Serum Folate and Cancer Mortality Among U.S. Adults: Findings from the Third National Health and Nutritional Examination Survey Linked Mortality File

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Abstract

Background: **The relation between folate status and cancer is controversial. Several epidemiologic studies have suggested that increased folate intake is associated with reduced risk of various cancers, others have found no such associations, and a few have suggested that high folate intake might increase the risk of certain cancers.**
Methods: **Using data from the Third National Health and Nutrition Examination Survey (NHANES III) Mortality File, a prospective cohort study of a nationally representative sample of 14,611 U.S. adults, we conducted Cox proportional hazards regression modeling to investigate the association of baseline serum folate concentrations and all-cancer mortality determined from linked death certificate data.**
Results: **Relative to the lowest quintile of serum folate (<3.0 ng/mL), the multivariable-adjusted hazard ratios across quintiles 2 to 5 were: 1.61 [95% confidence interval (95% CI), 1.11-2.32], 1.00 (95% CI, 0.65-1.49), 1.39 (95% CI, 0.96-2.03), and 0.85 (95% CI, 0.59-1.22). These findings did not differ substantially by age or sex, but the higher risk for those in the second quintile appeared limited to non-Hispanic whites.**
Conclusion: **These findings suggest that there may be a nonlinear relationship between folate status and the risk of all-cancer mortality such that persons with low, but not grossly deficient, serum blood folate concentrations may be at increased risk. Further study is needed to determine whether these findings are due to chance, and if not, to clarify their biological basis.** (Cancer Epidemiol Biomarkers Prev 2009;18(5):1439–47)

Introduction

Folate and folic acid are alternate forms of a B-vitamin that is essential for DNA synthesis, repair, and methylation. Alterations of these processes are thought to affect cancer risk (1-3). However, the association between folate (used herein as a collective term for folate and folic acid unless otherwise specified) and the risk of cancer is controversial. The results of many (4-12), but not all (13-21), prospective cohort studies have suggested that increased folate intake is associated with reduced risk of various cancers, such as acute lymphoblastic leukemia and colorectal, breast, pancreatic, esophageal, and gastric cancers, and a few studies have suggested that high folate intake may be associated with increased risk of certain cancers (21). Furthermore, a recent large, randomized, double-blind, placebo-controlled clinical trial of colorectal adenoma recurrence reported that daily supplementation with 1,000 μg of folic acid significantly increased the occurrence of multiple and advanced adenomas and total cancers (22, 23). Taken together, these studies imply a complex relationship between folate status and tumor development, whereby adequate folate intake may prevent the development of precancerous lesions, whereas excess folate, which usually results from taking high-dose folic acid supplements, may enhance tumor formation or growth (23-25).

Examining the relationship between folate intake and cancer risk is particularly important because enriched flour has been fortified with folic acid in the United States since 1998 to help reduce the risk of neural tube defects in newborns (26). Folic acid fortification has resulted in more than a doubling of mean blood folate concentrations in the U.S. population (27) and has been effective in reducing neural tube defect rates (28). However, folic acid fortification has also coincided temporally with an increase in colorectal cancer incidence rates in the United States (29).

Here we report an analysis of the association between serum folate concentration and cancer mortality among persons 20 years of age and older from death certificate data linked to the Third National Examination and Nutritional Health Survey (NHANES III) of a nationally representative sample of the noninstitutionalized U.S. population. Serum folate measurements for this study

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Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Table 1. Characteristics of cohort at baseline by quintiles of serum folate concentration, Third National Health and Nutrition Examination Survey Linked Mortality File 1988-2000

	Total population	Quintiles of serum folate concentration (ng/mL)*					P for trend†
		<3.0	3.0-<4.3	4.3-<6.1	6.1-<9.4	≥9.4	
Serum folate geometric mean in ng/mL (SE)	5.4 (0.12)	2.2 (0.02)	3.6 (0.01)	5.1 (0.01)	7.5 (0.02)	14.2 (0.17)	<0.001
Number of cancer deaths	403	62	99	85	86	71	—
Person-years of follow-up	125,179	25,240	30,602	26,023	22,967	20,346	—
Mean age in y (SE)	44.2 (0.47)	38.9 (0.37)	40.9 (0.43)	43.7 (0.58)	45.8 (0.65)	51.3 (0.76)	<0.001
% male (95% CI)	48.4 (47.4-49.3)	54.7 (50.5-56.8)	52.1 (49.5-54.7)	50.3 (47.6-52.9)	46.7 (44.0-49.5)	39.4 (36.5-42.3)	<0.001
Race or ethnicity, % (95% CI)							
Non-Hispanic white	76.6 (74.0-79.0)	71.7 (68.5-74.7)	71.4 (67.5-75.0)	73.8 (70.3-77.0)	79.2 (75.3-82.7)	86.3 (83.5-88.7)	—
Non-Hispanic black or African American	10.5 (9.4-11.8)	15.6 (13.6-17.9)	12.8 (11.1-14.7)	10.9 (9.6-12.4)	8.4 (7.1-9.9)	5.4 (4.5-6.4)	—
Hispanic American	5.2 (4.4-6.2)	6.2 (5.2-7.5)	6.8 (5.7-8.1)	5.9 (4.9-7.0)	4.5 (3.6-5.6)	2.7 (2.0-3.7)	—
Others	7.7 (6.2-9.5)	6.5 (5.0-8.4)	9.0 (6.7-12.0)	9.4 (7.1-12.5)	7.9 (5.8-10.8)	5.6 (4.0-7.9)	<0.001
Years of education, % (95% CI)							
<12	24.2 (22.2-26.3)	28.4 (25.3-31.8)	26.6 (24.0-29.3)	24.3 (21.0-27.9)	22.7 (19.9-25.8)	19.4 (16.7-22.3)	—
12-15	54.6 (52.9-56.2)	56.4 (53.9-59.0)	56.9 (53.8-59.8)	55.5 (51.9-59.0)	52.3 (49.2-55.4)	52.0 (48.5-55.5)	—
>15	21.2 (19.5-23.1)	15.2 (12.6-18.3)	16.7 (14.3-19.3)	20.2 (17.7-23.1)	25.0 (21.9-28.3)	28.6 (25.2-32.4)	<0.001
Smoking status, % (95% CI)							
Never	46.2 (44.6-47.8)	38.3 (35.3-41.3)	41.7 (38.6-45.0)	47.1 (43.5-50.7)	49.5 (46.1-52.8)	53.9 (51.0-56.7)	—
Former	25.2 (23.9-26.6)	17.1 (14.9-19.6)	22.1 (19.2-25.3)	26.6 (23.8-29.5)	27.6 (25.3-30.0)	32.2 (29.7-34.8)	—
Current	28.6 (26.8-30.4)	44.6 (41.5-47.8)	36.1 (32.5-39.9)	26.4 (23.7-29.2)	22.9 (20.6-25.4)	13.9 (11.8-16.3)	<0.001
Alcohol intake in drinks/wk, % (95% CI)							
None	46.5 (43.6-49.3)	45.5 (41.7-49.0)	42.9 (39.8-46.1)	45.8 (42.3-49.2)	45.6 (41.2-50.0)	52.5 (48.0-56.9)	—
<3	26.6 (25.0-28.2)	27.1 (24.5-29.8)	26.3 (23.4-29.5)	26.3 (23.5-29.2)	27.9 (24.6-31.5)	25.5 (21.9-29.5)	—
≥3-<7	12.2 (11.0-13.5)	12.9 (10.3-16.1)	13.1 (11.0-15.6)	12.4 (10.7-14.2)	11.6 (9.7-13.8)	10.9 (8.6-13.5)	—
≥7	14.7 (13.4-16.2)	14.2 (12.4-16.3)	17.7 (14.9-20.7)	15.5 (13.5-17.8)	14.9 (12.5-17.7)	11.2 (9.2-13.5)	0.001
NSAID use,‡ % (95% CI)							
None	42.6 (41.3-43.8)	44.4 (41.8-47.0)	43.9 (41.2-46.5)	42.9 (39.7-46.2)	41.9 (38.4-45.5)	39.9 (36.8-43.1)	—
Irregular (<15 times in last mo)	38.0 (36.4-39.7)	38.3 (35.2-41.4)	40.4 (37.6-43.2)	38.7 (34.9-42.7)	38.7 (35.5-42.1)	34.0 (30.6-37.6)	—
Regular (≥15 times in last mo)	19.4 (18.1-20.8)	17.4 (15.2-19.7)	15.8 (13.7-18.1)	18.4 (16.4-20.7)	19.4 (17.0-21.9)	26.1 (23.2-29.2)	0.001
Mean BMI in kg/m^2 (SE)	26.6 (0.12)	27.5 (0.25)	26.7 (0.15)	26.7 (0.18)	26.2 (0.23)	25.7 (0.18)	0.02
Physical activity in times/wk, % (95% CI)							
None	21.6 (19.7-23.6)	26.6 (23.8-29.5)	23.0 (20.2-26.0)	21.6 (19.0-24.5)	19.7 (17.0-22.8)	17.5 (15.0-20.4)	—
<5/wk	35.3 (33.9-36.8)	36.0 (32.9-39.3)	39.3 (36.3-42.5)	35.2 (32.4-38.1)	34.6 (31.3-38.0)	31.6 (29.0-34.3)	—
≥5/wk	43.1 (40.9-45.3)	37.5 (34.1-41.0)	37.7 (34.3-41.2)	43.1 (40.3-46.1)	45.7 (41.9-49.6)	50.9 (47.7-54.1)	<0.001
Total energy intake geometric mean in kcal/d (SE)	1,971 (17.1)	2,058 (26.8)	2,050 (28.8)	1,975 (28.1)	1,923 (31.7)	1,863 (32.7)	<0.001
Food folate intake geometric mean in μ/d (SE)	230 (2.3)	175 (3.1)	204 (4.1)	228 (4.2)	256 (5.6)	298 (6.0)	<0.001
Folic acid supplement users (%)	29.3 (27.7-30.9)	10.6 (8.8-12.7)	14.3 (12.0-17.0)	18.3 (16.2-20.6)	33.9 (30.5-37.5)	67.7 (65.0-70.2)	<0.001
Healthy Eating Index (SE)§	63.6 (0.29)	58.5 (0.38)	60.7 (0.34)	63.4 (0.37)	65.8 (0.34)	69.6 (0.43)	<0.001

*Mean and SE for continuous variables, and % and 95% CI for categorical variables presented by quintiles of serum folate concentration.
†P value for difference across quintiles of serum folate concentrations based on ANOVA for continuous variables and χ^2 test for categorical variables; all tests two-tailed.
‡Regular users (≥15 times in last mo) included all users of prescription NSAIDs.
§HEI is a score that ranges from 0 to 100 and contains information on the consumption of 10 subcomponents of the diet: grains, fruits, vegetables, dairy, meats, fats, saturated fat, cholesterol, sodium, and dietary variety. A higher score is indicative of a healthier eating pattern.

were taken prior to the implementation of mandatory folic acid fortification in the United States.

Materials and Methods

The Third National Health and Nutrition Examination Survey (NHANES III). NHANES III used a stratified, multistage probability design to obtain a nationally representative sample of the civilian, noninstitutionalized U.S. population. In NHANES III, each survey participant completed a household interview and underwent a physical examination (30). We restricted our analyses to participants who were ≥20 y of age. Of 18,800 participants who completed the NHANES III interview and examination from 1988 to 1994 and on whom complete mortality follow-up information was available, we excluded 780 participants who at baseline had been told by their physicians that they had cancer (other than skin cancer) and 2,552 participants on whom serum folate data were missing. We also excluded 857 participants on whom data on important covariates were missing, including 804 participants with no dietary intake information and 53 with missing information on other covariates. Thus, 14,611 NHANES III participants were available for the present analysis.

Serum Folate Measurement. Serum folate concentrations were determined by the National Center for Environmental Health at the Centers for Disease Control and Prevention using a commercially available radioprotein binding assay kit (Quantaphase II, Bio-Rad Laboratories; ref. 31).

Baseline serum folate concentrations were categorized according to study population quintiles to investigate their associations with cancer mortality. In NHANES III (1988-1994) approximately 20% of the U.S. population was characterized as folate deficient (blood folate <3 ng/mL; ref. 32). This cut point approximated our lowest quintile, which was used as the reference category for most of the data analyses reported below.

Baseline Covariates. The ages of participants were determined in years by self-report at the baseline examination. Race or ethnicity was classified as non-Hispanic white, non-Hispanic black (or African American), Hispanic American, or others. Educational attainment was classified as <12, 12-15, or >15 y of formal education. Body mass index (BMI) was calculated as kilograms of weight divided by meters of height squared (kg/m^2). History of cigarette smoking was categorized as never, former, or current. Alcohol consumption was classified as 0, <3, 3-<7, or ≥7 drinks/wk. Use of over-the-counter nonsteroidal anti-inflammatory drugs (NSAID) was classified as none, nonregular (<15 times/mo), or regular (≥15 times/mo) during the previous month; users of prescription NSAIDs were also included as regular users. Physical activity was categorized as 0, <5, or ≥5 times per week of walking, jogging or running, bicycling, swimming, aerobics or aerobic dancing, other dancing, calisthenics, gardening or yard work, or lifting weights during the previous month. During the household interview participants were asked about their use of dietary supplements, including single vitamins, multivitamins, minerals, and other nutritional substances. A participant was classified as a folic acid supplement user (yes/no) if she/he reported taking any folic acid–containing supplement in the past month. Total energy intake and folate intake from foods were estimated from one 24-h dietary recall administered during the baseline survey interview. We also included the Healthy Eating Index (HEI) in our analysis. The HEI is a score that ranges from 0 to 100 and contains information on the consumption of 10 subcomponents of the diet: grains, fruits, vegetables, dairy, meats, fats, saturated fat, cholesterol, sodium, and dietary variety (33). A higher HEI score indicates what is thought to be a healthier eating pattern.

Outcome Measures. NHANES III eligible participants for the linked mortality study were matched, using a probabilistic matching algorithm, to the National Death Index through December 31, 2000, to determine their mortality status. A complete, detailed description of the methodology used can be found at: http://www.cdc.gov/nchs/data/datalinkage/matching_methodology_nhanes3_final.pdf. The International Classification of

Table 2. Risk of all-cancer mortality overall and by age, sex, and race or ethnicity, Third National Health and Nutrition Examination Survey Linked Mortality File 1988-2000

	Quintiles of serum folate concentration (ng/mL)					*P**
	<3.0	3.0-<4.3	4.3-<6.1	6.1-<9.4	≥9.4	
Overall						
No. of cases	62	99	85	86	71	
HR adjusted for age, sex, and race only (95% CI)	1.0	1.44 (0.97-2.14)	0.82 (0.55-1.22)	1.09 (0.76-1.55)	0.63 (0.45-0.88)	<0.01
Fully-adjusted HR† (95% CI)	1.0	1.61 (1.11-2.32)	1.00 (0.65-1.49)	1.39 (0.96-2.03)	0.85 (0.59-1.22)	0.02
By race or ethnicity						
Non-Hispanic whites						
No. of cases	20	50	33	60	50	
HR adjusted for age and sex only (95% CI)	1.0	1.71 (1.05-2.79)	0.77 (0.46-1.31)	1.18 (0.77-1.82)	0.65 (0.44-0.96)	0.01
Fully adjusted HR‡ (95% CI)	1.0	1.91 (1.20-3.03)	0.92 (0.54-1.57)	1.51 (0.96-2.38)	0.87 (0.58-1.32)	<0.01
Non-Hispanic blacks						
No. of cases	26	30	29	17	12	
HR adjusted for age and sex only (95% CI)	1.0	0.85 (0.46-1.58)	1.10 (0.55-2.21)	0.81 (0.41-1.58)	0.72 (0.34-1.52)	0.76
Fully adjusted HR‡ (95% CI)	1.0	0.99 (0.53-1.82)	1.30 (0.68-2.45)	0.99 (0.49-1.99)	0.94 (0.39-2.27)	0.32
Hispanic Americans						
No. of cases	16	19	22	7	8	
HR adjusted for age and sex only (95% CI)	1.0	0.68 (0.25-1.85)	0.98 (0.39-2.44)	0.38 (0.09-1.53)	0.55 (0.16-1.87)	0.39
Fully adjusted HR‡ (95% CI)	1.0	0.80 (0.28-2.29)	1.16 (0.44-3.08)	0.49 (0.12-2.06)	0.81 (0.21-3.08)	0.61
By folic acid supplement use						
Nonusers						
No. of cases	59	88	75	66	29	
HR adjusted for age, sex, and race only (95% CI)	1.0	1.35 (0.88-2.06)	0.83 (0.53-1.29)	0.93 (0.61-1.42)	0.77 (0.53-1.13)	0.07
Fully adjusted HR† (95% CI)	1.0	1.51 (1.01-2.27)	1.01 (0.64-1.60)	1.23 (0.78-1.96)	0.97 (0.59-1.58)	0.23
Users						
No. of cases	3	11	10	20	42	
HR adjusted for age, sex, and race only (95% CI)	1.0	5.42 (1.09-27.0)	1.32 (0.39-4.45)	3.90 (0.85-17.9)	1.36 (0.30-6.13)	<0.01
Fully adjusted HR† (95% CI)	1.0	5.76 (1.09-30.6)	1.50 (0.45-5.02)	4.18 (0.94-18.6)	1.53 (0.34-6.83)	<0.01

*P for trend across quintiles of serum folate concentrations based on adjusted Satterthwaite F-test; all tests two-tailed.

† Adjusted for age, sex, race or ethnicity, smoking status, alcohol intake, BMI, education, NSAID use, physical activity, HEI, and total energy intake. P values for age by race or ethnicity interaction were 0.50 and 0.59 for age-, sex-, race/ethnicity- and multivariate-adjusted models, respectively, based on adjusted Satterthwaite F-test.

‡ Adjusted for age, sex, smoking status, alcohol intake, BMI, education, NSAID use, physical activity, HEI, and total energy intake.

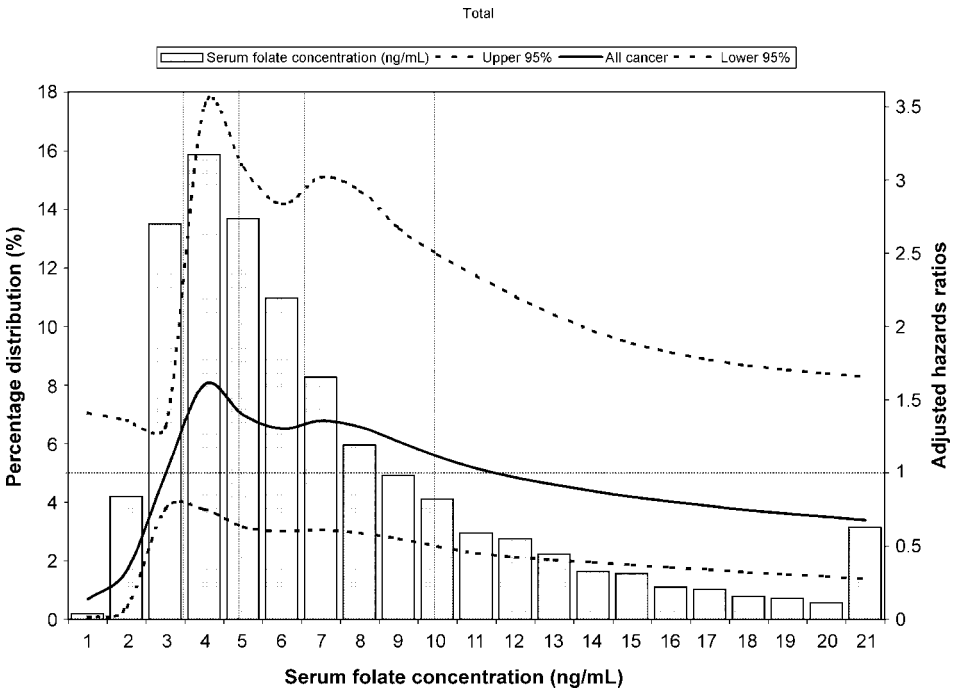

Figure 1. Adjusted HRs for all-cancer mortality by serum folate concentration. Solid line, adjusted HRs based on restricted cubic splines with six knots at the 10th, 20th, 40th, 60th, 80th, and 90th percentiles of serum folate concentration and the reference set at 2.0 ng/mL (5th percentile); dotted lines, 95% CIs; bars, percentage distribution of serum folate concentration in the population; vertical dotted lines, cut points of the serum folate quintiles used in the main analysis. The test for linearity of the dose-response curve indicated a significant departure from linearity ($P = 0.04$). HRs are adjusted for age, sex, race or ethnicity, smoking status, alcohol intake, BMI, education, NSAID use, physical activity, HEI, and total energy intake. NHANES III Linked Mortality File 1988-2000.

Disease, Tenth Revision (ICD-10) was used to identify subjects for whom any malignant neoplasm (ICD-10 codes C00-C97) was listed as the immediate or underlying cause of death. Follow-up of survival time continued until death due to cancer and was censored at the time of death among those who died from causes other than cancer. Participants who were not matched with a death record were considered alive through the entire follow-up period.

Statistical Analysis. We used ANOVA for continuous variables and the χ^2 test for categorical variables to assess possible associations between baseline quintiles of serum folate concentration and risk factors thought to be potential confounding variables. We used Cox proportional hazards regression methods to estimate the hazards ratios (HR; relative risks) and 95% confidence intervals (95% CI) for all-cancer mortality by different levels of serum folate concentration. We estimated age-sex-race/ethnicity-adjusted HRs, as well as multivariable-adjusted HRs. Covariates were selected *a priori* based on their suspected roles as confounders. Multivariable models were adjusted for age (5-year age groups), sex, race/ethnicity, educational attainment, BMI, smoking status, alcohol intake, NSAID use during the previous month, total energy intake, and HEI as potential confounders. Covariates were included in the final models if: (*a*) they were recognized predictors of risk of cancer as suggested by other studies (34); (*b*) they were associated with serum folate concentrations in the cohort, but not thought to be a consequence of exposure or cancer; or (*c*) the associated directed acyclic graph (a method to select potential confounders in epidemiologic studies) based on *a priori* considerations was consistent with confounding (35). Two risk factors that met these criteria, but were strong predictors of serum folate concentrations (use of supplements containing folic acid and food folate intake), were excluded from the final models. However, we included these latter variables in Tables 1 and 2 because of their importance in determining blood folate concentration. A P value for trend across the HRs for the quintiles was calculated using a Satterthwaite F-test.

We estimated HRs for all-cancer mortality for the whole cohort, as well as by race or ethnicity. Because several studies suggested that associations of certain cancers with folate intake may differ depending on the sources of folate (e.g., dietary folate, folic acid from dietary supplements, or a combination of the two; refs. 5, 17, 36), we conducted an analysis stratified by folic acid supplement use (users versus nonusers).

We tested for a possible dose-response association between serum folate and all-cancer mortality using log-transformed serum folate concentration as a continuous variable with six knots (at the 10th, 20th, 40th, 60th, 80th, and 90th percentiles of serum folate concentration) of the restricted cubic spline in the multivariate proportional hazards models (37). For this analysis we used <2.0 ng/mL (5th percentile) as the referent category for HR estimates. For ease of computation, we used SAS

Proc PHREG with the NORMALIZE option after the weight statement to rescale to the actual sample size for the dose-response analysis and adjusted the 95% CIs by assuming an average design effect of 1.3 (38).

All statistical tests using Cox proportional hazards analyses were based on adjusted Satterthwaite statistics, all tests were two-sided, and $P < 0.05$ was considered statistically significant. The proportional hazards assumptions of the Cox models were evaluated with Schoenfeld residuals, which revealed no significant departures from proportionality in hazards over time (39). We conducted our Cox proportional hazards analyses using SUDAAN (version 9.1) so that we could account for the complex sampling design (40), and our dose-response analysis using SAS Proc PHREG (version 9.1, SAS Institute Inc.).

Sensitivity Analyses. We conducted several sensitivity analyses to evaluate the estimated associations between serum folate and all-cancer mortality in more detail. In the analyses described above, we focused on people who developed incident cancers from which they died during follow-up and excluded people with any prevalent cancer at baseline. In our first sensitivity analysis (scenario 1), we included people who at baseline reported having been told by a physician that they had cancer. In our second sensitivity analysis (scenario 2), we focused solely on participants who had cancer at baseline to investigate the hypothesis that there is a dual role for folate intake in cancer risk (23-25). In our third sensitivity analysis (scenario 3), we included only cancer mortality that occurred after 3 y of follow-up to ensure that cancers reported during follow-up were more likely to be ''incident'' cancers that would not have affected folate measurements. We expected to see a higher risk of cancer mortality associated with higher blood folate concentrations under the conditions of scenario 2, and the reverse for scenario 3. In the fourth and fifth sensitivity analyses, we assessed the possible effects of mandatory folic acid fortification, which resulted in a doubling of the average serum folate concentration among the U.S. population, in two ways. First (scenario 4), we included the estimated number of servings of grain products (in quartiles) as a time-varying covariate in our multivariate proportional hazards models to partially control for changes in serum folate concentrations due to folic acid fortification since 1998. Second (scenario 5), we restricted our analyses to cancer mortality that occurred before folic acid fortification (i.e., prior to January 1, 1998).

Results

Among the 14,611 participants meeting our eligibility criteria, 403 cancer deaths over 125,179 person-years of follow-up (median follow-up, 8.6 years) were documented. There were 118 deaths from lung cancer, 46 from colorectal cancer, 22 from breast cancer, 28 from prostate cancer, and 189 from all other cancers combined.

The characteristics of the analysis cohort at baseline by quintiles of serum folate concentration (ng/mL) are summarized in Table 1. Higher serum folate

Table 3. Sensitivity analysis for associations of serum folate with cancer mortality, Third National Health and Nutrition Examination Survey Linked Mortality File 1988-2000

	Quintiles of serum folate concentration (ng/mL)					*P**
	<3.0	3.0-<4.3	4.3-<6.1	6.1-<9.4	≥9.4	
Scenario 1: including participants with cancer (excluding skin cancer) at baseline†						
No. of cases	72	115	105	110	93	
HR adjusted for age, sex, and race (95% CI)	1.0	1.37 (0.96-1.96)	0.87 (0.60-1.26)	1.09 (0.80-1.50)	0.65 (0.44-0.98)	<0.01
Fully adjusted HR‡ (95% CI)	1.0	1.55 (1.10-2.17)	1.07 (0.72-1.57)	1.41 (1.00-1.98)	0.90 (0.58-1.41)	0.02
Scenario 2: restricting analysis to participants with cancer (excluding skin cancer) at baseline§						
No. of cases	10	16	20	24	22	
HR adjusted for age, sex, and race (95% CI)	1.0	1.00 (0.34-2.91)	1.08 (0.44-2.65)	1.17 (0.48-2.87)	0.80 (0.29-2.20)	0.82
Fully adjusted HR‡ (95% CI)	1.0	1.13 (0.41-3.15)	1.21 (0.55-2.66)	1.45 (0.67-3.12)	1.23 (0.48-3.16)	0.90
Scenario 3: restricting analysis to cancer mortality that occurred after 3 y follow-up‖						
No. of cases	50	89	86	79	67	
HR adjusted for age, sex, and race (95% CI)	1.0	1.74 (1.07-2.83)	1.02 (0.62-1.68)	1.23 (0.80-1.88)	0.81 (0.54-1.21)	0.01
Fully adjusted HR‡ (95% CI)	1.0	1.95 (1.22-3.14)	1.22 (0.74-2.04)	1.57 (1.01-2.45)	1.09 (0.70-1.71)	0.03
Scenario 4: using average daily grain consumption as time-varying covariate to account for folic acid fortification beginning in 1998¶						
No. of cases	62	99	85	86	71	
HR adjusted for age, sex, and race (95% CI)	1.0	1.44 (0.95-2.18)	0.82 (0.52-1.29)	1.09 (0.72-1.66)	0.63 (0.40-0.99)	<0.01‖
Fully adjusted HR‡ (95% CI)	1.0	1.63 (1.07-2.48)	1.02 (0.64-1.61)	1.42 (0.92-2.19)	0.87 (0.55-1.38)	<0.01‖
Scenario 5: restricting analysis to cancer mortality that occurred before folic acid fortification (January 1, 1998)**						
No. of cases	43	63	57	64	51	
HR adjusted for age, sex, and race (95% CI)	1.0	1.10 (0.71-1.69)	0.60 (0.37-0.96)	0.90 (0.56-1.45)	0.48 (0.29-0.78)	<0.01
Fully adjusted HR‡ (95% CI)	1.0	1.24 (0.82-1.87)	0.73 (0.44-1.22)	1.16 (0.72-1.88)	0.65 (0.38-1.12)	0.05

*P for trend across quintiles of serum folate concentrations based on adjusted Satterthwaite F-test; all tests two-tailed.
† Scenario 1 included participants who at baseline had been told by a physician that they had cancer other than skin cancer.
‡ Adjusted for age, sex, race or ethnicity, smoking status, alcohol intake, BMI, education, NSAID use, physical activity, HEI, and total energy intake.
§ Scenario 2 included only participants who at baseline had been told by a physician that they had cancer other than skin cancer, and cancer mortality was restricted to that occurring within 3 y of follow-up.
‖ Scenario 3 excluded cancer mortality that occurred within 3 y of follow-up.
¶ Scenario 4 included average daily intakes of grain products as a time-varying covariate in the Cox proportional hazards models to adjust for possible effects of folic acid fortification mandated in 1998 in the United States. We used SAS Proc PHREG with the NORMALIZE option after a weight statement to rescale the weighted population to the actual sample size for the time-varying covariate analysis. P value based on Wald χ^2 test.
**Scenario 5 restricted cancer mortality to that that occurred before folic acid fortification began in the United States in January 1998.

concentrations were associated with older age, being non-Hispanic white, higher educational attainment, not being a current smoker or drinker of alcohol, taking NSAIDs, higher folate intake from foods, a higher HEI index, lower total energy intake, and greater physical activity.

As shown in Table 2, the incidence of cancer deaths was significantly higher among those with a serum folate concentration in the second quintile (3.0-4.3 ng/mL): the adjusted HR for those in this quintile relative to those in the lowest quintile (<3.0 ng/mL) was 1.61 (95% CI, 1.11-2.32). Risk estimates for those in quintiles 3 to 5 did not statistically significantly differ from 1.0. When participants with serum folate concentrations in the upper 5th percentile (>17 ng/mL), which included 16 who died of cancer, were compared with those with folate deficiency (<3.0 ng/mL), the HR was 0.50 (95% CI, 0.24-1.08; data not shown). Also, in an alternate analysis (data not shown), using the second quintile of serum folate concentrations (3.0-4.3 ng/mL) rather than the first quintile (<3.0 ng/mL) as the reference group, the adjusted HR for participants in the highest quintile of serum folate concentration was 0.49 (95% CI, 0.29-0.81). Associations between serum folate concentration and cancer risk by age group and sex were similar to those for the whole cohort (data not shown). There was no evidence of an association between serum folate and cancer risk among non-Hispanic blacks or African Americans or Hispanic Americans. We also conducted cancer site–specific analyses (data not shown), but beyond noting that our findings for lung and colorectal cancer were similar to those for all cancers combined, the sample sizes were too small to be reliable or to warrant further presentation or discussion.

Although folate and folic acid are commonly used interchangeably to refer to the B-vitamin, folate occurs naturally in foods whereas folic acid is a synthetic agent that is used in dietary supplements and fortified foods and is 1.7 times more effective than dietary folate in raising blood folate concentrations (41). To assess whether the serum folate–all-cancer mortality association depends on whether participants took a folic acid–containing supplement or not, we also stratified our analyses on folic acid supplement use (Table 2). Among participants who took a folic acid–containing supplement, all HRs for quintiles 2 to 5 were >1.0, and the HR for the second quintile was larger than that found for the overall population, whereas among participants who did not take a folic acid supplement, the pattern of risk for all-cancer mortality was similar to that found for the total population.

Figure 1 shows the results of the analysis to investigate more completely a possible dose-response association between serum folate and all-cancer mortality using log-transformed serum folate concentration as a continuous variable in the multivariate proportional hazards models. The results are displayed as HR (95% CI) using a referent serum folate concentration of <2.0 ng/mL superimposed on the distribution of serum folate concentration in the population. The risk estimates for all-cancer mortality tended to decline as blood folate concentration increased above the second quintile (3.0-4.3 ng/mL). The test for linearity of the dose-response curve indicated a significant departure from linearity ($P = 0.04$).

In the sensitivity analyses, the adjusted HRs remained largely unchanged when we included participants who had cancer at baseline (an additional 92 cancer deaths during follow-up; Table 3, scenario 1). We found no evidence of elevated or reduced cancer mortality in association with higher blood folate concentration among those who had cancer at baseline (scenario 2) or when restricting cancer mortality cases to those who died after three years of follow-up (scenario 3). Including a time-varying covariate for grain product intake did not materially change the estimated adjusted HRs (scenario 4). The pattern of adjusted HRs remained similar when we restricted our analysis to cancer mortality that occurred before 1998, the year mandatory folic acid fortification began in the United States (scenario 5), although the peak in the second quintile of serum folate concentration was not statistically significant, probably due to the smaller sample size. Finally, results similar to those reported in Table 2 were found when variables for the use of folic acid–containing supplements and for folate intake from foods were added to the multivariate models (data not shown).

Discussion

After prospectively following participants in this cohort of a nationally representative sample of U.S. adults for an average of 8.6 years, we observed a nonlinear association between serum folate concentration and risk of all-cancer mortality in which persons with low (3.0-4.5 ng/mL), but not classically deficient, serum folate concentrations were at statistically significantly higher risk of cancer mortality compared with participants with deficient serum folate concentrations [i.e., <3.0 ng/mL, a range that applied to 20% of Americans in NHANES III (1988-1994); ref. 27]. There was no substantial or statistically significant evidence of an association of serum folate concentrations >4.5 ng/mL and risk of all-cancer mortality.

Folate plays an important role in the synthesis of nucleotides that are essential for DNA replication and repair. Folate deficiency can lead to uracil misincorporation, disruption of DNA repair, and increased susceptibility of DNA to strand breaks (2). Folate is also required for synthesis of methionine, an important amino acid that is the precursor of *S*-adenosylmethionine, which is involved in DNA methylation and other methylation reactions. Inadequate folate status can lead to altered patterns of epigenetic DNA modification that are associated with carcinogenesis (42).

There are several inconsistencies in the literature regarding folate status and the risk of cancer. First, several (4-12), but not all (13-21), prospective cohort studies have found inverse associations between folate status, including dietary folate or folic acid or blood folate concentrations, and cancer risk. Second, contrary to these observational studies, a recent randomized, double-blind, placebo-controlled clinical trial ($n = 1,021$) of colorectal adenoma recurrence reported that daily supplementation with 1,000 μg of folic acid over 3 to 5 years significantly increased the recurrence of multiple and advanced adenomas and incidence of total cancers (22, 23); however, a similar trial ($n = 853$) using 500 μg of folic acid daily found no treatment effects on adenoma recurrence or cancer (44). Also, unmetabolized blood

folic acid may reduce natural killer cell cytotoxicity (45). Third, similar to our study, a nested case-control analysis of the population-based Northern Sweden Health and Disease Cohort found that plasma folate was significantly associated with the risk of colorectal cancer in a bell-shaped fashion, with an adjusted odds ratio of 2.0 (95% CI, 1.12-3.56) for those in the middle relative to those in the lowest quintile of plasma folate (46). Fourth, 5-fluorouracil, a mainstay of colon cancer chemotherapy, is a folate inhibitor, and a number of studies suggested that folate deficiency might inhibit, rather than enhance, the development of certain kinds of cancer among rats (47, 48). Our findings are most consistent with those from the Northern Sweden Health and Disease Cohort study (46). The latter study investigated colon cancer incidence, whereas the NHANES III Linked Mortality File upon which our analyses were conducted investigated all-cancer mortality (the NHANES III Linked Mortality File did not collect data on incident cancers). We do not know whether our observed association between cancer mortality and serum folate would hold for incident cancer in our study population. We did not find clear evidence for either increased or decreased risk of all-cancer mortality among participants with the highest concentrations of serum folate in our study. It is possible that, in contrast to the above noted clinical trial, the number of participants in our study with concentrations comparable with those achieved in the clinical trial (approximately 33 ng/mL) was too small to substantially affect the risk estimate. However, for all-cancer mortality, our findings taken together with those of other studies suggest that, although folate deficiency might inhibit cancer development and/or delay cancer mortality, moderately low folate status may be associated with higher risk.

Although the proposed mechanisms for a role for folate in carcinogenesis could be applicable to almost any cancer, the relative importance could vary substantially by cancer type; for example, it could be more relevant to cancers of tissues with more rapid cell turnover, such as the colon. Several meta-analyses have investigated associations between folate status and the risk of breast, esophageal, gastric, pancreatic, and colorectal cancer (36, 43, 49, 50). For breast cancer, higher folate status was statistically significantly associated with risk in the case-control studies but not in the cohort studies (49, 50). Higher dietary folate intake was associated with reduced risk of cancers of the esophagus, stomach, and pancreas, but the findings for gastric cancer were inconsistent (43). For colorectal cancer, higher dietary, but not total (dietary folate plus folic acid from supplements), folate intake was associated with reduced risk in the cohort studies, but the findings from case-control studies showed significant heterogeneity (36).

Our analyses stratified by race/ethnicity suggest that our overall findings might apply only to non-Hispanic whites; however, the sample size for other racial/ethnic groups was small, and the test for a multiplicative interaction was not statistically significant (P = 0.22). Consistent with our findings, several case-control studies reported no association between folate intake and risk of colorectal and breast cancer among blacks or African Americans (51, 52); however, other such studies found a slightly increased risk of colorectal cancer among those with total daily folate intakes <400 μg relative to those with intakes ≥400 μg (53). We are not aware of any population-based study of folate intake or blood folate concentration and cancer risk among Hispanic Americans. If there are race-specific effects of serum folate concentration on all-cancer mortality, the reason(s) is/are unknown. Other than limited sample size and statistical power, possible explanations for a null serum folate–all-cancer mortality association among racial/ethnicity groups other than non-Hispanic whites include sampling issues (e.g., differential matching rates, missed cancer deaths by race or ethnicity), residual confounding, and racial/ethnic differences in frequencies of variants in folate metabolic pathways genes (54).

Our findings that higher serum folate concentrations among persons who took folic acid–containing supplements tended to be associated with higher risk raises the possibility that attaining higher serum folate concentrations through folic acid supplementation rather than through folate from foods may not yield equivalent health effects. On the other hand, these findings may be due to unrecognized or incompletely accounted for confounding (i.e., factors associated with supplement taking, folate-rich diets, or cancer screening, diagnosis, and care) or, given the small sample size (especially in the referent group) for this analysis, chance.

In the pre–folic acid fortification era, approximately 90% of the U.S. population had a blood folate concentration <10 ng/mL, a level that was significantly less than the median (13.0 ng/mL) blood folate concentration after fortification (27). Whether folic acid fortification might prevent or promote cancer remains controversial (25, 55, 56). Our results suggest that the risk of cancer mortality in the U.S. population tended to decline with increasing serum folate concentrations above the 4.5 ng/mL range. These findings are consistent with those of some previous cohort studies (4-12, 43). A recent study reported a temporal association of folic acid fortification with an increase in colorectal cancer incidence rates in the United States (29). Another study suggested that folic acid fortification resulted in a change in the degree of expression of DNA methyltransferase in cells involved in cervical carcinogenesis that was consistent with an increased risk for cancer (57). Our scenario 2 sensitivity analysis, which was restricted to individuals with a history of cancer at baseline, yielded HRs >1.0 for those in the higher quintiles of serum folate. Although this finding is not robust because of the small number of participants included, it is consistent with concerns about the possible adverse effects of folate on cancer survival, possibly attributable to growth-promoting effects on metastases or to interference with drugs that target folate metabolism (23-25). Careful monitoring of changes in cancer incidence following folic acid fortification that take into consideration other factors that also changed in the United States in recent years is needed.

There are several limitations to our study. First, because of the limited number of deaths for each specific type of cancer, we primarily had to confine our analyses to all-cancer mortality. This would have implications if folate exposure is differentially important in the etiology of or survival from different cancers (24); it seems likely, however, that this would have tended to attenuate our estimated associations. Second, folate exposure was not randomly assigned, and was based on a single measurement of serum folate concentration at baseline. Serum folate concentrations are subject to

short-term fluctuations, and a single measurement might not reflect average, long-term levels. Third, the dietary information in NHANES III was derived from a single 24-hour dietary recall. Nutrient intake estimates derived from a single 24-hour recall may not reflect an individual's usual intake, and it is likely that adjustment for potential confounding by dietary covariates in our models was done with some degree of unmeasurable error. Fourth, because the mean age of our cohort at baseline (44 years) was younger than that in most other cohort studies of adult cancer (8, 10, 20, 21), one would expect fewer cancer deaths for any given follow-up period, especially for cancers that typically have a late age of onset, such as colorectal and prostate cancer. However, a younger cohort such as ours might have had less time to change their diet, thus providing less opportunity for selection bias and competing risks to affect study results (58). Fifth, several studies have suggested that alcohol use may modify the association of folate intake with certain cancers (7, 10), and although our test for a serum folate–all-cancer mortality interaction was not statistically significant (P = 0.16), we had insufficient statistical power to address this issue with confidence. Sixth, the NHANES III Linked Mortality File identified causes of death through the National Death Index, which is based on death certificates which are known to be subject to error in classification of the cause of death. Seventh, categorizing serum folate concentration into quintiles likely reduced the efficiency of our analyses. However, the pattern of risk across the quintiles in our primary analysis was consistent with that seen in our continuous dose-response curve analysis (Fig. 1). Eighth, the NHANES III Linked Mortality File included relatively few cancer deaths; thus the statistical power for analyses restricted to different races/ethnicities was limited. Finally, the last three years of follow-up in the present cohort were after mandatory folic acid fortification began in the United States; therefore, our assessment of folate exposure prior to fortification may not accurately reflect later exposure. Although we conducted several sensitivity analyses to address a possible influence of folic acid fortification on the risk of all-cancer mortality, these analyses could not fully address or rule out such an influence, if any.

On the other hand, our study also has several major strengths. These include the availability of serum folate measurements from a cohort of a nationally representative sample of the U.S. adult population, investigation of a large number of potential confounding variables, and ascertainment of cancer mortality over a reasonable length of time (a median of 8.6 years follow-up).

Based on this discussion, there seem to be several issues regarding folate and cancer that need to be addressed in future epidemiologic studies, including the need for large cohorts with substantial heterogeneity of folate intakes and large numbers of specific cancer cases (both incident cancers and cancer deaths); more time-integrative biomarkers of folate status (e.g., red blood cell folate) taken multiple times over several years; careful consideration of folate sources; valid assessment of other dietary variables and alcohol use; genetic variation; and timing of beginning (or ending) folic acid supplementation over the lifespan. It will also be important to consider that there may be an optimal range of folate intake for good health. Although folate deficiency may suppress cancer, there are other known adverse health consequences ranging from classical deficiency disorders to neural tube defects. Although large, supraphysiologic doses of folate, perhaps especially of folic acid rather than naturally occurring folate from foods, may not cause acute toxicities, they may increase long-term risk for chronic disease, such as cancer. This latter concern needs further investigation.

In summary, our findings suggest that there may be a nonlinear relationship between folate status and risk of all-cancer mortality such that persons with low, but not classically deficient, serum folate concentrations may be at increased risk. Further study is needed to determine whether these finding are due to chance or not, and if not, to clarify their biological basis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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