Dietary Folate Intake and Lung Cancer Risk in Former Smokers: A Case-Control Analysis[1]

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Abstract

No studies have focused on the role of dietary folate intake in risk of lung cancer in former smokers, in whom dietary folate intake is less likely confounded with current smoking. Therefore, we evaluated the association between dietary folate intake and risk of lung cancer in a population of 470 histopathologically confirmed incident lung cancer cases from M. D. Anderson Cancer Center and 472 cancer-free controls from enrollees at a community-based multispecialty physician practice, frequency-matched on age (5 years), sex, and ethnicity. Dietary folate intake levels were estimated from a National Cancer Institute standard food frequency questionnaire. Unconditional logistic regression analyses were used to calculate the crude and adjusted ORs and their 95% CIs. Dietary folate intake from natural food was significantly higher among the controls than among the cases ($P < 0.001$), and folate intake above the control median value was associated with a 40% decreased risk of lung cancer (adjusted OR, 0.60; 95% CI, 0.45–0.79). A significant inverse dose-response relationship between increasing dietary folate and decreasing risk of lung cancer was also evident (adjusted OR, 1.02; 95% CI, 0.71–1.47; OR, 0.67; 95% CI, 0.46–0.99; and OR, 0.53; 95% CI, 0.35–0.80 for the second, third, and fourth quartiles of average folate intake, respectively; P for trend <0.001). A more pronounced inverse association between dietary folate intake and lung cancer risk was observed among subjects who drank alcohol, had smoked relatively more, those who did not take supplemental folate, and those who reported a family history of lung cancer. Our data suggest that there is a possible protective role of dietary folate in lung carcinogenesis, a finding which may have implications in public health and cancer prevention.

Introduction

Lung cancer remains the leading cause of cancer-related death both in men and women in the United States with an estimated 171,900 new cases and 157,200 deaths in the year 2003 (1). It has been estimated that smoking is responsible for 87% of all lung cancers (1). Although quitting smoking can substantially decrease the risk of lung cancer, it is well documented that former smokers remain at higher risk of developing lung cancer as compared with never-smokers (2), probably because of persistent DNA damage from tobacco carcinogen exposure. Many epidemiological studies have provided evidence that high consumption of vegetables and fruits is associated with a reduced risk of lung cancer (2–4). Much effort has been focused on identifying specific components of these vegetables and fruits that have a protective effect on lung cancer risk (3). In the past, many of these studies focused on provitamin A carotenoids, particularly β-carotene (5–9). However, large intervention trials testing the effect of β-carotene supplementation have failed to confirm this hypothesis (10–12). Therefore, it is possible that other constituent compounds, such as folate, instead of β-carotene in vegetables and fruits, may also provide protection against lung cancer (12–14).

Reduced folate intake has been implicated (although not consistently) as a risk factor for cancer (15). There is an expanding body of evidence from laboratory studies that folate deficiency is associated with altered DNA methylation and synthesis, and disruption of DNA repair activities, which may be an underlying molecular mechanism for the association between folate deficiency and cancer risk (16). We noted in an accompanying article a dose-response relationship between decreased dietary folate intake and decreased DNA repair capacity (17). The role of folate in carcinogenesis has been best studied for colorectal cancer (18), and the main finding was that dietary folate intake is inversely associated with risk in a dose-dependent fashion (15, 19, 20). However, few studies have examined the association of dietary folate intake with lung cancer risk, and the results have been inconsistent. To date, three cohort studies (one in men and women, one in women only, and one in men only; Refs. 21–23), and two case-control studies (24, 25) have evaluated the association between dietary folate intake and lung cancer risk, and another two case-control studies have investigated the association between serum folate levels and lung cancer (26, 27). Whereas the case-control studies failed to find an association between lung cancer risk and either dietary or serum folate, two of the cohort studies observed an inverse association (21, 23). Both cohort studies suggested that folate and vitamin C might be better protective

agents against lung cancer than specific carotenoids such as α- and β-carotene (21, 23). However, no association was observed in the Nurses' Health Study, in which folate intake included supplement use (22).

Given the dietary associations and laboratory evidence observed in previous studies of lung cancer, one of the primary aims of this analysis was to evaluate the extensive dietary folate data we have generated and to relate dietary folate intake to the risk of lung cancer. Unlike previous studies, we not only calculated folate intake level from the food frequency questionnaire, but also collected detailed information on the use of vitamin supplements as well as the cereal grain foods with folic acid fortification after 1998 when such fortification was mandated in the United States. To minimize the residual confounding bias from smoking (28), we restricted our analysis to former smokers, controlling for other potential confounding factors, such as ethnicity, total energy intake, BMI,[3] family history of lung cancer, pack-years smoked, and alcohol consumption. Former smokers now constitute half of all incident lung cancer cases.

Materials and Methods

Study Subjects. The recruitment of cases and controls has been described previously (29). Between July 6, 1995, and July 17, 2001, a total of 1278 untreated patients with newly diagnosed lung cancer were consecutively recruited at The University of Texas M. D. Anderson Cancer Center. There were no age, ethnicity, histological, or stage restrictions, but all of the cases were histopathologically confirmed. We also created a pool of potential control subjects, recruited from the largest multispecialty physician organization in the Houston metropolitan area (30). During the same time period, 1043 subjects who had no previous history of malignancy (except for nonmelanoma skin cancers) were recruited and enrolled as control participants from this pool and frequency matched to the cases on age (± 5 years), sex, and smoking status (never, former, and current). The total response rate for participation was 77% for the cases and 73% for the controls. After exclusion of lung cancer cases who reported a previous history of cancer or those who were diagnosed >4 months before enrollment into the study, 987 (77.2%) cases and 857 (82.2%) controls were eligible. Of these eligible subjects, 470 lung cancer cases were former smokers for whom 472 suitable former-smoker controls were available. The definition of a former smoker was an individual who had smoked at least 100 cigarettes in his or her lifetime and had quit >12 months before the lung cancer diagnosis (for the cases) and before interview for control participants. Of these former-smoker cases, 216 were adenocarcinoma (46.0%), 85 were squamous cell carcinomas (18.1%), 69 were other non-small-cell carcinomas (including large cell carcinoma; 14.7%), 29 were small-cell carcinomas (6.2%), and 72 were carcinomas with unspecified histology (15.3%). Pack-years smoked were calculated from duration and amount of smoking. BMI was defined as weight in kg/height in m^2. The study protocol was approved by the M. D. Anderson and Kelsey Seybold Institutional Review Boards.

Dietary Folate Assessment. Trained interviewers collected dietary data using a modified version of the National Cancer Institute HHHQ (31). The HHHQ (1, 3) is a food frequency questionnaire developed for etiologic cancer research that in-

cludes a food frequency list, an open-ended food section, and other food-behavior questions pertaining to consumption of vitamin supplements, eating at restaurants, and food preparation methods, including folic acid-fortified food. The validity and reliability of this questionnaire are well documented in the DietSys Manual (32–34). The modified questionnaire used in this study lists 135 food and beverage items, including ethnic foods commonly consumed in the Houston area. Interviewers asked study participants about their diet during the previous year (controls) or prior to diagnosis (cases).

DietSys (version 4.01), the nutrient analysis program designed to accompany the HHHQ (33), was used for double key entry of all the completed questionnaires. Folate was fortified in the United States food supply as of January 1998 (35). Therefore, DietSys was used for dietary analysis of questionnaires completed before January 1998, because the nutrient file for this program version contained total folate values before the United States food supply folate fortification. For respondents who completed questionnaires after January 1998, dietary analysis was conducted using the DIETSYS+Plus dietary analysis program (Version 5.9 Block Dietary Data Systems, Berkeley CA, 1999), an updated version of DietSys that contained post-fortification total folate values. Postfortification values for natural folate and folic acid were obtained and added to the nutrient data file used by DIETSYS+Plus, because the software did not contain separate values for naturally occurring folate and folic acid supplied by food fortification. The primary source for postfortification folate values was the Nutrient Database for Standard Reference, Release 14 (SR14).[4] For multi-ingredient food items on the food frequency that were not available in SR14, appropriate recipes from the Continuing Survey of Food Intakes by Individuals, 1994–96, 1998 were used to derive natural folate, folic acid, and total folate using values obtained from SR14 for each recipe ingredient (36). As needed, recipe adjustments for moisture changes and nutrient loss because of cooking were also made. Details of procedures used to update folate values in a nutrient data file have been presented previously (37). Dietary folate intake was adjusted by daily calorie intake and expressed as dietary folate in $\mu g/1000$ kcal/day (27, 38).

Statistical Analysis. Standard statistical techniques for case-control studies were used. Pearson's χ^2 was used to test the differences in the distributions of demographic factors (age, sex, and ethnicity) between cases and controls. Nutrients were analyzed as either continuous or categorical variables. Student's t test was used to compare the differences in nutrients between the cases and controls. For logistic regression analysis, dummy variables of the median and quartile of folate intake were created to calculate the ORs and 95% CIs (with the lowest quartile as the reference category) as an estimate of the relative risk. The full models were adjusted for matching variables: age (continuous), sex, and other potential confounders including ethnicity, total energy intake, BMI, family history of lung cancer, pack-years smoked, and alcohol consumption. Trend tests for the ordered variables (*i.e.,* folate intake by quartiles) were performed by assigning the score j to the jth exposure level of the categorical variables (where j = 1,2, . . .) and treating the categorical variable as an interval predictor in an unconditional logistic regression model. Logistic regression analyses were also performed on the association between dietary folate intake

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[3] The abbreviations used are: BMI, body mass index; OR, odds ratio; CI, confidence interval; HHHQ, Health Habits and History Questionnaire.

Table 1 Distribution of select characteristics of lung cancer patients and cancer-free controls (frequency-matched former smokers)

Variable	Cases (n = 470)		Controls (n = 472)		P^a
	No.	%	No.	%	
Sex					
Male	272	57.9	291	61.7	0.237
Female	198	42.1	181	38.3	
Ethnicity					
Non-Hispanic whites	379	80.7	389	82.4	0.698
Blacks	58	12.3	50	10.6	
Hispanics	33	7.0	33	7.0	
Lung cancer history in first-degree relatives					
Yes	101	21.5	61	12.9	<0.001
No	369	78.5	411	87.1	
	Mean ± SD		Mean ± SD		
Age (years)	62.29 ± 9.15		61.96 ± 9.75		0.593
Body mass index (Kg/m^2)	26.44 ± 5.11		28.05 ± 4.41		<0.001
Pack-years smoked	48.07 ± 32.74		41.74 ± 30.53		0.002
Alcohol intake (g/day)	9.62 ± 23.55		10.20 ± 18.85		0.676
Total energy intake (kcal/day)	1989.23 ± 669.34		2018.91 ± 646.20		0.489
Protein intake (g/day)	72.48 ± 24.74		74.47 ± 24.53		0.216
Total fat intake (g/day)	86.71 ± 34.58		84.34 ± 33.55		0.285
Carbohydrate intake (g/day)	227.88 ± 79.71		237.25 ± 77.57		0.068

[a] Two-sided χ^2 test or Student t test.

Table 2 Distribution of caloric-adjusted (/1000kcal) dietary intake of folate from different sources by case/control status

Variables	Cases (n = 470)	Controls (n = 472)	P^a
	Means ± SD (median, range)	Means ± SD (median, range)	
Food folate (µg/day)	127.5 ± 42.3 (120.1, 49.3–344.6)	137.8 ± 45.4 (130.4, 58.3–289.3)	<0.001
Fortified folic acid (µg/day)	42.7 ± 44.8 (38.5, 0–340.8)	40.7 ± 40.9 (38.3, 0–270.9)	0.478
Supplemental folic acid (µg/day)	126.8 ± 251.3 (109.5, 0–3673.8)	104.3 ± 122.3 (104.9, 0–1364.5)	0.080
Total folate (µg/day)	297.1 ± 267.8 (270.1, 49.3–3865.6)	282.8 ± 140.9 (268.9, 65.9–1579.6)	0.305

[a] Two-sided t test or Wilcoxon's scores test (for fortification folate, supplement folate, and total tolate).

and lung cancer risk stratified by pack-years, alcohol drinking, supplemental folate intake, and family history of lung cancer.

All of the statistical tests were two-sided, and a P of ≤0.05 for any test or model was considered statistically significant. All of the analyses were performed with Statistical Analysis System Software (version 8.0; SAS Institute Inc., Cary, NC).

Results

Select characteristics of the 470 cases and 472 controls are summarized in Table 1. The lung cancer patients and control subjects appeared to be adequately matched on age, sex, and ethnicity. There were slightly higher percentages of female subjects among the cases (42.1%) than among the controls (38.3%; $P = 0.237$). Non-Hispanic whites made up 80.7% of the cases and 82.4% of the controls. Case subjects were significantly more likely than controls to report a family history of lung cancer in their first-degree relatives ($P < 0.001$). Compared with the controls, cases had lower BMI, and this difference was statistically significant. Although all of the cases and controls in this analysis were former smokers, cases reported significantly higher pack-years smoked than the controls (48.1 *versus* 41.7; $P = 0.002$). There were no significant differences between the cases and controls in mean dietary intake values for total energy, protein, total fat, total carbohydrate, and alcohol (Table 1).

Comparisons between cases and controls of calorie-adjusted mean and median intake values for food folate, fortified folate, supplemental folate, and total folate are presented in Table 2. The mean intake levels of food folate in the cases were significantly lower than those in the controls (127.5 *versus* 137.8 µg/day/1000 kcal; $P < 0.001$). However, the mean intake levels of fortified folate and supplemental folate, as well as total folate were slightly higher among the cases than among the controls, although these differences were not statistically significant. The median levels of these variables were much closer between cases and controls than their mean levels, because there were some extreme high values in some subjects, especially in the cases.

The associations between dietary folate intake and risk of lung cancer were evaluated by unconditional logistic regression analyses. As shown in Table 3, when the control median intake of food folate (130.4) was used as the cutoff value for calculating the ORs, only 39.8% (187 of 470) of the cases were above this median level. Dietary folate intake above the control median level was associated with a 40% decreased risk of lung cancer after adjustment for age, sex, ethnicity, total energy intake, BMI, family history of lung cancer, pack-year smoked, and alcohol consumption (adjusted OR, 0.60; 95% CI, 0.45–0.79). In addition, when the control quartiles of food folate intake were used to categorize risk, a significant inverse dose-response relationship between increasing food folate and decreasing risk of lung cancer was evident (ORs, 1.02, 0.67, and 0.53 for the second, third, and fourth quartiles of average folate intake, respectively; P for trend <0.001) after adjustment for these same variables (Table 3). For those subjects recruited after January 1998, we also evaluated the association between

Table 3   Associations between dietary folate intake and lung cancer risk in former smokers

Variables	Cases (n = 470)		Controls (n = 472)		Odds ratio (95% confidence interval)[a]	P
	No.	%	No.	%		
Food folate (μg/1000kcal/day)						
By median						
<130.4	283	60.2	236	50.0	1.00	0.002
≥130.4	187	39.8	236	50.0	0.60 (0.45–0.79)	
By quartile						
<103.5	136	28.9	118	25.0	1.00	<0.001
103.5–130.4	147	31.3	118	25.0	1.02 (0.71–1.47)	
130.4–162.7	102	21.7	118	25.0	0.67 (0.46–0.99)	
≥ 162.7	85	18.1	118	25.0	0.53 (0.35–0.80)	
Fortified folic acid[b]						
By median						
<52.6	154	48.4	159	49.8	1.00	0.721
≥52.6	164	51.6	160	50.2	1.11 (0.79–1.54)	
By quartile						
<37.5	81	25.5	79	24.8	1.00	0.544
37.5–52.6	73	23.0	80	25.1	0.92 (0.58–1.45)	
52.6–72.7	71	22.3	81	25.3	0.86 (0.54–1.38)	
≥72.7	93	29.2	79	24.8	1.27 (0.81–2.00)	
Supplemental folic acid						
No (= 0)	205	43.6	211	44.7	1.00	0.737
Yes (> 0)	265	56.4	261	55.3	1.06 (0.81–1.39)	
By tertile						
none	205	43.6	211	44.7	1.00	0.406
1–172.0	118	25.1	131	27.8	0.93 (0.66–1.32)	
≥172.0	147	31.3	130	27.5	1.21 (0.85–1.70)	

[a] Odds ratio (95% confidence interval) obtained from logistic regression model with adjustment for age, sex, ethnicity, total energy intake, body mass index, family-history of lung cancer, pack-year smoked, and alcohol consumption.
[b] Only for the subjects recruited since January, 1998 (cases = 318; controls = 319).

Table 4   Associations between dietary folate intake and lung cancer risk stratified by selected variables in former smokers

	Odds ratio (95% confidence interval)[a] for folate intake by quartile[b]				Trend test
	Lowest quartile (<25 percentile)	25–50 percentile	50–75 percentile	Highest Quartile (>75 percentile)	
Total (case = 470/control = 472)	1.00	1.02 (0.71–1.47)	0.67 (0.46–0.99)	0.53 (0.35–0.80)	<0.001
Alcohol drinker					
No (220/175)	1.00	0.73 (0.41–1.30)	0.58 (0.31–1.05)	0.69 (0.36–1.30)	0.161
Yes (250/297)	1.00	1.32 (0.81–2.12)	0.80 (0.48–1.33)	0.48 (0.28–0.82)	0.002
Pack-years smoked					
<36 (191/242)	1.00	1.08 (0.62–1.87)	1.13 (0.64–1.99)	0.89 (0.49–1.61)	0.749
≥36 (279/230)	1.00	1.02 (0.61–1.68)	0.45 (0.26–0.78)	0.34 (0.19–0.61)	<0.001
Supplement folate intake					
No (205/211)	1.00	0.74 (0.44–1.25)	0.52 (0.29–0.95)	0.33 (0.17–0.63)	0.001
Yes (265/261)	1.00	1.41 (0.84–2.37)	0.86 (0.51–1.46)	0.78 (0.45–1.34)	0.130
Family lung cancer history					
No (369/411)	1.00	1.12 (0.76–1.67)	0.89 (0.58–1.35)	0.60 (0.38–0.94)	0.016
Yes (101/61)	1.00	0.57 (0.19–1.69)	0.16 (0.05–0.48)	0.27 (0.09–0.85)	0.006

[a] Odds ratio (95% confidence interval) obtained from logistic regression model with adjustment for age, sex, ethnicity, total energy intake, body mass index, family history of lung cancer, pack-year smoked, and alcohol consumption.
[b] Quartile cutpoints were based on residual energy-adjusted intake among controls.

folate from fortified food and lung cancer risk. The distributions of fortified folate either by median or by quartile of controls were very similar between the cases and the controls, and no significant associations or dose-response relationships were observed. We found that 56.4% of the cases and 55.3% of the controls reported taking folic acid from supplements, and the association between supplemental folate and lung cancer risk was not significant (Table 3). We also performed conditional logistic regression analysis to evaluate the main effect of folate using a 1 to 1 matched case-control design, and the

results were consistent with the unconditional analysis (data not shown).

Additional stratified analyses were performed to evaluate the potential modification effects of selected variables on the association between food folate intake and lung cancer risk (Table 4). Among subjects who did not consume alcohol or who had been lighter smokers, natural food folate was only weakly but not significantly associated with a reduced risk of lung cancer (highest *versus* lowest quartile, OR, 0.69; 95% CI, 0.36–1.30 for nondrinkers and OR, 0.89; 95% CI, 0.49–1.61

for light smokers). However, a striking inverse association was observed for natural food folate intake among alcohol drinkers and former heavy smokers (highest *versus* lowest quartile, OR, 0.48; 95% CI, 0.28–0.82 and OR, 0.34; 95% CI, 0.19–0.61, respectively), suggesting a possible interaction, but tests for multiplicative interactions were not statistically significant (data not shown). As shown in Table 4, the protective effect associated with high food folate intake was also more evident among subjects with self-reported family history of lung cancer (highest *versus* lowest quartile, OR, 0.27; 95% CI, 0.09–0.85), although the sample size was small in this subgroup. In addition, a significant inverse dose-response relationship was observed among those who did not take folate supplement (OR, 0.74; 95% CI, 0.44–1.25; OR, 0.52; 95% CI, 0.29–0.95; OR, 0.33; 95% CI, 0.17–0.63 for the second, third, and fourth quartiles of average folate intake, respectively; *P* for trend <0.001). This pattern was less prominent among those taking folate supplements.

We also performed Pearson correlation analysis in this control population, and the results showed that daily intake of natural food folate, fortified folate, and supplemental folate were significantly correlated with total folate ($\gamma = 0.402$, $P < 0.001$ for natural food folate; $\gamma = 0.315$, $P < 0.001$ for fortified folate; and $\gamma = 0.897$, $P < 0.001$ for supplemental folate). There were no significant correlations between pack-years smoked and any folate variables, calories and alcohol intake, and only a modest correlation between alcohol and caloric intake was observed (data not shown).

Discussion

In this case-control study of former smokers, we provide evidence that dietary folate intake is inversely associated with the risk of lung cancer. In addition, this inverse association between dietary folate intake and lung cancer was particularly evident among alcohol drinkers, those who reported having been heavy smokers, those with a self-reported family history of lung cancer, and those who did not report taking supplemental folate. These findings in former smokers add new information about the role of dietary folate intake in lung carcinogenesis to the existing literature.

A growing body of epidemiological, clinical, and experimental evidence suggests that diminished folate status may be an important factor that predisposes individuals to the development of a variety of neoplasms including cervix, colorectum, lung, esophagus, brain, pancreas, and breast (15, 20), although the data are not entirely consistent. The important biological function of folate is to provide methyl groups required for intracellular methylation reactions and *de novo* deoxynucleotide triphosphate synthesis; therefore, folate deficiency is thought to be carcinogenic through disruption of DNA methylation and synthesis, and impaired DNA repair (15, 16). It has also been suggested that folic acid deficiency could affect the stability of cellular DNA/RNA at the chromosomal and molecular levels, which may facilitate activation of oncogenes and inactivation of some tumor suppressor genes (16). A previous small randomized folate chemoprevention study demonstrated that folate supplementation in conjunction with vitamin B_{12} supplementation could reverse purported precursors of bronchial squamous cell carcinoma of the lungs (38), thereby providing a biological basis for a protective effect of folate in reducing lung cancer risk. However, only a few epidemiological studies have been conducted to evaluate the effects of dietary folate in modifying the risk of lung cancer (21–26).

Our findings of an overall significant inverse association

of dietary folate intake with lung cancer risk, especially in former heavy smokers, are supported by two of the prospective studies (21–23). Bandera *et al.* (21) reported in the New York State Cohort Study that intake of folate, as well as vitamin C and carotenoids was inversely associated with lung cancer risk among men and that the protective effect of folate was more evident in heavy smokers. More recently, a significant protective effect of dietary folate on lung cancer incidence was also found in men in the Netherlands Cohort Study on Diet and Cancer (23), and this protective effect was also much stronger among current smokers than former smokers. However, in the Nurses' Health Study in middle-aged women, no association of total folate intake (including from supplements) and lung cancer risk was observed (22). In a nested case-control study of 300 lung cancer cases and 300 matched controls, Hartman *et al.* (26) reported that serum vitamin B_6 levels but not folate and vitamin B_{12} were significantly associated with decreased risk of lung cancer. In another case-control study of 332 lung cancer cases and 865 controls without matching on smoking, Le Marchand *et al.* (25) reported no association between dietary folate intake and lung cancer risk.

Although our analysis was performed only among former smokers, we noted that the inverse associations were stronger among former heavy smokers (>36 pack-years) as compared with former lighter smokers, a finding also noted by the Netherlands Cohort Study (23). It has been reported that poor dietary habits of smokers, along with associated alcohol consumption, could increase the risk for folate deficiency in smokers (27). Furthermore, direct adverse effects of components of cigarette smoke on folate and other B vitamins could lead to local deficiencies of these vitamins in the lungs of smokers (39). Therefore, sufficient folate intake is probably more important in heavy smokers than in light smokers. However, the results from different epidemiological studies remain inconsistent (3, 39, 40). Similarly, the inverse association was also more evident among alcohol drinkers in this study, suggesting that alcohol consumption modifies the association of dietary folate intake with lung cancer risk. This is probably because alcohol use reduces the absorption of dietary folate (21), similar to the interaction between dietary folate intake and alcohol use observed in colon (41) and breast cancer (42).

Our results also indicate that individuals with a family history of lung cancer who were in the highest quartile of folate intake may have a dramatically decreased risk of lung cancer as compared with those without family history, suggesting a possible interaction between folate deficiency and family history. It is biologically plausible that individuals with a family history of lung cancer are likely to have aberrations in DNA methylation or DNA repair because of inherited polymorphisms in related low-penetrance genes and are, therefore, more susceptible to dietary methyl deficiency.

In contrast to folate intake from natural foods, we found that there was no difference in folate intake from fortified food between cases and controls, and that folate supplementation was slightly higher among lung cancer cases than among controls, probably because of some extreme high values in the cases (cases range, 0–3674 and controls, 0–1364 μg/day/1000 kcal). It could be argued that folate supplementation can reduce cancer risk at the initial or mid-stage of carcinogenesis, but increase the risk at the later stage; highly bioavailable supplemented folate might rather enhance lung cancer development. Although the difference in supplementation between cases and controls was not statistically significant (126.8 ± 267.8 *versus* 104.3 ± 122.3; $P = 0.08$), given the bioavailability of supple-

mented folate, which is twice as high as natural food folate, the difference may be biologically relevant.

Because folic acid from fortified food was estimated in our database only on participants recruited after January 1998, we did not use folic acid from fortified food and total folate intake as exposure variables to perform risk assessment. Because as mentioned above folate status at early stages of disease onset may be especially relevant, assessment of folate supplements would be important. In fact, the proportion of the subjects reporting multiple vitamin use for >10 years in the cases (18.7%; 88 of 470) was borderline significantly lower than in controls (23.1%; 109 of 472), suggesting that long-term vitamin supplementation (including folate) may also play a protective role in lung carcinogenesis. However, this issue needs to be addressed far more rigorously than we were able to do with this data set.

Several methodological issues need to be addressed to interpret our findings appropriately. Like any other case-control study, potential selection and recall biases cannot be excluded. In this study, our lung cancer cases were enrolled from M. D. Anderson Cancer Center, and the controls were recruited from the enrollees of a managed care organization. We restricted our analysis to former smokers; thus, it is uncertain whether these results are generalizable. Because the questionnaire data were collected after the cases were diagnosed, the responses to questionnaires by the cases may be influenced by recent changes in diet. However, in this study, we asked about dietary intake in the previous year or before diagnosis (cases) and >80% of the cases were interviewed within 15 days after the diagnosis, thus reducing potential measurement errors attributable to recall bias as well as recent dietary changes after diagnosis. In addition, because dietary folate intake was significantly correlated with other protective nutrients such as vitamin C, vitamin B_6, and β-carotene (mostly from similar food resources), we cannot exclude the possibility that this protective effect from folate may in part have resulted from these nutrients. Another possible bias is the residual confounding bias by smoking, because many studies have shown that smokers, in particular current smokers, tend to have less "healthy" dietary habits (28, 39). However, in this study, we collected extensive information on smoking and restricted the analysis to former smokers whose dietary habits more closely resemble those of never-smokers than those of current smokers (28). In addition, in this analysis, pack-years of smoking was not correlated with any of the nutrient variables so that the difference of pack-years between cases and controls did not likely lead to bias. To exclude any residual confounding, we additionally adjusted pack-years smoked in the analyses. The strengths of our study include the very detailed information on folate intake (not only from natural food, but also from fortified food and from supplements), detailed data on many potential confounders [such as smoking (pack-years) and alcohol drinking], family history of lung cancer, and BMI. Nevertheless, we cannot exclude the possibility that the risk associated with folate might be influenced by other unmeasured confounding factors.

In summary, this study suggests that high dietary folate intake was significantly associated with a reduced risk of lung cancer in former smokers. This relationship was especially apparent among alcohol drinkers, former heavy smokers, those who did not report taking supplemental folate and subjects with a family history of lung cancer. Although the protective effect of high folate-containing food could be attributed to other nutrients that are abundant in these folate-containing foods, our findings may provide potential avenues for lung cancer prevention in populations at a relatively high risk (*e.g.,* smokers

including former smokers and those with family history of cancer) and help generate new hypotheses for mechanisms of folate metabolism in lung cancer development. Longitudinal studies that incorporate prediagnostic folate level assessments are needed to determine whether the observed protective association between dietary folate intake and its cofactors and lung cancer reflects a cause-effect relationship.

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