A Prospective Cohort Study on Antioxidant and Folate Intake and Male Lung Cancer Risk[1]

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Abstract

Many studies have reported inverse associations between vegetable and fruit consumption and lung cancer risk. The aim of the present study was to elucidate the role of several antioxidants and folate in this relationship. In the Netherlands Cohort Study on Diet and Cancer, 58,279 men of ages 55–69 years at baseline in 1986 returned a questionnaire including a 150-item food frequency questionnaire. After 6.3 years of follow-up, 939 male lung cancer cases were registered. A new Dutch carotenoid database was used to estimate intake of α-carotene, β-carotene, lutein + zeaxanthin, β-cryptoxanthin, and lycopene, completed with the antioxidant vitamins C and E and folate. Using case-cohort analysis, rate ratios were calculated, adjusted for age, smoking, educational level, and family history of lung cancer. Protective effects on lung cancer incidence were found for lutein + zeaxanthin, β-cryptoxanthin, folate, and vitamin C. Other carotenoids (α-carotene, β-carotene, and lycopene) and vitamin E did not show significant associations. After adjustment for vitamin C, only folate remained inversely associated, and after adjustment for folate, only β-cryptoxanthin and vitamin C remained significantly associated. Inverse associations were strongest among current smokers and weaker for former smokers at baseline. Inverse associations with carotenes, lutein + zeaxanthin, and β-cryptoxanthin seemed to be limited to small cell and squamous cell carcinomas. Only folate and vitamin C intake appeared to be inversely related to small cell and squamous cell carcinomas and adenocarcinomas. Folate, vitamin C, and β-cryptoxanthin might be better protective agents against lung cancer in smokers than α-carotene, β-carotene, lutein + zeaxanthin, and lycopene.

Introduction

Vegetables and fruits are associated with a reduced risk of cancers, especially lung cancer (1–4). Recent results from the Netherlands Cohort Study on Diet and Cancer on vegetable and fruit consumption and the association with cancer incidence at several sites have revealed reduced risks for lung cancer (5), less pronounced effects on colorectal cancer (6) and stomach cancer (7), and no effect on prostate cancer (8).

The possible protective compounds in vegetables and fruit include a wide variety of phytochemicals (9). Among them are the carotenoids, colorful compounds that are abundant as pigments in plants. The main carotenoids are α-carotene, β-carotene, lutein, zeaxanthin, β-cryptoxanthin, and lycopene. They are potent quenchers of free radicals, which are by-products of metabolic processes originating from environmental pollutants such as cigarette smoke. Some carotenoids can be metabolically converted into retinol (α-carotene, β-carotene, and β-cryptoxanthin). Case-control studies on the relationship between carotenoids in food and the incidence of lung cancer have shown inverse associations with β-carotene, α-carotene, and lutein + zeaxanthin but not with lycopene or β-cryptoxanthin (10, 11). In the past decade, eight prospective studies have been published concerning the association of dietary carotenoids and other antioxidants with lung cancer risk (12–19). Six of them presented RRs[3] using a summed variable for carotenoids (12, 14, 15, 17–19); one of them also presented mean daily intakes for the major carotenoids for cases and non-cases (12). Four studies presented RRs separately for β-carotene (13–16), and one recent study presented RRs for all major carotenoids (19). In the analyses, other antioxidants were included such as vitamin E (12, 16–19) and vitamin C (12–19), and two studies included folate (18, 19). The studies were performed in men (12, 14, 16), women (15, 19), or both (13, 17, 18). Most studies presented RRs for the respective food constituents by smoking status (12, 15, 17, 18) and/or by histological type of lung cancer (15, 18).

We have recently developed a database containing data on the most important carotenoids based on chemical analyses of the main vegetables and literature values (20). This gave us the opportunity to perform analyses on lung cancer incidence and carotenoid intake in the Netherlands Cohort Study on Diet and Cancer and to evaluate in more detail possible mechanisms underlying the inverse associations found between vegetable and fruit consumption and the incidence of lung cancer (6). In addition to the carotenoids covered by the database (α-carotene, β-carotene, lutein + zeaxanthin, β-cryptoxanthin, and lycopene), folate and the antioxidant vitamins C and E were in-

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[3] The abbreviations used are: RR, rate ratio; CI, confidence interval.

cluded in the analyses. With over 900 incident male lung cancer cases, analyses could be performed for separate strata of smoking status and histology.

Materials and Methods

Subjects. The Netherlands Cohort Study on diet and cancer is a prospective cohort study that started in September 1986. The study design has been reported in detail elsewhere (21). The cohort included 58,279 men of ages 55–69 years at the beginning of the study. The study population originated from 204 municipalities with computerized population registries.

Data Collection. At baseline, the cohort members completed a mailed, self-administered questionnaire on dietary habits, lifestyle, smoking, personal and family history of cancer, and demographics. The dietary section of the questionnaire was a 150-item semiquantitative food frequency questionnaire. The questionnaire concentrated on the habitual consumption of foods and beverages during the year preceding the start of the study. The principal nutrients of interest in the design of the questionnaire were energy, protein, fat, cholesterol, carbohydrates, dietary fiber, alcohol, calcium, vitamin A, β-carotene, and vitamin C. The questionnaire was validated against a 9-day diet record (22).

Information on dietary supplement use was collected with an open-ended question with space for four different supplements at most. Participants were asked whether they used vitamin tablets, drops, or other preparations during the 5 years before baseline.

Food Composition Tables. Mean daily nutrient intakes were calculated using the computerized Dutch food composition table (23). For calculation of the intake of specific carotenoids, an additional food composition table has been constructed recently (20), providing information on the most important carotenoids: α- carotene, β-carotene, lutein + zeaxanthin, lycopene, and β-cryptoxanthin. Briefly, foods that are the main sources of carotenoids (*e.g.,* vegetables) were sampled and analyzed for α-carotene, β-carotene, lutein, zeaxanthin, and lycopene. Some other foods, such as margarines, were also analyzed to check data supplied by manufacturers. Values for all other foods mostly were derived from recent literature based on the same methods of analysis as we used. For mixed dishes, carotenoids were estimated based on the recipe of preparation. Vegetables were sampled in two to three periods across a year, and at each occasion a pooled sample derived from seven different retailers was analyzed as described previously (20). In the carotenoid food composition table, lutein and zeaxanthin had to be taken together because most of the literature sources used did not provide separate values for each of these carotenoids. Vegetables, however, contain primarily lutein and only minor quantities of zeaxanthin. Folate content was mainly derived from the food composition tables of McCance and Widdowson (24).

Record Linkage. The method of record linkage to obtain information on cancer incidence has been described previously (25). In short, follow-up for incident cancer was established by computerized record linkage with all regional cancer registries in the Netherlands and with PALGA, a computerized national database of pathology reports. Completeness of follow-up of cancer was at least 96% (26). After 6.3 years of follow-up, *i.e.,* from September 1986 to December 1992, 1096 incident male lung cancer cases were identified.

Data Analysis. The case-cohort approach was used for data analysis (27). This means that cases are enumerated for the entire cohort, whereas the person-years at risk for the entire cohort are estimated from a random subcohort sample. After baseline exposure measurement, a subcohort of 1688 men was randomly sampled from the cohort and followed up biennially for vital status information. No subcohort members were lost to follow-up over the 6.3 years of follow-up.

After exclusion of lung cancer cases who reported prevalent cancer other than skin cancer at baseline, lung cancer cases without microscopically confirmed cancer, cases with lung cancer other than carcinoma (sarcoma, lymphoma, and unspecified morphology), or with *in situ* lung carcinoma, 1050 incident lung cancer cases were identified. From the subcohort, prevalent cancer cases other than skin cancer were excluded as well. Subjects with incomplete or inconsistent dietary data were also excluded from analyses. These included subjects who: *(a)* left blank 60 (of 150) items in the questionnaire and reported to eat <35 items at least once a month or more; or *(b)* subjects who left one or more item blocks (grouping of items, *e.g.,* beverages) blank. More details are given in a separate report (22). Eventually, 939 male lung cancer cases and 1525 male subcohort members were available for analyses.

Analyses were performed for the carotenoids α-carotene, β-carotene, lutein + zeaxanthin, lycopene, and β-cryptoxanthin, vitamin C, vitamin E, and folate. Mean intakes and SDs were calculated for cases and subcohort members. For different strata of age, smoking status, educational level, and family history of lung cancer, mean intakes were calculated for the subcohort. Pearson correlation coefficients were calculated for the food constituents of interest and energy intake. RRs of lung cancer and their 95% CIs were computed using the GLIM statistical package (28). Exponentially distributed survival times were assumed in the follow-up period. Because standard software was not available for case-cohort analysis, specific macros were developed to account for the additional variance introduced by sampling from the cohort instead of using the entire cohort (29). Age, level of education, family history of lung cancer, current cigarette smoking (yes/no), number of cigarettes per day, and years of smoking cigarettes were considered as potential confounders for which the RRs were adjusted. Because energy consumption is frequently considered to confound RRs, an additional model was used that included energy. Additionally, models were used adjusting for either vitamin C or folate content, because these nutrients were found to be highly correlated to (some of the) carotenoids. Tests for trends in the RRs were based on two-sided likelihood ratio tests. Intakes of food constituents were classified into quintiles based on the distribution in the subcohort. For vitamin C, however, the validation study had pointed out that quintiles 2 and 3 and quintiles 4 and 5 could not be distinguished; therefore, we reduced vitamin C intake to three categories (22). RRs for quintiles/categories of intake were also computed separately in never-smokers, former smokers, and current smokers. Because the number of cases among never-smokers was small, tertiles of intake were used instead of quintiles. Also, subgroup analyses were performed for the most common histological types of lung cancer: small cell carcinoma, squamous cell carcinoma, and adenocarcinoma.

Results

For both lung cancer cases and subcohort members, mean daily intakes of the major carotenoids, vitamin C, vitamin E, and folate are presented in Table 1. In general, intakes were lower among cases than in the subcohort. Mean daily intakes of the subcohort are presented for different categories of age, smoking

Table 1 Daily intake of carotenoids, vitamins C and E, and folate in male lung cancer cases and subcohort members: Netherlands Cohort Study 1986–1992

	Unit	Cases[a] (n = 939)	Subcohort[a] (n = 1525)
α-Carotene	μg/day	644 (486)	691 (555)
β-Carotene	μg/day	2813 (1383)	2976 (1555)
Lutein + Zeaxanthin	μg/day	2455 (1152)	2554 (1149)
β-Cryptoxanthin	μg/day	138 (159)	152 (160)
Lycopene	μg/day	983 (1517)	1050 (1560)
Vitamin C	mg/day	92 (42)	98 (42)
Vitamin E	mg/day	14.4 (6.4)	14.7 (6.6)
Folate	μg/day	291 (82)	303 (80)
Vitamin C supplement use	% yes	6.3%	8.2%
Vitamin E supplement use	% yes	4.3%	4.8%

[a] Mean (SD).

status, educational level, and family history of lung cancer, which are potential confounders of the association between intake data and lung cancer incidence (Table 2). In the higher age categories, intake of carotenes and folate tended to be lower and intakes of β-cryptoxanthin and vitamin C tended to be higher (Table 2). Current smokers had the lowest intakes for all carotenoids and vitamins of interest, and subjects who had never smoked had the highest intakes (except for vitamin E). For educational level, intake data did not show clear trends, and subjects with a family history of lung cancer tended to have lower intakes of most carotenoids and vitamins of interest.

Because the carotenoids and vitamins partly originated from the same food sources (vegetables and fruit), intakes could be highly correlated. To illustrate this, Pearson correlation coefficients were calculated for subcohort members (Table 3). Correlations were high between α-carotene and β-carotene (0.93), between lutein + zeaxanthin and β-carotene (0.68), between vitamin C and β-cryptoxanthin (0.77), and between folate and either β-carotene (0.62), lutein + zeaxanthin (0.66), or vitamin C (0.66). Also, correlation coefficients with energy intake were generally low with the exception of vitamin E (0.53) and folate (0.58).

RRs for lung cancer were calculated with different models. The most straightforward model, including age, smoking, socioeconomic status, and family history of lung cancer, revealed significant inverse associations with lung cancer incidence for lutein + zeaxanthin, β-cryptoxanthin, vitamin C, and folate (RRs for highest *versus* lowest category: 0.72, 0.71, 0.63, and 0.70, respectively; 95% CI all excluding 1.00; and P-trends <0.05; Table 4). No significant effects were found for α-carotene, β-carotene, lycopene, or vitamin E, although for the carotenes RRs were <1.00 for all quintiles above reference. The use of supplements containing vitamin C or vitamin E was not associated with the incidence of lung cancer. Including energy intake in the model did not change RRs importantly. Additional inclusion of vitamin C, however, reduced the effects found previously, leaving only folate with a significant negative trend. Adjustment for folate only left significant negative associations for β-cryptoxanthin and vitamin C.

Cases and subcohort members were classified based on smoking status at baseline: never-smokers (35 cases), former smokers (312 cases), and current smokers (487 cases; Table 5). In current smokers, significant inverse associations were found for β-carotene, lutein + zeaxanthin, β-cryptoxanthin, vitamin C, and folate, and the associations were stronger than those found in unstratified analyses. In former smokers, significant inverse trends were found only for lutein + zeaxanthin; all other RRs were closer to 1.00 than for current smokers. However, RR estimates for the carotenes, folate, and vitamin C remained <1.00 for all quintiles/categories above reference. With only 35 lung cancer cases among never-smokers, estimates were unstable, despite the use of tertiles of intake instead of quintiles. No significant trends were found for the carotenoids and vitamins of interest. For β-cryptoxanthin, vitamin C, and vitamin E, estimates were <1.00 for all categories of intake above reference, and for the carotenes, they were >1.00. In analogy with data presented in Table 4, additional adjustments were made for either vitamin C or folate (results not shown). For current smokers, inverse relationships with both carotenes and lutein/zeaxanthin disappeared, whereas negative associations with β-cryptoxanthin, vitamin C, and folate remained intact. Additional adjustment for vitamin C or folate left the negative associations with lutein/zeaxanthin and folate in former smokers unchanged. In never-smokers, additional adjustment for either vitamin C or folate resulted in a stronger positive association with β-carotene (results not shown).

Lutein + zeaxanthin, β-cryptoxanthin, vitamin C, and folate showed significant inverse associations with both risk of small cell carcinomas (157 cases) and squamous cell carcinomas (377 cases; Table 6). Nonsignificant inverse associations were found for α-carotene and β-carotene with small cell carcinomas. Adenocarcinomas (143 cases) seemed to be inversely associated only with folate intake and (borderline) with vitamin C but not with the carotenoids. Similar to analyses for all lung cancer cases taken together, additional adjustment for vitamin C resulted in a loss of inverse associations with lutein + zeaxanthin and β-cryptoxanthin, with the exception of β-cryptoxanthin in squamous cell carcinomas, which stayed significantly negative. The inverse association with folate disappeared for small cell carcinomas but not squamous cell and adenocarcinomas. Similar to analyses for the whole group, adjustment for folate weakened the inverse associations for small and squamous cell carcinomas with lutein + zeaxanthin but not with β-cryptoxanthin. The inverse associations found with vitamin C were weaker for all histological types of carcinomas after adjustment for folate. β-Carotene turned out to have a significant positive relationship with adenocarcinomas (results not shown).

Because folate and vitamin C were highly correlated, additional analyses were performed with combinations of tertiles of folate consumption with categories of vitamin C intake (Table 7). For men who had high intake of both nutrients compared with men who had a low intake, the RR was 0.56 (95% CI, 0.44–0.72). In the highest tertile of folate intake, a higher vitamin C intake led to a lower RR, and *vice versa*.

Discussion

In the Netherlands Cohort Study on Diet and Cancer, the associations were evaluated between lung cancer incidence and intakes of several carotenoids, vitamin C, vitamin E, and folate. After adjustment for age, smoking history, education level, and family history of lung cancer, statistically significant protective effects on lung cancer incidence were found for the carotenoids lutein + zeaxanthin and β-cryptoxanthin as well as for folate and vitamin C. For other carotenoids (α-carotene, β-carotene, and lycopene) and vitamin E, no such inverse associations were found. Use of supplements containing vitamin C or vitamin E did not influence lung cancer incidence. In our previous report on vegetable and fruit consumption and lung cancer risk, consumption of vegetables and fruits appeared to be inversely associated with lung cancer (5). The protective effect on lung

Table 2 Mean dietary intake of carotenoids, vitamins C and E, and folate for several characteristics in the subcohort (n = 1525): Netherlands Cohort Study 1986–1992

	n	α-Carotene[a] μg/day	β-Carotene[a] μg/day	Lutein + Zeaxanthin[a] μg/day	β-Cryptoxanthin[a] μg/day	Lycopene[a] μg/day	Vitamin C[a] mg/day	Vitamin E[a] mg/day	Folate[a] μg/day
Age (yr)									
55–59	582	713 (595)	3037 (1625)	2576 (1105)	140 (149)	992 (1171)	95 (40)	14.8 (6.9)	307 (83)
60–64	534	689 (544)	2972 (1561)	2536 (1155)	152 (161)	1167 (2063)	98 (41)	14.9 (6.6)	303 (79)
65–69	409	663 (507)	2896 (1440)	2546 (1204)	170 (173)	979 (1244)	102 (44)	14.2 (6.3)	296 (77)
Smoking status									
Never	204	823 (711)	3264 (1936)	2581 (1225)	173 (164)	1135 (1492)	103 (43)	14.7 (6.6)	312 (88)
Former smoker	778	694 (555)	2991 (1541)	2568 (1114)	154 (162)	1073 (1723)	100 (42)	14.9 (6.6)	305 (79)
Current smoker	543	639 (476)	2847 (1393)	2525 (1171)	141 (156)	985 (1320)	94 (41)	14.3 (6.7)	296 (77)
Highest educational level[b]									
Primary school	393	699 (583)	2997 (1722)	2581 (1323)	133 (164)	938 (1490)	92 (43)	14.2 (6.2)	299 (86)
Lower vocational	321	687 (483)	2960 (1446)	2571 (1192)	140 (137)	938 (1323)	96 (41)	15.6 (7.1)	311 (84)
Secondary/medium vocational	530	684 (607)	2964 (1600)	2571 (1104)	170 (170)	1093 (1780)	103 (43)	14.7 (6.5)	303 (78)
University/higher vocational	271	707 (481)	3006 (1328)	2464 (900)	159 (159)	1256 (1442)	101 (39)	14.3 (6.7)	299 (69)
Family history of lung cancer									
No	138	696 (566)	2984 (1557)	2550 (1124)	151 (159)	1052 (1580)	98 (42)	14.7 (6.7)	303 (79)
Yes	141	648 (431)	2896 (1537)	2590 (1379)	161 (169)	1025 (1351)	97 (42)	14.2 (5.9)	300 (87)

[a] Mean (SD).
[b] Totals do not add to 1525 because of missing values.

Table 3 Pearson correlation coefficients of carotenoids, other antioxidant vitamins, and folate in men from the subcohort of the Netherlands Cohort Study 1986–1992

	α-Carotene	β-Carotene	Lutein/Zeaxanthin	β-Cryptoxanthin	Lycopene	Vitamin C	Vitamin E	Folate	Energy
α-Carotene		0.93	0.39	0.13	0.16	0.30	0.16	0.44	0.24
β-Carotene			0.68	0.16	0.26	0.42	0.23	0.62	0.31
Lutein/Zeaxanthin				0.17	0.15	0.47	0.22	0.66	0.22
β-Cryptoxanthin					0.08	0.77	0.07	0.34	0.05
Lycopene						0.23	0.11	0.20	0.06
Vitamin C							0.23	0.66	0.23
Vitamin E								0.42	0.53
Folate									0.58

cancer was not limited to a specific group of vegetables or fruits, but the effect was strongest for vegetables of the *Brassica* genus and for citrus fruit. The present analyses on carotenoids, other antioxidants, and folate suggest that neither α-carotene nor β-carotene is mainly responsible for the inverse associations found, but that contributions of lutein + zeaxanthin, β-cryptoxanthin, folate, and vitamin C are more important. The RRs for these food constituents, comparing the highest category of intake with the lowest one, are of the same magnitude as the ones found for vegetable and fruit intake. The relatively low RR for vitamin C (0.63; 95% CI, 0.49–0.83) is in line with the lower RRs for oranges and fresh orange juice, as published before.

There may be some limitations to this study which could have influenced the results. With 6.3 years of follow-up and 939 male lung cancer cases, the power of the study is such that the probability for overall observed results to be caused by chance is small. The prospective nature of a cohort study together with completeness of follow-up, as has been achieved in this study, reduced the potential for selection bias to a minimum. Information bias, *i.e.*, a change in (reported) dietary habits of lung cancer cases because of the disease, is also largely avoided in a prospective study because dietary habits were reported before the disease was diagnosed. A change in dietary habits of subjects with latent lung cancer at the time of completing the baseline questionnaire remains, however, a possibility, although this is much less likely than in subjects with gastrointestinal cancer, for example. Repeating analyses excluding cases diagnosed during the first year of follow-up hardly affected RRs (results not presented).

Intake data were based on the baseline questionnaire in 1986, departing from the assumption that dietary and other habits would not change considerably during the 6.3 years of follow-up. Because participants were 55–70 years of age at entry, important changes were not expected. In a random sample of the cohort, the reproducibility of the questionnaire was tested during the first 5 years of follow-up, which led to the conclusion that the potential of a single food frequency questionnaire measurement to rank subjects according to nutrient intake dropped only slightly over time (30). Measurement error attributable to use of the carotenoid database will be relatively small, because analyses were performed in those vegetables known to be mainly responsible for carotenoid intake in the Dutch diet.

A potentially more realistic threat to the interpretation of the observed inverse associations is residual confounding by risk factors for lung cancer that happen to be associated with the intake of carotenoids, vitamin C, vitamin E, or folate. The most important risk factor in this respect is cigarette smoking. Unhealthy habits, such as smoking and a diet low in vegetables and fruit, tend to cluster in the same subjects in most populations (31); therefore, insufficient control of one factor will confound the association between the other factor and lung cancer. In this way, it might happen that an observed inverse association between intake of food constituents and lung cancer was attributable to residual confounding by smoking. Evidence of an

Table 4 RRs[a] (95% CI) for quintiles of dietary intake of carotenoids, vitamins C and E, and folate for lung cancer, according to different models: Netherlands Cohort Study 1986–1992

	Median	Age, family history, smoking, SES[b]	Age, family history, smoking, SES, energy	Age, family history, smoking, SES, vit C (cat[b]), energy (cont[b])	Age, family history, smoking, SES, folate (quintiles), energy (cont)
α-Carotene	μg/day				
Quintile 1[c]	198	1.00	1.00	1.00	1.00
Quintile 2	378	0.60 (0.44–0.82)	0.60 (0.44–0.83)	0.63 (0.45–0.87)	0.63 (0.46–0.88)
Quintile 3	565	0.69 (0.51–0.95)	0.70 (0.51–0.96)	0.74 (0.53–1.02)	0.88 (0.57–1.09)
Quintile 4	809	0.83 (0.61–1.13)	0.85 (0.62–1.15)	0.90 (0.66–1.24)	0.96 (0.69–1.32)
Quintile 5	1300	0.85 (0.62–1.16)	0.89 (0.65–1.23)	1.01 (0.71–1.43)	1.00 (0.71–1.40)
P-trend		0.77	0.90	0.29	0.20
β-Carotene	μg/day				
Quintile 1[c]	1480	1.00	1.00	1.00	1.00
Quintile 2	2148	0.72 (0.53–0.98)	0.73 (0.54–1.00)	0.79 (0.57–1.09)	0.83 (0.60–1.14)
Quintile 3	2675	0.79 (0.58–1.09)	0.81 (0.59–1.12)	0.90 (0.64–1.27)	1.00 (0.72–1.39)
Quintile 4	3351	0.83 (0.61–1.13)	0.86 (0.63–1.18)	0.99 (0.68–1.45)	1.14 (0.80–1.62)
Quintile 5	4729	0.81 (0.59–1.11)	0.85 (0.63–1.18)	1.02 (0.68–1.53)	1.11 (0.76–1.60)
P-trend		0.21	0.48	0.38	0.09
Lutein/Zeaxanthin	μg/day				
Quintile 1[c]	1388	1.00	1.00	1.00	1.00
Quintile 2	1897	0.78 (0.57–1.06)	0.78 (0.58–1.06)	0.81 (0.59–1.12)	0.87 (0.64–1.19)
Quintile 3	2376	0.71 (0.52–0.97)	0.72 (0.53–0.99)	0.81 (0.56–1.17)	0.85 (0.61–1.18)
Quintile 4	2853	0.72 (0.53–0.99)	0.74 (0.54–1.01)	0.86 (0.57–1.29)	0.97 (0.68–1.39)
Quintile 5	3857	0.72 (0.53–0.99)	0.75 (0.54–1.03)	0.88 (0.57–1.38)	0.96 (0.65–1.43)
P-trend		0.001	0.005	0.39	0.91
β-Cryptoxantin	μg/day				
Quintile 1[c]	12	1.00	1.00	1.00	1.00
Quintile 2	46	0.77 (0.57–1.04)	0.78 (0.58–1.06)	0.84 (0.61–1.15)	0.79 (0.58–1.07)
Quintile 3	103	0.64 (0.47–0.87)	0.64 (0.47–0.88)	0.73 (0.50–1.05)	0.68 (0.49–0.93)
Quintile 4	194	0.64 (0.47–0.88)	0.66 (0.48–0.88)	0.80 (0.49–1.30)	0.72 (0.52–1.00)
Quintile 5	356	0.71 (0.52–0.96)	0.72 (0.53–0.98)	0.91 (0.33–1.74)	0.82 (0.59–1.13)
P-trend		<0.0001	0.0002	0.22	0.02
Lycopene	μg/day				
Quintile 1[c]	132	1.00	1.00	1.00	1.00
Quintile 2	251	0.82 (0.60–1.12)	0.83 (0.61–1.23)	0.85 (0.62–1.17)	0.87 (0.63–1.19)
Quintile 3	720	1.01 (0.74–1.38)	1.02 (0.75–1.39)	1.14 (0.82–1.57)	1.09 (0.79–1.49)
Quintile 4	1087	1.07 (0.79–1.47)	1.07 (0.79–1.47)	1.21 (0.87–1.69)	1.18 (0.85–1.62)
Quintile 5	2035	0.91 (0.67–1.25)	0.93 (0.67–1.27)	1.12 (0.77–1.61)	1.05 (0.75–1.46)
P-trend		0.81	0.72	0.04	0.14
Vitamin C[d]	mg/day				
Category 1[c]	51	1.00	1.00		1.00
Category 2	82	0.69 (0.53–0.90)	0.70 (0.59–0.83)		0.79 (0.59–1.05)
Category 3	138	0.63 (0.49–0.83)	0.64 (0.54–0.77)		0.77 (0.54–1.08)
P-trend		<0.0001	<0.0001		0.03
Vitamin E	mg/day				
Quintile 1[c]	7	1.00	1.00	1.00	1.00
Quintile 2	10	1.19 (0.87–1.63)	1.23 (0.90–1.69)	1.31 (0.93–1.83)	1.34 (0.98–1.85)
Quintile 3	13	1.13 (0.83–1.55)	1.21 (0.88–1.67)	1.25 (0.90–1.74)	1.31 (0.94–1.82)
Quintile 4	17	1.01 (0.74–1.38)	1.08 (0.78–1.51)	1.14 (0.81–1.62)	1.16 (0.83–1.64)
Quintile 5	24	1.02 (0.74–1.41)	1.15 (0.80–1.65)	1.21 (0.83–1.76)	1.29 (0.88–1.89)
P-trend		0.62	0.64	0.42	0.24
Folate	μg/day				
Quintile 1[c]	212	1.00	1.00	1.00	
Quintile 2	259	0.71 (0.52–0.96)	0.70 (0.52–0.96)	0.77 (0.52–1.14)	
Quintile 3	295	0.75 (0.55–1.02)	0.74 (0.54–1.03)	0.86 (0.51–1.44)	
Quintile 4	331	0.49 (0.36–0.68)	0.49 (0.35–0.68)	0.58 (0.32–1.05)	
Quintile 5	400	0.70 (0.51–0.95)	0.69 (0.47–0.99)	0.83 (0.39–1.75)	
P-trend		<0.0001	<0.0001	0.03	
Vitamin C supplement use					
No[c]		1.00	1.00	1.00	1.00
Yes		0.90 (0.61–1.33)	0.90 (0.61–1.33)	0.92 (0.61–1.38)	0.93 (0.63–1.38)
P-trend		0.45	0.44	0.54	0.63
Vitamin E supplement use					
No[c]		1.00	1.00	1.00	1.00
Yes		1.13 (0.68–1.88)	1.13 (0.68–1.87)	1.10 (0.65–1.87)	1.16 (0.70–1.93)
P-trend		0.48	0.48	0.59	0.40

[a] Adjusted for current smoking, years of smoking cigarettes, number of cigarettes per day, highest educational level, family history of lung cancer, and age.
[b] SES, socioeconomic status; cat, categories; cont, continuous.
[c] Reference category.
[d] Category 1, quintile 1; Category 2, quintiles 2 and 3; Category 3, quintiles 4 and 5.

Table 5 RRs[a] (95% CIs) for quintiles (Q) or tertiles (T) of dietary intake of carotenoids, vitamins C and E, and folate, among never-smokers, former smokers, and current smokers: Netherlands Cohort Study 1986–1992

Quintiles/ tertiles	Never-smokers[b]	Former smokers[b]	Current smokers[b]
	Cases: $n = 35$ Subcohort: $n = 203$	Cases: $n = 312$ Subcohort: $n = 728$	Cases: $n = 487$ Subcohort: $n = 476$
α-Carotene			
Q/T 1[c]	1.00	1.00	1.00
Q/T 2	1.32 (0.46–3.79)	0.63 (0.38–1.03)	0.58 (0.38–0.90)
Q/T 3	1.61 (0.61–4.21)	0.53 (0.32–0.88)	0.79 (0.52–1.21)
Q 4		0.74 (0.46–1.19)	0.83 (0.54–1.28)
Q 5		0.96 (0.60–1.56)	0.76 (0.49–1.17)
P-trend	0.27	0.94	0.41
β-Carotene			
Q/T 1[c]	1.00	1.00	1.00
Q/T 2	2.35 (0.83–6.65)	0.62 (0.39–1.00)	0.82 (0.54–1.25)
Q/T 3	1.98 (0.75–5.26)	0.72 (0.44–1.19)	0.83 (0.54–1.27)
Q 4		0.77 (0.48–1.24)	0.79 (0.51–1.24)
Q 5		0.83 (0.51–1.34)	0.74 (0.47–1.16)
P-trend	0.14	0.58	0.05
Lutein/Zeaxanthin			
Q/T 1[c]	1.00	1.00	1.00
Q/T 2	0.83 (0.29–2.35)	0.83 (0.52–1.33)	0.74 (0.49–1.13)
Q/T 3	1.35 (0.56–3.26)	0.59 (0.36–0.96)	0.84 (0.54–1.29)
Q 4		0.88 (0.54–1.44)	0.64 (0.42–0.99)
Q 5		0.66 (0.39–1.09)	0.69 (0.45–1.07)
P-trend	0.42	0.05	0.003
β-Cryptoxantin			
Q/T 1[c]	1.00	1.00	1.00
Q/T 2	0.50 (0.18–1.41)	0.89 (0.55–1.42)	0.69 (0.45–1.05)
Q/T 3	0.86 (0.36–2.06)	0.68 (0.41–1.13)	0.61 (0.40–0.93)
Q 4		0.76 (0.47–1.24)	0.58 (0.37–0.90)
Q 5		1.08 (0.67–1.73)	0.51 (0.33–0.79)
P-trend	0.76	0.98	<0.0001
Lycopene			
Q/T 1[c]	1.00	1.00	1.00
Q/T 2	0.92 (0.32–2.65)	0.92 (0.57–1.49)	0.72 (0.47–1.09)
Q/T 3	1.54 (0.61–3.90)	1.21 (0.75–1.96)	0.89 (0.58–1.37)
Q 4		0.97 (0.59–1.60)	1.08 (0.71–1.66)
Q 5		1.06 (0.64–1.74)	0.81 (0.53–1.23)
P-trend	0.26	0.68	0.74
Vitamin C[d]			
Category 1[c]	1.00	1.00	1.00
Category 2	0.72 (0.26–1.99)	0.75 (0.50–1.12)	0.70 (0.49–1.01)
Category 3	0.67 (0.25–1.79)	0.84 (0.56–1.27)	0.54 (0.38–0.78)
P-trend	0.39	0.38	<0.0001
Vitamin E			
Q/T 1[c]	1.00	1.00	1.00
Q/T 2	0.80 (0.32–2.02)	1.39 (0.85–2.29)	1.19 (0.78–1.82)
Q/T 3	0.67 (0.26–1.67)	1.06 (0.64–1.76)	1.20 (0.78–1.85)
Q 4		0.98 (0.59–1.64)	1.12 (0.72–1.72)
Q 5		1.19 (0.71–1.98)	0.97 (0.62–1.53)
P-trend	0.32	0.94	0.79
Folate			
Q/T 1[c]	1.00	1.00	1.00
Q/T 2	1.04 (0.39–2.77)	0.72 (0.45–1.15)	0.71 (0.47–1.07)
Q/T 3	1.09 (0.44–2.72)	0.79 (0.50–1.26)	0.75 (0.48–1.16)
Q 4		0.79 (0.49–1.29)	0.37 (0.24–0.58)
Q 5		0.72 (0.45–1.17)	0.63 (0.41–0.98)
P-trend	0.82	0.16	<0.0001

[a] Adjusted for highest educational level, family history of lung cancer, and age. For current smokers and former smokers, also adjusted for years of smoking cigarettes and number of cigarettes per day (continuous).
[b] RR (95% CI).
[c] Reference category.
[d] Category 1, quintile 1; Category 2, quintiles 2 and 3; Category 3, quintiles 4 and 5.

association between smoking and intake of several nutrients was also found in the Netherlands Cohort Study of Diet and Cancer, although it was not very strong and not very consistent. For example, never-smokers had higher intakes of all carotenoids, vitamin C, and folate than former smokers, and current smokers had the lowest intakes for all food constituents of interest. To minimize residual confounding attributable to smoking, we made an attempt to model cigarette smoking habits such that they best explained lung cancer. This resulted in a model including number of years smoked, habitual number of cigarettes smoked per day, both as continuous variables, and an indicator variable for current smoking. When we added the smoking variables to an age-adjusted model, the RR estimates for food constituents and lung cancer changed only slightly (data not shown). We believe, therefore, that the inverse association observed between the food constituents of interest and lung cancer was not entirely attributable to residual confounding by smoking, although we cannot exclude some influence.

In this study, adjustment for either vitamin C or folate intake reduced the effects of carotenoids importantly. This led to the conclusion that vitamin C and folate might be more important in terms of protection against lung cancer incidence than the carotenoids. Using combinations of men high in intake of both nutrients *versus* men with a low intake, we could conclude that the effects of vitamin C and folate are independent of each other.

In the past decade, five cohort studies have reported RRs for β-carotene, three showing nonsignificant inverse associations with lung cancer (14, 15, 19), one a nonsignificant inverse association for women but no association for men (13), and one a significant inverse trend only for subjects with stable high intakes over a 10-year period (16). One cohort study recently published data on individual dietary carotenoids, showing a statistically significant inverse association for α-carotene only. For total carotenoids, significant negative associations have been found for men only in the New York State Cohort Study (18) and for nonsmoking men in the Finnish Mobile Clinic Health Survey (12). Nonsignificant negative associations have been found in policy holders with the Lutheran Brotherhood Insurance Society (14) and in the First National Health and Nutrition Examination Survey Epidemiological Follow-up Study (17). Two cohort studies have recently reported RRs for vitamin C of the same magnitude as those we found (RR 0.63 for the highest *versus* the lowest category; Refs. 17 and 18). Others found inverse nonsignificant (14, 15) or nonconsistent (16) RRs for vitamin C, one only found a nonsignificant inverse association for women but no association for men (13), and one study found a strong inverse relationship for nonsmokers only (12). A statistically significant positive association for vitamin C was found recently in the Nurses' Health Study (19), but supplements were included, resulting in much higher intakes in the highest quintiles than in our study. No data were presented excluding supplement use. As in our study, no effect was found for vitamin E in all five studies that included this nutrient (12, 16–19). In our study, among the nutrients included, folate appeared to be the strongest and most consistent protective factor against lung cancer incidence. A highly significant inverse association for folate was found in the New York State cohort as well (18), but the Nurses' Health Study found no association (19). Again, however, in the latter study, supplements were included. Intake levels of the fourth quintile in the NHS were comparable with the fifth quintile level in our study (about 400 μg/day), and the RR of this fourth quintile of the NHS (compared with the first) was 0.7 (95% CI, 0.5–0.9). A negative association with folate may be an important finding,

Table 6 RRs[a] (95% CI) for quintiles of dietary intake of carotenoids, vitamins C and E, and folate, stratified by histological type of lung cancer: Netherlands Cohort Study 1986–1992

	Small cell carcinoma (157 cases)		Squamous cell carcinoma (377 cases)		Adenocarcinoma (143 cases)	
	n	RR (95% CI)	*n*	RR (95% CI)	*n*	RR (95% CI)
α-Carotene						
Quintile 1[b]	47	1.00	95	1.00	25	1.00
Quintile 2	23	0.42 (0.24–0.73)	61	0.56 (0.38–0.82)	30	0.98 (0.55–1.75)
Quintile 3	26	0.55 (0.32–0.93)	64	0.66 (0.45–0.97)	30	1.14 (0.64–2.03)
Quintile 4	36	0.69 (0.42–1.14)	80	0.77 (0.53–1.12)	38	1.41 (0.81–2.44)
Quintile 5	25	0.59 (0.34–1.02)	77	0.93 (0.64–1.35)	20	0.90 (0.48–1.69)
P-trend		0.17		0.83		0.63
β-Carotene						
Quintile 1[b]	41	1.00	96	1.00	21	1.00
Quintile 2	31	0.68 (0.40–1.15)	67	0.63 (0.44–0.93)	35	1.53 (0.85–2.76)
Quintile 3	26	0.63 (0.36–1.09)	72	0.76 (0.52–1.10)	29	1.39 (0.76–2.56)
Quintile 4	32	0.71 (0.42–1.21)	64	0.64 (0.43–0.94)	35	1.72 (0.95–3.11)
Quintile 5	27	0.69 (0.40–1.19)	78	0.88 (0.60–1.27)	23	1.18 (0.62–2.24)
P-trend		0.17		0.37		0.49
Lutein/Zeaxanthin						
Quintile 1[b]	41	1.00	1.00	1.00	33	1.00
Quintile 2	39	1.00 (0.60–1.65)	74	0.76 (0.53–1.10)	20	0.63 (0.34–1.14)
Quintile 3	30	0.80 (0.47–1.35)	72	0.75 (0.52–1.09)	30	0.95 (0.55–1.63)
Quintile 4	20	0.54 (0.30–0.98)	55	0.63 (0.42–0.93)	32	1.12 (0.65–1.91)
Quintile 5	27	0.64 (0.37–1.12)	76	0.75 (0.52–1.08)	28	0.85 (0.49–1.49)
P-trend		0.009		0.03		0.77
β-Cryptoxantin						
Quintile 1[b]	50	1.00	118	1.00	41	1.00
Quintile 2	27	0.66 (0.39–1.12)	71	0.71 (0.49–1.01)	30	0.83 (0.49–1.40)
Quintile 3	23	0.53 (0.30–0.91)	58	0.56 (0.38–0.82)	21	0.55 (0.31–0.97)
Quintile 4	28	0.65 (0.38–1.11)	64	0.62 (0.42–0.90)	20	0.54 (0.30–0.97)
Quintile 5	29	0.61 (0.36–1.04)	66	0.59 (0.41–0.85)	31	0.81 (0.48–1.36)
P-trend		0.04		0.0002		0.12
Lycopene						
Quintile 1[b]	44	1.00	86	1.00	35	1.00
Quintile 2	29	0.72 (0.42–1.22)	80	1.00 (0.69–1.45)	20	0.57 (0.31–1.03)
Quintile 3	28	0.84 (0.49–1.43)	72	1.09 (0.74–1.60)	25	0.86 (0.49–1.51)
Quintile 4	24	0.72 (0.41–1.27)	72	1.10 (0.75–1.61)	35	1.30 (0.77–2.20)
Quintile 5	32	0.86 (0.51–1.45)	67	0.93 (0.63–1.37)	28	0.90 (0.52–1.56)
P-trend		0.54		0.90		0.38
Vitamin C[c]						
Category 1[b]	54	1.00	99	1.00	40	1.00
Category 2	46	0.46 (0.30–0.73)	154	0.85 (0.62–1.16)	52	0.68 (0.43–1.07)
Category 3	57	0.54 (0.35–0.83)	124	0.65 (0.47–0.89)	51	0.65 (0.41–1.02)
P-trend		0.003		0.001		0.06
Vitamin E						
Quintile 1[b]	34	1.00	75	1.00	27	1.00
Quintile 2	32	0.99 (0.58–1.70)	87	1.21 (0.83–1.77)	27	1.03 (0.58–1.85)
Quintile 3	31	0.94 (0.55–1.63)	77	1.06 (0.72–1.56)	37	1.40 (0.81–2.42)
Quintile 4	33	0.92 (0.53–1.57)	73	0.96 (0.65–1.41)	26	1.03 (0.58–1.86)
Quintile 5	27	0.83 (0.47–1.47)	65	0.95 (0.64–1.41)	26	1.15 (0.64–2.08)
P-trend		0.45		0.36		0.62
Folate						
Quintile 1[b]	41	1.00	112	1.00	42	1.00
Quintile 2	36	0.88 (0.53–1.47)	72	0.64 (0.44–0.92)	27	0.64 (0.38–1.10)
Quintile 3	31	0.84 (0.49–1.43)	62	0.61 (0.42–0.90)	24	0.63 (0.36–1.09)
Quintile 4	19	0.40 (0.22–0.73)	62	0.51 (0.35–0.74)	27	0.63 (0.37–1.07)
Quintile 5	30	0.74 (0.44–1.27)	69	0.63 (0.44–0.91)	23	0.59 (0.33–1.03)
P-trend		0.02		0.0003		0.04

[a] Adjusted for current smoking, years of smoking cigarettes, and number of cigarettes per day, highest educational level, family history of lung cancer, and age.
[b] Reference category.
[c] Category 1, quintile 1; Category 2, quintiles 2 and 3; Category 3, quintiles 4 and 5.

because folate possibly has a role in carcinogenesis, given its involvement in DNA synthesis, methylation, and repair (32). However, thus far, it has rarely has been associated with the incidence of lung cancer.

As summarized by Van Poppel and Goldbohm (33), cohort studies using plasma concentrations of β-carotene have found remarkably consistent negative associations with lung cancer. This could be in line with our results, because plasma β-carotene can be considered to be a marker for folate, vitamin C, or other carotenoids. In a prospective study published recently, levels of several carotenoids and other antioxidants in blood were linked to lung cancer risk (34). Protective effects on lung

Table 7 RR[a] estimates (95% CI) for combinations of tertiles of dietary folate and categories of dietary vitamin C intake: Netherlands Cohort Study 1986–1992

Folate tertiles	Vitamin C categories[b]		
	1	2	3
1	1.00[c]	0.74 (0.57–0.96)	0.81 (0.53–1.23)
2	0.87 (0.58–1.30)	0.60 (0.46–0.78)	0.67 (0.51–0.90)
3	0.88 (0.33–2.32)	0.72 (0.52–0.98)	0.56 (0.44–0.72)

[a] Adjusted for current smoking, years of smoking cigarettes, number of cigarettes per day, highest educational level, family history of lung cancer, and age.
[b] Category 1, quintile 1; Category 2, quintiles 2 and 3; Category 3, quintiles 4 and 5.
[c] Reference category.

cancer were found for β-cryptoxanthin, β-carotene, and lutein + zeaxanthin. Nonsignificant protective effects were found for α-carotene and ascorbic acid. No effect was found for lycopene, α-tocopherol, and selenium.

Negative associations were strongest among current smokers at baseline and weaker for former smokers. Only 35 cases were never-smokers, and no significant associations with the carotenoids and vitamins mentioned could be found. These results are contradictory to results in Finnish men (117 lung cancer cases), where inverse gradients were found for summed carotenoids (α- carotene, β-carotene, γ-carotene, lycopene, and lutein + zeaxanthin), vitamin E (nonsignificant), vitamin C, and lung cancer incidence only in nonsmokers. However, in that study, nonsmokers included both former smokers (18 cases) and never-smokers (6 cases). Among smokers (93 cases), no associations were found (12). More in line with our results are those from the National Health and Nutrition Examination Survey Epidemiological Follow-up Study, showing a protective effect of carotenoids and vitamin C only among current smokers, not in nonsmokers (never-smokers + former smokers). In that study, significant inverse associations were found only in the lowest tertiles of pack-years of smoking for carotenoids, vitamin C, and vitamin E (17). In the Iowa Women's Health Survey, separate analyses were made on β-carotene for current smokers (81 cases), ex-smokers (38 cases), and never-smokers (19 cases). Because of the small number of cases, conclusions were hard to draw, but the association seemed to be stronger in former than in current smokers (15). In the New York State Cohort, only analyses have been presented for light and heavy smokers separately. Risk estimates for vitamin C and carotenoids were of similar magnitude for light and heavy smokers. The effect of folate appears to be the strongest in heavy smokers (18). As shown in this study, estimates change after correction for either vitamin C or folate. Comparisons with other studies have to be made without further adjustment, because other groups have not presented adjusted results. In summary, results of different studies are hard to compare because analyses were based on different definitions of smoking strata. In general, results on never-smokers are not relevant thus far in cohort studies, because insufficient cases had been diagnosed to do proper analyses. However, as summarized by Ziegler *et al.* (4), case-control studies that focused on never smokers found effects of carotenoids, vegetables, and fruit similar in magnitude to those generally observed among active smokers.

In the present study, negative associations with carotenoids seemed to be limited to small cell carcinomas and squamous cell carcinomas. Both folate and vitamin C intake appeared to be inversely related to small cell, squamous cell, and adenocarcinomas. Two other cohort studies have presented results stratified by histological type of lung cancer. The New York State Cohort Study analyses were performed separately for squamous cell carcinomas and adenocarcinomas, indicating significant inverse associations of vitamin C and folate with squamous cell carcinomas only (18). In the Iowa Women's Health Study, analyses on β-carotene were performed separately for adenocarcinomas, small cell carcinomas, squamous cell carcinomas, and large cell carcinomas, revealing only a (nonsignificant) negative association for small cell carcinomas (37 cases) and large cell carcinomas (12 cases). Because of the small numbers of cases, these latter results might be less accurate (15). Results of the New York State Cohort Study are in line with our results, although it should be stressed that in our study, additional adjustment for either folate or vitamin C importantly influenced risk estimates. A lack of effect of carotenoids, vitamin C, and folate on adenocarcinomas might be attributable to the fact that these are less strongly related to smoking (18).

By comparing our findings with available results of cohort studies, we conclude that carotenoids, especially β-carotene, and vitamin E have only marginal inverse associations with lung cancer incidence. This is in line with results of the three major published intervention trials that have not found a reduction in lung cancer incidence in subjects given vitamin E (35), vitamin A (36), or β- carotene (35–37). However, our study has indicated that lutein + zeaxanthin and β-cryptoxanthin, as well as vitamin C and folate, might be more promising food components in this matter, although associations with both carotenoids did not persist after further adjustment with vitamin C or folate.

Results from this prospective cohort study indicate that α-carotene and β-carotene appear not to be the food constituents responsible for a lower lung cancer incidence with higher vegetable and/or fruit consumption. The role of other important carotenoids (if any) may be limited to lutein + zeaxanthin and β-cryptoxanthin, and more studies are required to allow definite conclusions to be drawn. Because both vitamin C and folate appear to have a stronger protective impact on lung cancer risk, these should be included in future analyses. It remains possible, however, that inverse associations with lung cancer risk are a generalized fruit and vegetable effect and cannot be attributed to one or more specific nutrients.

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