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Dietary carotenoids, vegetables, and lung cancer risk in women: the Missouri Women's Health Study (United States)

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Abstract

Objective: To examine the effect of specific dietary carotenoids and their primary plant food sources on lung cancer risk in a population-based case–control study of women.
Methods: Data were available for 587 incident primary lung cancer cases and 624 controls frequency matched to cases based on age. A modified version of the 100-item NCI-Block food-frequency questionnaire was used to obtain information concerning usual diet 2–3 years prior to interview.
Results: In models adjusted for age, total calorie intake, pack-years of smoking, and education, β-carotene, β-cryptoxanthin, lutein + zeaxanthin, and total carotenoid intake were each associated with a significantly lower risk of lung cancer. Several vegetable groups were predictive of lower lung cancer risk, particularly the frequency of total vegetable intake. Individual and total carotenoids were no longer significantly associated with lower lung cancer risk in models adjusted for total vegetable intake. However, total vegetable intake remained significantly inversely associated with risk in models adjusted for total carotenoids.
Conclusions: These results indicate that consumption of a wide variety of vegetables has a greater bearing on lung cancer risk in a population of smoking and nonsmoking women than intake of any specific carotenoid or total carotenoids.

Introduction

Lung cancer is the leading cause of cancer death, and the second most commonly diagnosed cancer, among women in the United States [1]. The age-adjusted mortality rate from this disease is still increasing in women, although the rate of increase has slowed considerably over the past decade [1]. Incidence rates continue to increase among females. Despite advances in diagnosis and treatment, overall 5-year survival rates remain low at 14% [1]. Although cigarette smoking accounts for the vast majority of lung cancer cases among women, dietary factors may also play a role, either as distinct etiologic agents or as mediators of the relationship between smoking and lung cancer.

Increased vegetable and fruit consumption has been associated with a lower risk of lung cancer in many observational studies [2]. However, the specific phytochemical(s) responsible for this protection continue to elude researchers. Carotenoids are a class of phytochemicals with proven antioxidant activity *in vitro* and *in vivo* in animal models [3]. β-Carotene has been the most extensively studied carotenoid, and the vast majority of observational studies show a significant inverse association between consumption of this carotenoid and lung cancer risk. However, supplementary β-carotene failed to lower lung cancer risk in clinical trials, and even increased risk among smokers in two trials [4, 5]. Research directed at more promising phytochemicals, including the other major carotenoids found in US diets (α-carotene, β-cryptoxanthin, lutein + zeaxanthin, and lycopene) is currently under way. No single dietary

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carotenoid has consistently been associated with a lower risk of lung cancer in observational studies [6–12], few of which were conducted exclusively in women. There are, in fact, few data on lung cancer and carotenoids in women, particularly for those residing in rural America. There are also limited data on whether individual carotenoids or actual foods are more important predictors of lung cancer risk in women.

The present study was part of a larger investigation of indoor radon, diet, and lung cancer risk among Missouri women [13, 14]. The purpose of this analysis is to elucidate which carotenoids, if any, are associated with lung cancer risk in females (most of whom are smokers), and whether these phytochemicals are more or less strongly associated with risk than their primary plant food sources. Associations will also be evaluated according to smoking status and histologic type.

Materials and methods

Study population

A detailed description of the study methodology has been described in a previous publication [14]. Briefly, a population-based case–control study was conducted among women residing in Missouri. The Missouri Cancer Registry was utilized to identify women between the ages of 35 and 84 with incident primary lung cancer diagnosed between 1 January 1993 and 31 January 1994. Of the 783 women identified in this manner, 34 were determined to be ineligible because they did not have primary lung cancer, seven were excluded because they did not permanently reside in the state, and 32 were later disqualified because they were younger than 65 years of age and lacked a valid driver's license at the time of diagnosis. The latter exclusion was made to ensure comparability with selection of controls. A brief telephone interview was completed by 665 women or a proxy respondent (94% of all eligibles). Afterwards, 610 women (86% of all eligible cases) agreed to complete in-person interviews designed to ascertain detailed dietary information. Several women were excluded due to implausible dietary information ($n = 16$; see below for criteria) or missing information regarding important confounders ($n = 7$). A total of 587 cases (83% of eligibles) were included in the final analyses. Three pathologists simultaneously used a multi-head microscope to verify the histologic classification of tissue slides from all 587 cases.

Controls less than 65 years of age were selected randomly from Missouri driver's license files; those between the ages of 65 and 84 were selected randomly from US Health Care Financing Administration lists. Of the 4592 potential controls identified for an initial screening interview, 3386 were determined to be eligible (age, race, and smoking status used as screening variables) and 730 were ultimately selected for study interviews (see below for sampling strategy). A brief telephone interview was completed by 700 women (96% of eligible controls), and 624 went on to complete in-depth dietary interviews. Thus, 85% of all eligible controls were included in the final analyses. All controls were frequency matched to cases based on 5-year age strata.

A two-stage randomized recruitment process was utilized among controls in order to prevent a large discrepancy in smoking habits between the case and control groups. The sampling strategy has been presented elsewhere [15]. Briefly, Missouri cancer registry records from 1993 were used to estimate the percentage of lung cancer cases that were lifetime nonsmokers, former smokers, current light-to-moderate smokers, or current heavy smokers. Disease rates among these smoking categories were then utilized to develop sampling probabilities for control selection. The randomization procedure was carried out separately among whites and non-whites, and all eligible current heavy smokers in both race categories were invited to participate. Appropriate selection probabilities for the remaining smoking–race categories were determined, and a corresponding percentage of eligible screened controls in each group were invited to complete full interviews.

Data collection

Trained interviewers administered a modified version of the 100-item NCI-Block food-frequency questionnaire (FFQ) [16] to subjects or proxies in their homes. The modified FFQ was designed to more thoroughly capture vegetable and fat consumption, and included expanded food and food preparation lists, as well as an open-ended section. For all dietary questions, participants were asked to describe their usual frequency of consumption and corresponding portion size 2–3 years prior to interview. The Dietary Analysis Personal Computer System (DIETSYS version 3.7C) was used to process all questionnaires [17]. Estimates of α-carotene, β-carotene, β-cryptoxanthin, lutein + zeaxanthin, and lycopene intake were derived from the USDA/NCI carotenoid database [18]. Individuals who failed to provide portion size estimates, reported implausibly extreme amounts of total food eaten (*i.e.* less than four food items per day or more than 30 food items per day), or skipped more than 15% of the food items on the FFQ were excluded.

Statistical analysis

All statistical analyses were performed using the SAS System for Windows version 8.0 (SAS Institute Inc., Cary, NC). Carotenoid intakes were categorized into quintiles based on the distribution among control subjects. Intake of total carotenoids was calculated by summing individual carotenoids (on a molar basis) and then dividing into quintiles based on the controls. Crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) were estimated for each quintile of intake relative to the reference level (quintile 1) via multiple logistic regression. All adjusted models included age (continuous), total energy intake (quintiles), pack-years of smoking (continuous), and education level (tertiles). Inclusion of terms for residential radon exposure, smoking status, saturated fat intake, red meat consumption, and ingestion of heterocyclic amines did not appreciably alter model estimates. Adjustment for total energy intake was evaluated with the standard multivariate, nutrient density, and nutrient residual methods, all of which yielded comparable results. Tests for linear trend across quintiles of intake were carried out by taking the median values of each quintile and modeling as a continuous variable. This method seemed particularly appropriate since distributions of carotenoid intakes are typically highly skewed. Subgroup analyses were carried out by stratifying risk estimates according to smoking status (never/former; current) and histologic type (adenocarcinoma; small cell/squamous cell carcinoma).

We used likelihood ratio tests to determine which fruit and vegetable groups had the greatest impact on the fit of the model predicting lung cancer risk. Logistic regression models were constructed with case–control status as the dependent variable and age, total energy intake, pack-years of smoking, and education as independent variables. Food groups were added one at a time to the base model, and the resulting change in deviance between the two models (likelihood ratio statistic) was estimated (see Appendix for a detailed listing of food group components). All carotenoid models were subsequently adjusted for the food group with the largest likelihood ratio statistic.

Results

In the Missouri Women's Health Study population, cases and controls were similar with respect to age, but not with respect to pack-years of smoking, smoking status, and education (Table 1). In general, cases smoked more extensively and were less well educated than controls. Direct interviews were obtained from the vast majority of controls (99%), whereas proxy interviews were obtained for a significant proportion of cases (39%). Adenocarcinoma was the most common histologic subtype, followed by small cell carcinomas, squamous cell carcinomas, and "other" lung cancer subtypes. With regard to dietary intakes, controls tended to ingest significantly greater amounts of β-carotene, β-cryptoxanthin, lutein + zeaxanthin, total carotenoids, and total vegetables than cases. Controls also ingested greater amounts of α-carotene and lycopene, although not significantly so. Cases consumed marginally higher amounts of total kilocalories than controls.

Increased consumption of α-carotene, β-carotene, β-cryptoxanthin, lutein + zeaxanthin, and total carotenoids was associated with a significantly lower risk of lung cancer in age-adjusted models (Table 2). In models further adjusted for pack-years of smoking, total calorie intake, and education, β-carotene, β-cryptoxanthin, lutein + zeaxanthin, and total carotenoids remained significantly associated with lower risk (for highest *versus* lowest quintile of intake, $OR_{\beta\text{-carotene}}$: 0.58, 95% CI: 0.39–0.86, *p* trend: 0.03; $OR_{\beta\text{-cryptoxanthin}}$: 0.64, 95% CI: 0.43–0.96, *p* trend: 0.003; $OR_{\text{lutein+zeaxanthin}}$: 0.52, 95% CI: 0.35–0.78, *p* trend: 0.0005; $OR_{\text{total carotenoids}}$: 0.61, 95% CI: 0.41–0.91, *p* trend: 0.02). α-Carotene and lycopene were inversely associated with risk in multivariate models, although not significantly so. These results, as well as those presented below, are based on analyses of the full study population since risk estimates did not vary according to interview type (direct versus proxy; data not shown).

Several plant food groups were strong predictors of lower lung cancer risk in this case–control study (Table 3). Likelihood ratio analysis revealed that weekly frequency of total vegetable intake had the greatest impact on the fit of models predicting lower lung cancer risk (all one degree of freedom; ΔG^2: 16.5, *p*-value: <0.00005). Risk estimates derived from multivariate models indicated a significant inverse association between increased consumption of total vegetables and lung cancer risk (data not shown; for highest *versus* lowest quintile of intake, OR: 0.45, 95% CI: 0.30–0.69, *p* trend: 0.0007). Other significant predictors of risk (all modeled as weekly frequency of intake) included raw vegetables; total vegetables, fruits, and fruit juices; dark green and deep yellow vegetables; and dark green, deep yellow, and cruciferous vegetables. The remaining plant food groups, including most fruit categories, were not significantly associated with lower lung cancer risk.

Associations between carotenoids and lung cancer risk varied according to smoking status and histologic type. β-Carotene, lutein + zeaxanthin, and total carotenoids were inversely associated with lung cancer risk in

Table 1. Selected characteristics of the Missouri Women's Health Study population (n = 1211)

Characteristic	Controls (n = 624): Mean (SD)	Cases (n = 587): Mean (SD)	*p*-Value[a]
Age (years)	66.2 (10.0)	66.4 (10.3)	0.68
Pack-years of smoking[b]	30 (26)	48 (34)	<0.0001
	% (n)	% (n)	
Smoking status			<0.0001
Lifetime nonsmoker	13.1 (82)	7.8 (46)	
Former smoker[c]	29.2 (182)	26.4 (155)	
Current light smoker[d]	47.6 (297)	46.3 (272)	
Current heavy smoker[e]	10.1 (63)	19.4 (114)	
Education level (years)			<0.0001
<12	26.0 (162)	36.6 (215)	
12	46.5 (290)	45.0 (264)	
>12	27.6 (172)	18.4 (108)	
Histology			
Squamous cell carcinoma	NA[f]	19.8 (116)	
Small cell carcinoma	NA	23.2 (136)	
Adenocarcinoma	NA	31.7 (186)	
Other	NA	25.4 (149)	
Interview type			<0.0001
Direct	99.0 (618)	60.7 (356)	
Total proxy	1.0 (6)	39.4 (231)	
	Mean (SD)	Mean (SD)	
Dietary intake[g] of:			
Energy (total kilocalories)	1562 (598)	1635 (690)	0.05
α-Carotene (μg/day)	161 (123)	148 (148)	0.10
β-Carotene (μg/day)	1648 (1152)	1482 (1014)	0.008
β-Cryptoxanthin (μg/day)	81 (53)	69 (46)	<0.0001
Lutein + zeaxanthin (μg/day)	1416 (1382)	1255 (1283)	0.04
Lycopene (μg/day)	573 (503)	532 (487)	0.15
Total carotenoids (μM/day)	7.1 (4.7)	6.4 (4.2)	0.005
Total vegetables (frequency/week)	14.7 (6.5)	13.2 (4.8)	<0.0001

[a] *p*-Value derived from *t*-tests (continuous variables) or chi-square tests (categorical variables) for significant differences between cases and controls.
[b] Information based on 617 controls and 571 cases.
[c] Former smokers stopped smoking 3 or more years before diagnosis (cases) or interview date (controls).
[d] Current light smokers smoke less than 30 cigarettes per day.
[e] Current heavy smokers smoke 30 or more cigarettes per day.
[f] NA = not applicable.
[g] Based on food-frequency questionnaire (FFQ) data.

never/former smokers and current smokers (Table 4), although results were significant only among current smokers (for highest *versus* lowest quintile of intake, $OR_{\beta\text{-carotene}}$: 0.54, 95% CI: 0.32–0.90, *p* trend: 0.02; $OR_{\text{lutein+zeaxanthin}}$: 0.48, 95% CI: 0.29–0.81, *p* trend: 0.001; $OR_{\text{total carotenoids}}$: 0.55, 95% CI: 0.32–0.93, *p* trend: 0.01). β-Cryptoxanthin was also inversely associated with risk in both smoking strata, although associations did not reach statistical significance. In analyses stratified by smoking intensity (pack-year tertiles: 0–22.5; 22.6–47.0; 47.1+; data not shown), intakes of β-carotene, β-cryptoxanthin, lutein + zeaxanthin, and total carotenoids were significantly inversely associated with lung cancer risk among subjects in the highest pack-year tertile (for highest *versus* lowest quintile of intake, $OR_{\beta\text{-carotene}}$: 0.42, 95% CI: 0.21–0.85, *p* trend: 0.05; $OR_{\beta\text{-cryptoxanthin}}$: 0.40, 95% CI: 0.20–0.79, *p* trend: 0.003; $OR_{\text{lutein+zeaxanthin}}$: 0.47, 95% CI: 0.23–0.94, *p* trend: 0.06; $OR_{\text{total carotenoids}}$: 0.47, 95% CI: 0.24–0.94, *p* trend: 0.05). In addition, a significant dose–response relationship between consumption of lutein + zeaxanthin and lung cancer risk was observed among subjects in the lowest and middle pack-year tertiles (*p* trend: <0.05).

Table 2. Odds ratios (OR) and 95% confidence intervals (CI) for lung cancer risk according to dietary intake of specific carotenoids: Missouri Women's Health Study

Carotenoid quintile	Cases (no.)	Controls (no.)	Age-adjusted OR (95% CI)	Multivariate OR[a] (95% CI)
α-Carotene (μg/day)				
<58.90	132	124	1.0	1.0
58.90–102.04	119	125	0.89 (0.63–1.3)	0.96 (0.66–1.4)
102.05–156.63	141	125	1.1 (0.75–1.5)	1.2 (0.81–1.7)
156.64–242.68	103	125	0.77 (0.54–1.1)	0.87 (0.59–1.3)
>242.68	92	125	0.69 (0.48–0.99)	0.82 (0.55–1.2)
p Trend			0.02	0.20
β-Carotene (μg/day)				
<823.58	166	124	1.0	1.0
823.58–1145.95	115	125	0.69 (0.49–0.97)	0.71 (0.49–1.0)
1145.96–1526.06	100	125	0.60 (0.42–0.85)	0.60 (0.41–0.87)
1526.07–2323.54	110	125	0.65 (0.46–0.92)	0.71 (0.48–1.1)
>2323.54	96	125	0.57 (0.40–0.81)	0.58 (0.39–0.86)
p Trend			0.007	0.03
β-Cryptoxanthin (μg/day)				
<30.92	142	124	1.0	1.0
30.92–59.74	151	125	1.1 (0.75–1.5)	1.0 (0.72–1.5)
59.75–89.77	137	125	0.95 (0.68–1.3)	1.0 (0.69–1.4)
89.78–115.38	73	125	0.51 (0.35–0.74)	0.60 (0.40–0.90)
>115.38	84	125	0.58 (0.40–0.84)	0.64 (0.43–0.96)
p Trend			0.0001	0.003
Lutein + zeaxanthin (μg/day)				
<567.14	143	124	1.0	1.0
567.14–839.90	125	125	0.87 (0.62–1.2)	0.84 (0.58–1.2)
839.91–1267.40	141	125	0.98 (0.70–1.4)	1.0 (0.71–1.5)
1267.41–1907.11	93	125	0.65 (0.45–0.93)	0.66 (0.45–0.97)
>1907.11	85	125	0.59 (0.41–0.85)	0.52 (0.35–0.78)
p Trend			0.001	0.0005
Lycopene (μg/day)				
<175.80	127	124	1.0	1.0
175.80–314.30	112	125	0.88 (0.61–1.3)	0.94 (0.64–1.4)
314.31–552.26	131	125	1.0 (0.72–1.5)	1.1 (0.74–1.6)
552.27–962.27	124	125	0.97 (0.68–1.4)	0.95 (0.64–1.4)
>962.27	93	125	0.73 (0.50–1.1)	0.73 (0.48–1.1)
p Trend			0.12	0.11
Total Carotenoids (μM/day)				
<3.75	158	123	1.0	1.0
3.75–5.199	125	125	0.79 (0.56–1.1)	0.80 (0.55–1.2)
5.20–6.78	109	125	0.69 (0.49–0.98)	0.71 (0.49–1.0)
6.79–9.52	95	124	0.61 (0.43–0.87)	0.62 (0.42–0.93)
>9.52	100	125	0.64 (0.45–0.90)	0.61 (0.41–0.91)
p Trend			0.008	0.02

[a] Odds ratios adjusted for age, total calorie intake, pack-years of smoking, and education.

In models stratified by histologic type of lung cancer (Table 5), β-carotene and total carotenoids were more strongly inversely associated with squamous/small cell carcinoma risk (for highest *versus* lowest quintile of intake, $OR_{\beta\text{-carotene}}$: 0.45, 95% CI: 0.26–0.79, *p* trend: 0.01; $OR_{\text{total carotenoids}}$: 0.48, 95% CI: 0.27–0.83, *p* trend: 0.01), while lutein + zeaxanthin was more strongly inversely associated with adenocarcinoma risk (for highest *versus* lowest quintile of intake, OR: 0.37, 95% CI: 0.20–0.68, *p* trend: 0.0007). β-Cryptoxanthin was significantly inversely associated with squamous/small cell carcinoma risk (for highest *versus* lowest quintile of intake, OR: 0.49, 95% CI: 0.28–0.87, *p* trend: 0.002), but not with lower adenocarcinoma risk.

Associations between selected fruit and vegetable groupings and lung cancer risk also varied according

Table 3. Likelihood ratio analysis of selected fruit and vegetable groupings and lung cancer risk: Missouri Women's Health Study

Model	−2 log-likelihood	ΔG^2	*p*-Value[a]
Null model[b]	1645.1	NA[c]	NA
Base model[d]	1523.8	121.3	2.8E-25
Total vegetables	1507.3	16.5	4.8E-05
Raw vegetables	1512.1	11.8	0.0006
Total vegetables, fruits, and fruit juices	1513.0	10.8	0.001
Dark green and deep yellow vegetables	1514.2	9.6	0.002
Dark green, deep yellow, and cruciferous vegetables	1518.2	5.6	0.02
Citrus fruits	1520.3	3.5	0.06
Citrus fruits and juices	1520.4	3.4	0.07
Total cruciferous vegetables	1521.7	2.1	0.14
Total fruits and fruit juices	1522.4	1.4	0.23
Fruit juices	1523.4	0.44	0.51
Low vitamin A cruciferous vegetables	1523.5	0.33	0.57
Other fruits (excluding citrus)	1523.8	<0.05	0.90

[a] *p*-Values correspond to goodness-of-fit statistics comparing base model with individual food group models (ΔG^2), and are based upon a chi-square distribution with one degree of freedom.
[b] Intercept only.
[c] NA = not applicable.
[d] Adjusted for age, total calorie intake, pack-years of smoking, and education.

to smoking status and histologic type. Increased consumption of total vegetables was inversely associated with lung cancer risk in never/former smokers and current smokers (Table 4), although associations were significant only in the latter group (for highest *versus* lowest quintile of intake, OR: 0.41, 95% CI: 0.24–0.71, *p* trend: 0.001). Other vegetable groupings – including dark green and deep yellow vegetables; raw vegetables; and dark green, deep yellow, and cruciferous vegetables – were also significantly inversely associated with risk in current smokers (OR: <1 for highest *versus* lowest quintile of intake, 95% CI excludes unity, *p* trend: <0.05) and inversely associated with risk among never/former smokers, although not significantly so (data not shown). In analyses stratified by smoking intensity (data not shown), increased consumption of total vegetables was strongly inversely associated with lung cancer risk among subjects in the middle (22.6–47.0 pack-years) and highest (47.1+ pack-years) pack-year tertiles (for 22.6–47.0 pack-years, highest *versus* lowest quintile of intake, OR: 0.41, 95% CI: 0.20–0.85, *p* trend: 0.009; for 47.1+ pack-years, highest *versus* lowest quintile of intake, OR: 0.32, 95% CI: 0.16–0.65, *p* trend: 0.009), but not among subjects in the lowest pack-year tertile (for <22.5 pack-years, highest *versus* lowest quintile of intake, OR: 0.76, 95% CI: 0.36–1.60, *p* trend: 0.60).

Increased consumption of total vegetables was significantly inversely associated with risk of both histologic types of lung cancer (Table 5), although associations were slightly stronger for squamous/small cell carcinomas (for highest *versus* lowest quintile of intake, OR: 0.34, 95% CI: 0.18–0.61, *p* trend: 0.0004) than for adenocarcinomas (for highest *versus* lowest quintile of intake, OR: 0.41, 95% CI: 0.23–0.76, *p* trend: 0.006). Increased consumption of dark green and deep yellow vegetables was also strongly inversely associated with risk of both histologic types of cancer (data not shown). Citrus fruits; fruit and fruit juices; and raw vegetables were each significantly inversely associated with risk of squamous/small cell carcinoma but not with risk of adenocarcinoma; consumption of cruciferous vegetables was associated with a significantly lower risk of adenocarcinoma, but not with risk of squamous/small cell carcinoma (data not shown).

When selected plant food groups were adjusted for total carotenoids (Table 6a), total vegetables; raw vegetables; and total vegetables, fruits, and fruit juices remained significantly inversely associated with lung cancer risk (OR: <1, 95% CI excludes unity, *p* trend: <0.05). Dark green and deep yellow vegetables, as well as dark green, deep yellow, and cruciferous vegetables, also remained inversely associated with risk, although not significantly so.

When individual and total carotenoids were each adjusted for weekly frequency of total vegetable intake (Table 6b), all carotenoid odds ratios became nonsignificant (95% CI includes unity, *p* trend: ≥ 0.05). Total vegetable intake remained significantly associated with lower lung cancer risk in these models. β-Cryptoxanthin was less affected by vegetable adjustment than the other carotenoids, as expected, since β-cryptoxanthin is predominantly derived from citrus fruits [19].

Table 4. Odds ratios (OR) and 95% confidence intervals (CI) for lung cancer risk according to dietary intake of specific carotenoids and total vegetables, stratified by smoking status: Missouri Women's Health Study

	Never/former smokers			Current smokers		
	Cases (no.)	Controls (no.)	Multivariate OR[a] (95% CI)	Cases (no.)	Controls (no.)	Multivariate OR[a] (95% CI)
α-Carotene (μg/day)						
<58.90	40	47	1.0	92	77	1.0
58.90–102.04	40	44	1.2 (0.63–2.3)	79	81	0.85 (0.54–1.3)
102.05–156.63	43	51	1.2 (0.63–2.3)	98	74	1.1 (0.72–1.8)
156.64–242.68	36	60	0.96 (0.50–1.8)	67	65	0.79 (0.49–1.3)
>242.68	42	62	0.89 (0.47–1.7)	50	63	0.74 (0.44–1.2)
p Trend			0.41			0.23
β-Carotene (μg/day)						
<823.58	53	48	1.0	113	76	1.0
823.58–1145.95	33	56	0.55 (0.29–1.0)	82	69	0.79 (0.50–1.3)
1145.96–1526.06	35	52	0.55 (0.29–1.1)	65	73	0.59 (0.37–0.95)
1526.07–2323.54	41	50	0.76 (0.40–1.4)	69	75	0.67 (0.41–1.1)
>2323.54	39	58	0.62 (0.33–1.2)	57	67	0.54 (0.32–0.90)
p Trend			0.51			0.02
β-Cryptoxanthin (μg/day)						
<30.92	44	38	1.0	98	86	1.0
30.92–59.74	47	52	0.77 (0.41–1.4)	104	73	1.2 (0.77–1.9)
59.75–89.77	48	57	0.73 (0.40–1.4)	89	68	1.2 (0.75–1.9)
89.78–115.38	32	66	0.45 (0.23–0.86)	41	59	0.71 (0.42–1.2)
>115.38	30	51	0.62 (0.32–1.2)	54	74	0.65 (0.40–1.1)
p Trend			0.07			0.03
Lutein + zeaxanthin (μg/day)						
<567.14	50	51	1.0	93	73	1.0
567.14–839.90	46	58	0.79 (0.44–1.4)	79	67	0.88 (0.55–1.4)
839.91–1267.40	46	57	0.87 (0.48–1.6)	95	68	1.2 (0.74–1.9)
1267.41–1907.11	28	50	0.66 (0.35–1.3)	65	75	0.66 (0.41–1.1)
>1907.11	31	48	0.64 (0.33–1.2)	54	77	0.48 (0.29–0.81)
p Trend			0.18			0.001
Lycopene (μg/day)						
<175.80	48	54	1.0	79	70	1.0
175.80–314.30	39	52	0.92 (0.49–1.7)	73	73	0.98 (0.60–1.6)
314.31–552.26	47	61	0.91 (0.50–1.7)	84	64	1.2 (0.73–2.0)
552.27–962.27	40	51	0.79 (0.41–1.5)	84	74	1.1 (0.67–1.8)
>962.27	27	46	0.74 (0.36–1.5)	66	79	0.74 (0.43–1.3)
p Trend			0.38			0.19
Total carotenoids (μM/day)						
<3.75	52	49	1.0	106	74	1.0
3.75–5.199	38	61	0.58 (0.31–1.1)	87	64	0.96 (0.60–1.5)
5.20–6.78	37	44	0.90 (0.48–1.7)	72	81	0.62 (0.39–0.99)
6.79–9.52	35	57	0.61 (0.32–1.2)	60	67	0.62 (0.37–1.0)
>9.52	39	52	0.71 (0.37–1.4)	61	73	0.55 (0.32–0.93)
p Trend			0.51			0.01
Total vegetables (frequency/week)						
<9.2	49	46	1.0	91	76	1.0
9.2–11.2	30	51	0.54 (0.28–1.0)	82	62	1.2 (0.73–1.9)
11.3–14.7	48	60	0.80 (0.43–1.5)	91	79	1.0 (0.63–1.6)
14.8–19.6	46	54	0.76 (0.41–1.4)	80	64	1.1 (0.65–1.8)
>19.6	28	53	0.51 (0.25–1.0)	42	79	0.41 (0.24–0.71)
p Trend			0.19			0.001

[a] Odds ratios adjusted for age, total calorie intake, pack-years of smoking, and education.

Table 5. Odds ratios (OR) and 95% confidence intervals (CI) for lung cancer risk according to dietary intake of specific carotenoids and total vegetables, stratified by histologic type: Missouri Women's Health Study

	Adenocarcinoma		Squamous/small cell carcinoma	
	Cases (no.)	Multivariate OR[a] (95% CI)	Cases (no.)	Multivariate OR[a] (95% CI)
α-Carotene (μg/day)				
<58.90	41	1.0	63	1.0
58.90–102.04	40	1.1 (0.63–1.8)	46	0.72 (0.44–1.2)
102.05–156.63	45	1.2 (0.71–2.0)	58	0.97 (0.60–1.6)
156.64–242.68	32	0.91 (0.52–1.6)	45	0.72 (0.43–1.2)
>242.68	28	0.83 (0.47–1.5)	40	0.66 (0.39–1.1)
p Trend		0.37		0.17
β-Carotene (μg/day)				
<823.58	57	1.0	68	1.0
823.58–1145.95	30	0.54 (0.32–0.92)	54	0.82 (0.51–1.3)
1145.96–1526.06	37	0.72 (0.43–1.2)	41	0.55 (0.32–0.93)
1526.07–2323.54	34	0.73 (0.42–1.3)	52	0.77 (0.46–1.3)
>2323.54	28	0.50 (0.28–0.89)	37	0.45 (0.26–0.79)
p Trend		0.08		0.01
β-Cryptoxanthin (μg/day)				
<30.92	47	1.0	60	1.0
30.92–59.74	41	0.84 (0.51–1.4)	74	1.1 (0.67–1.7)
59.75–89.77	44	1.0 (0.61–1.6)	56	0.91 (0.56–1.5)
89.78–115.38	24	0.58 (0.33–1.0)	31	0.59 (0.34–1.0)
>115.38	30	0.70 (0.40–1.2)	31	0.49 (0.28–0.87)
p Trend		0.14		0.002
Lutein + zeaxanthin (μg/day)				
<567.14	51	1.0	55	1.0
567.14–839.90	41	0.82 (0.49–1.4)	52	0.85 (0.51–1.4)
839.91–1267.40	46	0.93 (0.56–1.5)	64	1.3 (0.77–2.0)
1267.41–1907.11	28	0.61 (0.35–1.1)	41	0.69 (0.41–1.2)
>1907.11	20	0.37 (0.20–0.68)	40	0.57 (0.33–0.99)
p Trend		0.0007		0.02
Lycopene (μg/day)				
<175.80	35	1.0	63	1.0
175.80–314.30	37	1.1 (0.61–1.8)	53	1.1 (0.65–1.8)
314.31–552.26	43	1.2 (0.68–2.1)	46	0.81 (0.49–1.4)
552.27–962.27	35	1.1 (0.59–1.9)	57	0.93 (0.56–1.5)
>962.27	36	1.0 (0.55–1.9)	33	0.58 (0.32–1.0)
p Trend		0.89		0.05
Total carotenoids (μM/day)				
<3.75	54	1.0	69	1.0
3.75–5.199	33	0.65 (0.38–1.1)	54	0.82 (0.50–1.3)
5.20–6.78	43	0.82 (0.50–1.4)	43	0.57 (0.34–0.96)
6.79–9.52	26	0.54 (0.31–0.97)	48	0.68 (0.40–1.1)
>9.52	30	0.53 (0.30–0.95)	38	0.48 (0.27–0.83)
p Trend		0.04		0.01
Total vegetables (frequency/week)				
<9.2	50	1.0	58	1.0
9.2–11.2	32	0.76 (0.45–1.3)	51	0.93 (0.56–1.6)
11.3–14.7	50	0.93 (0.57–1.5)	63	1.0 (0.62–1.6)
14.8–19.6	32	0.75 (0.44–1.3)	54	0.89 (0.53–1.5)
>19.6	22	0.41 (0.23–0.76)	26	0.34 (0.18–0.61)
p Trend		0.006		0.0004

[a] Odds ratios adjusted for age, total calorie intake, pack-years of smoking, and education.

Table 6a. Odds ratios (OR) and 95% confidence intervals (CI) for lung cancer risk according to consumption (continuous) of specific plant food groups, before and after adjusting for total carotenoid intake: Missouri Women's Health Study

Food group (weekly frequency)	Multivariate OR[a] (95% CI)	*p* Trend	Carotenoid-adjusted OR[b] (95% CI)	*p* Trend
Total vegetables	0.67 (0.55–0.82)	<0.0001	0.70 (0.56–0.87)	0.001
Raw vegetables	0.74 (0.62–0.88)	0.0007	0.77 (0.64–0.92)	0.003
Total vegetables, fruits, and fruit juices	0.73 (0.60–0.88)	0.001	0.77 (0.63–0.96)	0.02
Dark green and deep yellow vegetables	0.84 (0.75–0.94)	0.002	0.87 (0.75–1.0)	0.07
Dark green, deep yellow, and cruciferous vegetables	0.82 (0.69–0.97)	0.02	0.90 (0.72–1.1)	0.36

[a] Odds ratios are based upon a difference corresponding to the interquartile range (IQR) of exposure among the controls, and are adjusted for age, total calorie intake, pack-years of smoking, and education
[b] Odds ratios further adjusted for total carotenoid intake (continuous).

Table 6b. Odds ratios (OR) and 95% confidence intervals (CI) for lung cancer risk according to intake (continuous) of specific dietary carotenoids, before and after adjusting for total vegetable consumption: Missouri Women's Health Study

Carotenoid (μg/day)	Multivariate OR[a] (95% CI)	*p* Trend	Vegetable-adjusted OR[b] (95% CI)	*p* Trend
α-Carotene	0.94 (0.82–1.1)	0.40	1.1 (0.93–1.2)	0.36
β-Carotene	0.85 (0.74–0.98)	0.03	0.97 (0.83–1.1)	0.68
β-Cryptoxanthin	0.75 (0.63–0.90)	0.002	0.83 (0.68–1.0)	0.05
Lutein + zeaxanthin	0.90 (0.81–0.99)	0.03	0.97 (0.87–1.1)	0.54
Lycopene	0.91 (0.78–1.1)	0.19	0.94 (0.81–1.1)	0.40
Total carotenoids (μM/day)	0.83 (0.72–0.96)	0.01	0.94 (0.81–1.1)	0.47

[a] Odds ratios are based upon a difference corresponding to the interquartile range (IQR) of exposure among the controls, and are adjusted for age, total calorie intake, pack-years of smoking, and education.
[b] Odds ratios further adjusted for weekly frequency of total vegetable intake (continuous).

Discussion

Increased consumption of β-carotene, β-cryptoxanthin, lutein + zeaxanthin, and total carotenoids was associated with a significantly lower risk of lung cancer in this case–control study. Each of these carotenoids – with the exception of β-cryptoxanthin – was significantly inversely associated with risk in current smokers; inverse associations were not statistically significant among never/former smokers. Lower risk of adenocarcinoma was most strongly associated with increased consumption of lutein + zeaxanthin, whereas β-carotene, β-cyptoxanthin, and total carotenoids were most strongly associated with lower risk of squamous/small cell carcinoma. Total vegetable intake – the strongest plant food predictor of lung cancer risk – was associated with lower risk in current smokers, and was significantly inversely associated with both histologic types of lung cancer, although associations were more pronounced for squamous/small cell carcinomas. Total vegetable intake was more strongly associated with lower lung cancer risk than intake of any individual carotenoid or total carotenoids.

Other data on lung cancer and carotenoids in women are not entirely consistent with our results or each other; these discrepancies may be partially attributable to differences in study populations. Dietary α-carotene was the only carotenoid significantly associated with lower lung cancer risk in the Nurses Health Study cohort [6]. However, increased consumption of β-carotene, lutein + zeaxanthin, lycopene, and total carotenoids was inversely associated with risk in that study, and their respective relative risks all approached statistical significance. Data from a case–control study conducted among Spanish women showed no significant associations between increased consumption of individual carotenoids and lower risk of lung cancer, although most associations were inverse [10]. In a Hawaiian population, strong inverse associations between β-carotene, α-carotene, and lutein + zeaxanthin and risk of lung cancer among women were observed (*p* trend: <0.05) [9]. Results from a nested case–control analysis of serum carotenoids and lung cancer risk in Washington County, Maryland, were similar to our own, and indicated that serum α-carotene, β-carotene, β-cryptoxanthin, and lutein + zeaxanthin were each inversely associated with lung cancer risk in women [20]. In addition, serum β-cryptoxanthin was more strongly inversely associated with lung cancer risk than any other individual carotenoid. A recent study conducted among men in China also showed that high serum levels of β-cryptoxanthin, but not other individual carotenoids, were significantly inversely associated with lung cancer risk [21].

Most observational studies support a protective effect of increased vegetable and/or fruit consumption on lung cancer risk [2]. In our study, consumption of several vegetable groups strongly predicted overall lung cancer risk, but consumption of fruits did not. Our overall and stratum-specific vegetable and fruit results are comparable to recent findings obtained from the Nurses Health Study and the Netherlands Cohort Study on Diet and Cancer [8, 22, 23], although statistical significance of various food groups varied between studies.

Very few epidemiologic studies have used statistical significance testing to formally examine whether individual and/or total carotenoids are more or less strongly associated with lung cancer risk than their primary plant food sources, and none has been conducted exclusively in women. Results from a study conducted among men and women in Hawaii indicated that total vegetable intake was more strongly inversely associated with lung cancer risk than intake of total carotenoids [9]. In a study of Finnish men, adjustment for individual and total carotenoids did not affect the association between intake of root vegetables and lung cancer risk [7]. Our study, which represents one of the first to address this question in a population of women, supports these findings.

Case–control studies are often prone to selection bias, information bias, and residual confounding. Preferential participation of health-conscious controls and differential recall of diet according to case–control status are possible limitations of the present study. Another possible limitation arises from the potential (although unlikely) effects of preclinical lung disease on dietary habits, in which case intake 2–3 years prior to interview might not reflect usual adult diet. Residual confounding by smoking is a concern in most case–control studies of diet and lung cancer. Probability sampling of controls and adjustment for pack-years of smoking in multivariate models were utilized to address this issue. Nevertheless, the number of lifetime nonsmokers was small, and we were unable to assess associations between carotenoids and lung cancer risk in this subgroup. Results from studies carried out among lifetime nonsmokers are rare, yet informative. A study of nonsmoking women in Florida indicated that increased consumption of α-carotene, β-carotene, β-cryptoxanthin, and total carotenoids was significantly inversely associated with lung cancer risk in this population [12].

One of the principal strengths of this study is the large number of cases, which increases the power to detect modest associations and allows for analysis based on quintiles rather than quartiles. Other strengths include use of a food-frequency questionnaire designed to accurately capture vegetable intake, use of an updated national carotenoid database, strong external validity due to recruitment of population rather than hospital-based controls, high participation rates among cases and controls, relatively small numbers of proxy respondents, and use of incident and histologically confirmed lung cancer cases.

The present study indicates that consumption of a wide variety of vegetables is more strongly associated with a lower risk of lung cancer in women than consumption of any single food item or phytochemical. Although carotenoids may play a role in lung cancer prevention, they clearly are not the only etiologic agents found in plant foods. Other phytochemicals, including ones that have yet to be discovered, or interactions among various nutrients (including carotenoids) may be more important in terms of lung cancer prevention. Screening approaches for asymptomatic lung cancer have not been demonstrated to reduce lung cancer mortality to date, and prognosis is often poor after diagnosis. Therefore, smoking and diet modification represent the most feasible alternatives for lowering lung cancer risk. Smoking is the largest preventable cause of lung cancer, but dietary practices also seem quite important. The public health implications of our study suggest that women should eat a wide variety of vegetables in order to lower their risk of lung cancer; current smokers should pay particular attention to this message because smoking cessation efforts are sometimes unsuccessful. Unfortunately, the vast majority of women in the United States consume less than the five-plus recommended daily servings of fruits and vegetables [24].

Appendix: food group constituents

Total vegetables: string beans, green or yellow snap; peas; black-eyed peas; corn; cabbage/sauerkraut; winter squash; raw tomatoes; salsa, red chili sauce; broccoli; cauliflower, brussel sprouts; raw spinach; cooked spinach; collards, kale, greens; coleslaw; carrots, mixed vegetables with carrots; green salad; french fries, fried potatoes; sweet potatoes; other potatoes; other vegetables; beef stew, pot pie; vegetable soup; other beans (baked, pintos, kid); chili with beans; tomato juice or V-8; raw carrots; carrot juice; olives; cucumber; jalapeno peppers; celery; garlic; onions; asparagus; raw red/green pepper; avocado; beets; pickles; canned tomatoes; rutabaga; turnips; mushrooms.

Cruciferous vegetables: cabbage/sauerkraut; broccoli; cauliflower, brussel sprouts; raw spinach; cooked spinach; collards, kale, greens; coleslaw; beets; rutabaga; turnips.

Dark green and deep yellow vegetables: winter squash; broccoli; raw spinach; cooked spinach; collards, kale, greens; carrots, mixed vegetables with carrots; sweet potatoes; raw carrots; carrot juice.

Dark green, deep yellow, and cruciferous vegetables: cabbage/sauerkraut; winter squash; broccoli; cauliflower, brussel sprouts; raw spinach; cooked spinach; collards, kale, greens; coleslaw; carrots, mixed vegetables with carrots; sweet potatoes; raw carrots; carrot juice; beets; rutabaga; turnips.

Low vitamin A cruciferous vegetables: cabbage/sauerkraut; cauliflower, brussel sprouts; coleslaw; beets; rutabaga; turnips.

Raw vegetables: raw tomatoes; raw spinach; coleslaw; green salad; raw carrots; carrot juice; olives; cucumber; jalapeno peppers; celery; raw red/green pepper; avocado; pickles.

Fruit and fruit juices: apples, applesauce, pears; bananas; peaches, apricots, nectarines, fresh or canned; cantaloupe, in season; cantaloupe, out of season; watermelon, in season; strawberries, in season; oranges, tangerines; orange juice, grapefruit juice; grapefruit; Kool-Aid or fruit drinks with added vitamin C; other fruits; raisins; prunes; lemonade; cranberry juice cocktail; lemon juice; rhubarb.

Citrus fruits and juices: oranges, tangerines; orange juice, grapefruit juice; grapefruit; lemonade; lemon juice.

Citrus fruits: oranges, tangerines; grapefruit.

Fruit juices: orange juice, grapefruit juice; Kool-Aid or fruit drinks with added vitamin C; lemonade; cranberry juice cocktail; lemon juice.

Other fruits (excluding citrus): apples, applesauce, pears; bananas; peaches, apricots, nectarines, fresh or canned; cantaloupe, in season; cantaloupe, out of season; watermelon, in season; strawberries, in season; Kool-Aid or other fruit drinks with added vitamin C; other fruits; raisins; prunes; lemonade; cranberry juice cocktail; rhubarb.

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References

1. Greenlee RT, Hill-Harmon MB, Murray T, Thun M (2001) Cancer statistics, 2001. *CA Cancer J Clin* **51**: 15–36.
2. Ziegler RG, Mayne ST, Swanson CA (1996) Nutrition and lung cancer. *Cancer Causes Control* **7**: 157–177.
3. Rock CL (1997) Carotenoids: biology and treatment. *Pharmacol Ther* **75**: 185–197.
4. Alpha-Tocopherol and Beta-Carotene Cancer Prevention Study Group (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. *N Engl J Med* **330**: 1029–1035.
5. Omenn GS, Goodman GE, Thornquist MD, *et al.* (1996) Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* **334**: 1150–1155.
6. Michaud DS, Feskanich D, Rimm EB, *et al.* (2000) Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am J Clin Nutr* **72**: 990–997.
7. Knekt P, Jarvinen R, Teppo L, Aromaa A, Seppanen R (1999) Role of various carotenoids in lung cancer prevention. *J Natl Cancer Inst* **91**: 182–184.
8. Ziegler RG, Colavito EA, Hartge P, *et al.* (1996) Importance of alpha-carotene, beta-carotene, and other phytochemicals in the etiology of lung cancer. *J Natl Cancer Inst* **88**: 612–615.
9. Le Marchand L, Hankin JH, Kolonel LN, Beecher GR, Wilkens LR, Zhao LP (1993) Intake of specific carotenoids and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* **2**: 183–187.
10. Garcia-Closas R, Agudo A, Gonzalez CA, Riboli E (1998) Intake of specific carotenoids and flavonoids and the risk of lung cancer in women in Barcelona, Spain. *Nutr Cancer* **32**: 154–158.
11. Voorrips LE, Goldbohm RA, Brants HA, *et al.* (2000) A prospective cohort study on antioxidant and folate intake and male lung cancer risk. *Cancer Epidemiol Biomarkers Prev* **9**: 357–365.
12. Candelora EC, Stockwell HG, Armstrong AW, Pinkham PA (1992) Dietary intake and risk of lung cancer in women who never smoked. *Nutr Cancer* **17**: 263–270.
13. Alavanja MC, Lubin JH, Mahaffey JA, Brownson RC (1999) Residential radon exposure and risk of lung cancer in Missouri. *Am J Public Health* **89**: 1042–1048.
14. Swanson CA, Brown CC, Sinha R, Kulldorff M, Brownson RC, Alavanja MC (1997) Dietary fats and lung cancer risk among women: the Missouri Women's Health Study (United States). *Cancer Causes Control* **8**: 883–893.
15. Alavanja MC, Brownson RC, Berger E, Lubin J, Modigh C (1996) Avian exposure and risk of lung cancer in women in Missouri: population based case-control study. *BMJ* **313**: 1233–1235.
16. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L (1986) A data-based approach to diet questionnaire design and testing. *Am J Epidemiol* **124**: 453–469.
17. Smucker R, Block G, Coyle L, Harvin A, Kessler L (1989) A dietary and risk factor questionnaire and analysis system for personal computers. *Am J Epidemiol* **129**: 445–449.
18. Chug-Ahuja JK, Holden JM, Forman MR, Mangels AR, Beecher GR, Lanza E (1993) The development and application of a carotenoid database for fruits, vegetables, and selected multi-component foods. *J Am Diet Assoc* **93**: 318–323.
19. Panel on Dietary Antioxidants and Related Compounds, Food and Nutrition Board, Institute of Medicine (2000) *Dietary*

Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: National Academy Press.

20. Comstock GW, Alberg AJ, Huang HY, *et al*. (1997) The risk of developing lung cancer associated with antioxidants in the blood: ascorbic acid, carotenoids, alpha-tocopherol, selenium, and total peroxyl radical absorbing capacity. *Cancer Epidemiol Biomarkers Prev* **6**: 907–916.
21. Yuan JM, Ross RK, Chu XD, Gao YT, Yu MC (2001) Prediagnostic levels of serum beta-cryptoxanthin and retinol predict smoking-related lung cancer risk in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* **10**: 767–773.
22. Feskanich D, Ziegler RG, Michaud DS, *et al*. (2000) Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *J Natl Cancer Inst* **92**: 1812–1823.
23. Voorrips LE, Goldbohm RA, Verhoeven DT, *et al*. (2000) Vegetable and fruit consumption and lung cancer risk in the Netherlands Cohort Study on diet and cancer. *Cancer Causes Control* **11**: 101–115.
24. National Center for Chronic Disease Prevention and Health Promotion (2001) *Behavioral Risk Factor Surveillance System, Year 2000*. Survey data. Centers for Disease Control and Prevention, US Department of Health and Human Services.