 1 y and 8 mo, with 50% of subjects having follow-up time in the range of 9.5 mo to 3 y.

Laboratory Assays

Serum and Plasma Analyses. Plasma concentrations of tocopherols, retinoids, and carotenoids were determined by high-pressure liquid chromatography with photo diode array detection slightly modified from our earlier protocol (14, 15). In brief, we used 0.3-mL plasma followed by partitioning into hexane, drying, and redissolving in 0.15 mL of the high-pressure liquid chromatography mobile phase. Twenty microliters were injected onto a Spherex C18 analytic column (150 mm × 3.2.mm, 3 μm; Phenomenex) coupled to a Spherex C18 precolumn (4 mm × 3.0 mm, 10 μm) using isocratic elution with a mobile phase of 665 mL methanol/218 mL dichloromethan/117 mL acetonitrile/2 mL aq. bis-tris propane (0.5M; pH 6.8) and containing 0.25 g/L butylated hydroxytoluene at 0.3 mL/min. Carotenoids and tocopherols (α-, γ + β, δ-tocopherol) were quantitated by absorbance at 450 and 295 nm, respectively. β-Tocopherol and γ-tocopherol were not separated and quantitated together. Serum carotenoids are presented as individual compound concentrations plus as the addition of all individual compound concentrations (''total carotenoids''); combining them in molar units would show only very minor differences because the molecular weights are very similar.

The procedures and associated quality control practices for analyzing serum selenium level adjusted for sodium via neutron activation analysis have recently been described (16). After being individually placed in the top-center position of a ''shuttle rabbit,'' each sample was irradiated for 7 s in the Row II position. Then, after a decay of 15 s, each sample was real-time counted for 30 s using a high-resolution γ-ray spectrometer. The 161.9 keV γ-ray from the decay of Se-77m was used to determine Se concentrations by standard comparison.

Urinary Analyses. 15-Isoprostane F_{2t} was measured using a competitive ELISA kit from Oxford Biomedical Research, Inc. Urine samples were thawed and mixed with 4 μL glucuronidase (250,000 units/mL), Oxford Biomedical Research, Inc., and incubated for 2 h at 37°C and then centrifuged for 2 min at 2,000 rpm in a microfuge. Standards and samples (100 μL) are added in duplicate to 96-well plates, followed by addition of 100 μL of diluted F_{2t} horseradish peroxidase conjugate and incubated for 2 h at room temperature. After washing to remove any unbound substances, 200 μL of substrate solution is added to each well and color was allowed to develop proportionate to the amount of isoprostane present. The color development is stopped with the addition of 50 μL 3N H_2SO_4 and the microplate then read at 450 nm and also at 590 nm as a background control. Plots of log concentration versus absorbance for standards are prepared and concentrations of unknown samples extrapolated from the standard curve using a four-parameter fit and adjusted for any dilution of urine and reported as pg/mL. After measurement of urinary creatinine, isoprostane is then calculated and reported as ng/mg creatinine.

The mean intra-assay coefficients of variation were 7.7% for α-tocopherol, 7.4% for β + γ-tocopherol, 7.8% for total tocopherol, 5.7% for β-cryptoxanthin, 6.5% for β-carotene, 6.5% for lutein + zeaxanthin, 6.4% for lycopene, 4.7% for total carotenoids, 10.0% for retinol, 5.1% for selenium, and 11.9% for 15-isoprostane F_{2t}.

Statistical Analyses. To estimate the association between the biomarkers and lung cancer risk, we used conditional logistic regression models separately for men and women to compute odds ratios (OR) and 95%

Table 2. Levels of antioxidants among lung cancer cases and controls, by sex

	Men		Women	
	Cases n = 136	Controls n = 272	Cases n = 71	Controls n = 142
Antioxidants, median (interquartile range)				
From plasma (ng/mL)				
α-Tocopherol	13,898 (9,612-17,881)	13,132 (10,227-17,011)	13,266 (10,436-17,948)	13,912 (11,154-20,354)
β + γ-Tocopherol	1,464 (688-2,724)	1,484 (680-2,397)	1,635 (857-2,718)	1,510 (826-2,753)
Total tocopherols	15,878 (12,457-20,606)	15,491 (12,365-18,511)	15,481 (13,030-20,703)	16,268 (13,637-21,079)
β-Cryptoxanthin	136 (82-223)	175 (111-288)	174 (90-315)	178 (99-310)
α-Carotene	35 (22-61)	53 (34-83)	45 (24-97)	49 (30-87)
β-Carotene	149 (73-273)	213 (130-429)	214 (126-422)	212 (126-391)
Lutein + zeaxanthin	373 (275-520)	398 (297-552)	406 (261-535)	371 (271-499)
Lycopene	261 (171-362)	320 (212-432)	268 (174-362)	248 (173-359)
Total carotenoids	1,202 (895-1,633)	1,502 (1,096-1,853)	1,317 (863-1,977)	1,307 (957-1,722)
Retinol	1,211 (926-1,655)	1,191 (996-1,563)	1,085 (777-1,427)	1,113 (871-1,516)
From serum				
Selenium (μg/g of sodium)	0.13 (0.12-0.15)	0.13 (0.12-0.15)	0.13 (0.12-0.15)	0.13 (0.12-0.14)
From urine				
15-Isoprostane F_{2t} (ng/mg of creatinine)	4.27 (3.55-5.52)	3.77 (2.93-4.86)	5.14 (4.22-6.68)	4.94 (3.83-6.53)

Table 3. ORs and 95% CIs for risk of lung cancer *among men* across tertiles of plasma tocopherols, carotenoids, and retinol, serum selenium, and urinary 15-isoprostane F_{2t}

Variable	Tertiles of concentration levels			P_{trend}*
	1	2	3	
Plasma micronutrients (ng/mL)				
α-Tocopherol				
Median level	9,135	13,249	20,212	
No. of cases/no. of controls †	45/72	29/87	42/75	
Base model OR ‡	1.00	0.54 (0.28-1.02)	1.00 (0.54-1.85)	0.72
Fully adjusted OR§	1.00	0.57 (0.30-1.08)	1.00 (0.54-1.87)	0.76
β + γ-Tocopherol				
Median level	560	1,482	2,972	
No. of cases/no. of controls †	41/75	32/85	43/74	
Base model OR ‡	1.00	0.75 (0.39-1.45)	0.98 (0.52-1.84)	0.98
Fully adjusted OR§	1.00	0.77 (0.39-1.51)	0.96 (0.50-1.83)	0.96
Total tocopherols				
Median level	11,368	15,715	22,012	
No. of cases/no. of controls †	40/77	34/83	42/74	
Base model OR ‡	1.00	0.89 (0.48-1.65)	1.21 (0.65-2.26)	0.51
Fully adjusted OR§	1.00	0.95 (0.51-1.77)	1.18 (0.63-2.22)	0.59
β-Cryptoxanthin				
Median level	82	162	353	
No. of cases/no. of controls †	51/66	35/82	30/86	
Base model OR ‡	1.00	0.56 (0.30-1.03)	0.32 (0.15-0.70)	0.005
Fully adjusted OR§	1.00	0.58 (0.31-1.08)	0.33 (0.15-0.73)	0.007
α-Carotene				
Median level	24	45	100	
No. of cases/no. of controls †	56/60	35/83	25/91	
Base model OR ‡	1.00	0.36 (0.18-0.70)	0.22 (0.10-0.47)	0.0006
Fully adjusted OR§	1.00	0.38 (0.19-0.74)	0.24 (0.11-0.53)	0.002
β-Carotene				
Median level	87	202	497	
No. of cases/no. of controls †	55/61	34/84	27/89	
Base model OR ‡	1.00	0.41 (0.22-0.79)	0.28 (0.13-0.57)	0.002
Fully adjusted OR§	1.00	0.46 (0.24-0.90)	0.30 (0.15-0.64)	0.004
Lutein + zeaxanthin				
Median level	250	393	623	
No. of cases/no. of controls †	48/69	37/80	31/85	
Base model OR ‡	1.00	0.70 (0.37-1.30)	0.42 (0.20-0.87)	0.02
Fully adjusted OR§	1.00	0.68 (0.36-1.28)	0.45 (0.21-0.94)	0.04
Lycopene				
Median level	164	296	463	
No. of cases/no. of controls †	46/70	43/75	27/89	
Base model OR ‡	1.00	0.88 (0.47-1.63)	0.35 (0.17-0.71)	0.004
Fully adjusted OR§	1.00	0.93 (0.50-1.74)	0.36 (0.18-0.75)	0.007
Total carotenoids				
Median level	908	1,377	2,030	
No. of cases/no. of controls †	52/65	36/80	28/89	
Base model OR ‡	1.00	0.45 (0.25-0.84)	0.32 (0.15-0.65)	0.002
Fully adjusted OR§	1.00	0.48 (0.26-0.89)	0.32 (0.15-0.68)	0.003
Retinol				
Median level	890	1,202	1,804	
No. of cases/no. of controls †	36/81	40/77	40/76	
Base model OR ‡	1.00	1.06 (0.54-2.07)	1.08 (0.50-2.34)	0.85
Fully adjusted OR§	1.00	1.22 (0.62-2.40)	1.26 (0.57-2.77)	0.61
Serum selenium (μg/grams of sodium)				
Median level	0.12	0.13	0.15	
No. of cases/no. of controls †	49/85	44/91	40/94	
Base model OR ‡	1.00	0.77 (0.42-1.40)	0.73 (0.39-1.36)	0.34
Fully adjusted OR§	1.00	0.74 (0.40-1.38)	0.70 (0.37-1.33)	0.30
Urinary 15-isoprostane F_{2t} (ng/mg of creatinine)				
Median level	2.83	3.92	5.89	
No. of cases/no. of controls †	18/76	31/62	36/58	
Base model OR ‡	1.00	2.52 (1.12-5.66)	2.30 (1.06-4.99)	0.09
Fully adjusted OR§	1.00	2.31 (1.02-5.25)	2.16 (0.98-4.78)	0.12

*Linear dose-response in the logit of risk was tested by a Wald test for each biomarker modeled as a trend variable assigned the median value of the appropriate category.

† Two controls were matched to each case on geographic area, male sex, ethnicity, year of birth, date and time of specimen collection, fasting status, and smoking status (never, former, current). Cases with missing covariate data were excluded from the analyses (n = 3 for selenium, n = 20 for other antioxidants, n = 51 for 15-isoprostane F_{2t}).

‡ Adjusted by conditional logistic regression with matched sets as strata for age at specimen collection, fasting hours before blood draw, pack-years, and pack-years squared.

§Further adjusted for years of schooling and family history of lung cancer.

Table 4. ORs and 95% CIs for risk of lung cancer *among women* across tertiles of plasma tocopherols, carotenoids, and retinol, serum selenium, and urinary 15-isoprostane F_{2t}

Variable	Tertiles of concentration levels			P_{trend}*
	1	2	3	
Plasma micronutrients (ng/mL)				
α-Tocopherol (μg/mL)				
Median level	9,346	13,678	22,756	
No. of cases/no. of controls †	25/40	21/45	19/45	
Base model OR ‡	1.00	0.75 (0.33-1.70)	0.76 (0.33-1.75)	0.58
Fully adjusted OR§	1.00	0.74 (0.31-1.76)	0.79 (0.32-1.94)	0.70
β + γ-tocopherol				
Median level	640	1,520	3,261	
No. of cases/no. of controls †	20/45	23/43	22/42	
Base model OR ‡	1.00	0.93 (0.38-2.27)	0.98 (0.40-2.40)	0.99
Fully adjusted OR§	1.00	0.93 (0.37-2.39)	0.88 (0.35-2.24)	0.80
Total tocopherols				
Median level	12,400	16,113	24,161	
No. of cases/no. of controls †	25/40	21/45	19/45	
Base model OR ‡	1.00	1.30 (0.57-2.95)	0.83 (0.36-1.89)	0.54
Fully adjusted OR§	1.00	1.32 (0.56-3.11)	0.88 (0.37-2.12)	0.66
β-Cryptoxanthin				
Median level	82	177	413	
No. of cases/no. of controls †	22/42	21/46	22/42	
Base model OR ‡	1.00	0.94 (0.39-2.26)	1.32 (0.54-3.26)	0.50
Fully adjusted OR§	1.00	1.02 (0.41-2.55)	1.58 (0.59-4.23)	0.32
α-Carotene				
Median level	22	48	109	
No. of cases/no. of controls †	24/41	16/49	25/40	
Base model OR ‡	1.00	0.64 (0.24-1.74)	2.01 (0.77-5.26)	0.11
Fully adjusted OR§	1.00	0.59 (0.20-1.73)	1.52 (0.53-4.38)	0.29
β-Carotene				
Median level	100	214	508	
No. of cases/no. of controls †	22/43	20/46	23/41	
Base model OR ‡	1.00	1.02 (0.45-2.31)	1.55 (0.62-3.90)	0.30
Fully adjusted OR§	1.00	0.99 (0.41-2.39)	1.33 (0.49-3.61)	0.53
Lutein + zeaxanthin				
Median level	236	376	563	
No. of cases/no. of controls †	22/43	18/47	25/40	
Base model OR ‡	1.00	1.12 (0.46-2.73)	1.94 (0.76-4.94)	0.15
Fully adjusted OR§	1.00	0.89 (0.35-2.26)	2.23 (0.79-6.26)	0.11
Lycopene				
Median level	144	263	401	
No. of cases/no. of controls †	22/43	20/45	23/42	
Base model OR ‡	1.00	0.98 (0.41-2.32)	1.72 (0.69-4.27)	0.23
Fully adjusted OR§	1.00	0.84 (0.33-2.13)	1.94 (0.72-5.22)	0.15
Total carotenoids				
Median level	818	1,308	2,091	
No. of cases/no. of controls †	22/43	21/45	22/42	
Base model OR ‡	1.00	1.11 (0.47-2.63)	1.75 (0.68-4.52)	0.23
Fully adjusted OR§	1.00	0.96 (0.38-2.42)	1.78 (0.62-5.08)	0.25
Retinol				
Median level	777	1,107	1,712	
No. of cases/no. of controls †	23/42	23/43	19/45	
Base model OR ‡	1.00	0.69 (0.28-1.69)	0.63 (0.25-1.61)	0.36
Fully adjusted OR§	1.00	0.78 (0.30-2.01)	0.77 (0.29-2.06)	0.63
Serum selenium (μg/g of sodium)				
Median level	0.12	0.13	0.15	
No. of cases/no. of controls †	24/46	21/51	26/45	
Base model OR ‡	1.00	0.67 (0.29-1.58)	1.00 (0.45-2.25)	0.89
Fully adjusted OR§	1.00	0.64 (0.26-1.60)	0.98 (0.42-2.29)	0.91
Urinary 15-isoprostane F_{2t} (ng/mg of creatinine)				
Median level	3.75	4.96	7.51	
No. of cases/no. of controls †	15/42	19/41	19/38	
Base model OR ‡	1.00	1.29 (0.47-3.53)	0.98 (0.35-2.76)	0.87
Fully adjusted OR§	1.00	1.29 (0.46-3.61)	1.19 (0.40-3.49)	0.82

*Linear dose-response in the logit of risk was tested by a Wald test for each biomarker modeled as a trend variable assigned the median value of the appropriate category.

† Two controls were matched to each case on geographic area, female sex, ethnicity, year of birth, date and time of specimen collection, fasting status, and smoking status (never, former, current). Cases with missing covariate data were excluded from the analyses (n = 6 for tocopherols, carotenoids, and retinol; n = 18 for 15-isoprostane F_{2t}).

‡ Adjusted by conditional logistic regression with matched sets as strata for age at specimen collection, fasting hours before blood draw, pack-years, and pack-years squared.

§Further adjusted for years of schooling and family history of lung cancer.

Table 5. Percentage distribution of lung cancer cases by cell type and sex

Cell type	Male cases n = 133	Female cases n = 71
Large cell	2 (2%)	2 (3%)
Small cell	16 (12%)	2 (3%)
Squamous cell	31 (23%)	11 (15%)
Adenocarcinoma	52 (39%)	36 (51%)
Other	32 (24%)	20 (28%)

NOTE: Missing histology for 2% (3/136) of male cases.

CIs, where the matched case-controls sets were the strata. Biomarker variables were categorized into tertiles based on the sex-specific distribution of cases and controls combined and were represented in the models as two dummy variables (with the lowest tertile as the reference group). Linear dose-response in the logit of risk was tested by a Wald test for each biomarker modeled as a trend variable assigned the median value of the appropriate category. The category of total carotenoids was created to encompass plasma levels of the individual micronutrients of β-cryptoxanthin, α-carotene, β-carotene, lutein + zeaxanthin, and lycopene, as well as α-cryptoxanthin, anhydrolutein, and dihydro-lycopene.

The initial base models were adjusted for pack-years of cigarette smoking (as a linear variable and a quadratic term) and for the matching variables of age at blood draw and hours of fasting before blood draw (as continuous variables to account for any variation within matched sets). Fully adjusted models were created to further adjust the risk estimates for years of schooling and family history of lung cancer. Body mass index, physical activity, and long-term supplement use were also considered as potential confounders, but did not materially affect the main associations. Models were also considered excluding the subset of subjects who were diagnosed within 1 y after blood draw (68 cases and their 136 matched controls). We created models with separate terms for the biomarkers by smoking status (never versus ever), histologic type (adenocarinoma versus nonadenocarcinoma), and long-term supplement use (defined as use of vitamin A, vitamin, C, vitamin E, β-carotene, calcium, selenium, iron, or multivitamin supplements for >1 y versus no use or use for <1 y) and examined effect modification using the likelihood ratio test. The sample size with the current follow-up was too small to allow for ethnic-specific analyses. Polytomous logistic regression and a Wald test was used to compare risks by histologic type.

Results

Overall, cases averaged a larger number of pack-years than controls (38 versus 20 for men, 27 versus 12 for women) and were somewhat less likely to be long-term supplement users than controls (44% versus 48% for men, 45% versus 51% for women), but were similar to controls in terms of physical activity and body mass index (Table 1). Male cases were more likely than controls to have an education level of high school or less (47% versus 37%) or to have a family history of lung cancer (13% versus 6%), whereas female cases were *less* likely than controls to have only a high school education (40% versus 47%) or to have a family history of lung cancer (4% versus 12%). In comparing analyte levels, male cases had lower levels of all measured carotenoids and higher levels of isoprostane than their matched controls, but were similar to their matched controls for tocopherols, retinol, and selenium (Table 2). Female cases were generally similar to their matched controls for all analytes.

For men, strong monotonic reductions in risk were seen with increasing tertiles of all plasma carotenoids, with the ORs for individuals in the third tertile, compared with those in the first tertile, ranging from 0.24 to 0.45 (multivariate adjusted P for trends as follows: 0.007 for β-cryptoxanthin, 0.002 for α-carotene, 0.004 for β-carotene, 0.04 for lutein + zeaxanthin, 0.007 for lycopene, and 0.003 for total carotenoids; Table 3). A doubling in risk of lung cancer was seen for male cases in the second and third tertiles of urinary 15-isoprostane F_{2t} (OR, 2.31; 95% CI, 1.02-5.25; and OR, 2.16; 95% CI, 0.98-4.78) but the trend was not monotonic (P_{trend} = 0.12). No associations were observed in males between lung cancer risk and circulating levels of tocopherols, retinol, or selenium. None of the associations among men substantially changed when cases diagnosed with lung cancer less than one year after blood draw were excluded.

For women, we observed no significant associations between lung cancer risk and circulating concentrations of tocopherols, carotenoids, retinol, or selenium, or with urinary 15-isoprostane F_{2t} (Table 4). The test for an interaction with sex was statistically significant for total plasma carotenoids (P = 0.002) but not significant for urinary levels of isoprostane (P = 0.23). When cases who were diagnosed <1 year after blood draw were excluded, there was a strong suggestion that women in the second and third tertiles of isoprostane had an almost 4-fold increase in risk of lung cancer, compared with those in the first tertile. The resulting multivariate-adjusted ORs were 4.07 (95% CI, 0.93-17.8) and 3.78 (95% CI, 0.77-18.4), respectively.

As an exploratory analysis to attempt to understand the difference in the association of plasma carotenoids and lung cancer risk by sex, we stratified by histologic type (adenocarcinoma versus not adenocarcinoma) among all study subjects, as the female cases in our study were more likely to have adenocarinomas than the male cases (51% versus 39%, respectively; see Table 5). In sex-adjusted analyses, we found that the trend of decreasing risk with increasing plasma levels of carotenoids was seen primarily in the nonadenocarcinoma group, and a Wald test found that the difference in the association with lung cancer risk by histologic type was significant for β-cryptoxanthin (P = 0.03), β-carotene (P = 0.05), and total carotenoids (P = 0.007), of borderline significance for α-carotene (P = 0.08), and not significant for lutein + zeaxanthin (P = 0.17) or lycopene (P = 0.19; data not shown). However, the differing histology distributions did not account for the difference in risk among men and women, as within each histologic group, there was still a significant interaction between sex and both α-carotene and β-carotene ($P_{interaction}$ = 0.01 for both adenocarinomas and nonadenocarcinomas).

Stratification by smoking status (ever versus never) or by long-term supplement use (>1 y) did not provide evidence for effect modification (data not shown).

Discussion

In this nested case-control study, we found strong inverse associations between lung cancer risk and total plasma carotenoid levels, as well as individual levels of β-cryptoxanthin, α-carotene, β-carotene, lutein + zeaxanthin, and lycopene, for men only. We observed more than a doubling of risk of lung cancer for men with urinary isoprostane levels in the second or third tertiles, compared with the first. We saw no associations of carotenoid or isoprostane levels with lung cancer risk in women, or with tocopherols, retinol, or selenium levels and lung cancer risk in either sex. When we stratified by histologic type, the inverse associations with carotenoids were stronger for lung cancers not classified as adenocarcinomas (i.e., squamous cell, large cell, small cell, and other) than for lung adenocarcinomas, but this still did not explain the difference in associations by sex. When cases diagnosed with lung cancer <1 year after blood draw were excluded, we found a strong suggestion of an increase in risk for women in the second and third tertiles of urinary isoprostane, similar to what we found in men, but the numbers for this analysis were small (30 cases) and the estimates not statistically significant.

In a recent meta-analysis of prospective studies of serum carotenoids and lung cancer risk for both sexes combined, suggestions of inverse associations were found between all five carotenoids analyzed in our study—β-cryptoxanthin, α-carotene, β-carotene, lutein, and lycopene—as well as for total carotenoids, but a significant dose-response trend was found only with lycopene (5). Half of the studies included in the review included men and women combined and the review did not present results stratified by sex. In contrast, we found significant associations with plasma carotenoids in men only.

In one of the vitamin supplementation prevention trials that was limited to men, the α-Tocopherol, β-Carotene Cancer Prevention Study (ATBC; ref. 9), baseline serum β-carotene was found to have a significant inverse dose-response association with lung cancer risk (P_{trend} = 0.02), such as found in our study (P_{trend} = 0.004). However, the ATBC study also found significant inverse associations with baseline serum retinol (P_{trend} < 0.0001), the only other biomarker measured in the study, whereas we found no association with plasma retinol (P_{trend} = 0.61), even when limiting our analyses to male smokers (P_{trend} = 0.92), as in the ATBC. However, during chronic inflammation or infection, circulating retinol levels are known to decrease (17), and so we reanalyzed the association excluding the cases diagnosed <1 year after blood draw (those likely to have preclinical disease at that time), but again found no association with plasma retinol (data not shown). The cohort members in the present study were at least 10 years older, on average, than participants in the ATBC study, but this did not translate into an appreciably larger average number of pack-years.

Participants in the β-Carotene and Retinol Efficacy Trial were also at least 10 years younger than the subjects in the present study, and inverse associations between baseline serum carotenoid levels and lung cancer risk were also observed (8). Interestingly, these relationships were strongest among women in the study, in contrast to our findings limited to men only. It is important to note that women in the β-Carotene and Retinol Efficacy Trial study were required to be at high risk for lung cancer, based on a minimum of 20 pack-years of cigarette smoking, and, consequently, the mean pack-years of smoking was 58 for cases and 49 for controls for both sexes combined (sex-specific averages were not given). In contrast, only 31% (66 of 213) of our female participants reported 20 or more pack-years of smoking, and the mean pack-years of smoking for women in our study was 27 for cases and 12 for controls (34 for cases and 17 for controls for both sexes combined). Thus, our female participants were at lower risk than those of the β-Carotene and Retinol Efficacy Trial trial. It is also possible that urinary isoprostane levels are more reflective of overall antioxidant status than plasma carotenoid levels because a general increase in risk for both men and women was observed in the second and third tertiles of this measure of total lipid peroxidation.

Our findings of a null association between plasma α-tocopherol and lung cancer risk is consistent with two previously published prospective studies (18, 19), whereas two other prospective studies reported an inverse association (8, 20). Two of these four studies (one that found an overall inverse association and one that found an overall null association) saw the strongest inverse relationships between α-tocopherol and lung cancer when limiting their population to men under the age of 60 years (19, 20). In our study population, only 7% (27 of 408) of men were ages <60 years, so we were not able to examine this group separately. As for circulating γ-tocopherol, the two studies that examined its relationship with lung cancer risk found no association (8, 19), similar to our null finding for plasma β + γ tocopherol, although these relationships are complicated by the fact that inflammation and smoking cause an increase in plasma γ-tocopherol levels that may mask any protective effect of γ-tocopherol (21).

In the present study, higher levels of serum selenium were not significantly associated with reduced risk of lung cancer in either men or women. These results are similar to the findings in a recent meta-analysis, which reported a summary relative risk of 0.80 (95% CI, 0.58-1.10), comparing highest level to lowest level of circulating selenium with lung cancer risk among six nested case-control studies with predominantly male participants (22). Additionally, the Selenium and Vitamin E Cancer Prevention Trial recently published their results, which indicated no effect of selenium supplementation on lung cancer risk (23).

Although 15-isoprostane F_{2t} has been acknowledged as a marker of oxidative stress that can be measured in urine and is chemically stable (24), few investigators have examined its association with cancer risk. Results of a Taiwanese nested case-control study revealed that individuals in the second and third tertiles of urinary

15-isporostane F_{2t} levels, compared with those in the first tertile, had statistically significant 4- and 6-fold increases in risk, respectively, for hepatocellular carcinoma (25). A recent study of breast cancer and 15-isoprostane F_{2t} levels in Long Island found almost a doubling in risk for those in the third and fourth quartiles compared with the lowest quartile (OR, 1.53; 95% CI, 0.99-2.35; and OR, 1.88; 95% CI, 1.23-2.88, respectively; ref. 26). Although we were unable to find any studies of lung cancer risk and 15-isporostane F_{2t} levels, our finding of a doubling of risk for men in the second and third tertiles is consistent with our findings of a decrease in risk with the antioxidant carotenoids, and with the findings of the two studies noted above. Furthermore, we found a strong suggestion of an increased risk of lung cancer for women in the second and third tertiles of 15-isporostane F_{2t} levels when excluding the early incident cases.

Indeed, the positive association with isoprostane combined with the inverse association with total carotenoids increases our confidence in the validity of the findings of our analysis. And yet, residual confounding by smoking, which cannot be completely excluded, could partly explain these associations. However, this seems unlikely because we carefully matched cases and controls on smoking status (never, former, current), and additionally adjusted for pack-years of smoking. Other limitations include our lack information on passive smoking, which could be responsible for residual confounding, as well as a small sample size, particularly when stratifying by sex, and the relatively short follow-up period. It is also worth considering that it may not be carotenoids per se that are protective but foods rich in carotenoids, and that those typically include other phytochemicals that may be protective. In fact, several dietary modification trials with fruits and vegetables have shown that these measures of plasma antioxidants are good markers of fruit and vegetable intake generally (27, 28). As with our positive findings, our null findings between serum and urinary markers of antioxidants and lung cancer risk among women should have relevance to an elderly population only.

This study supports the previously observed association between increasing levels of serum carotenoids and a reduced risk of lung cancer in men, and adds to the limited current literature regarding urinary 15-isoprostane F_{2t} as a marker of cancer risk. Future research examining the possible relationship between isoprostanes and lung cancer is warranted, as are studies of antioxidants, particularly among high-risk women (e.g., smokers of at least 20 pack-years) and possibly women of younger age.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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