

Plasma Folate, Vitamin B₆, Vitamin B₁₂, and Homocysteine and Pancreatic Cancer Risk in Four Large Cohorts

Eva Schernhammer,^{1,7,8} Brian Wolpin,³ Nader Rifai,⁴ Barbara Cochrane,⁹ Jo Ann Manson,^{1,2,5} Jing Ma,^{1,5} Ed Giovannucci,^{1,5,6} Cynthia Thomson,^{10,11} Meir J. Stampfer,^{1,5,6} and Charles Fuchs^{1,2,3}

¹Channing Laboratory, Department of Medicine, and ²Division of Preventive Medicine, Brigham and Women's Hospital and Harvard Medical School; ³Department of Adult Oncology, Dana-Farber Cancer Institute; ⁴Department of Laboratory Medicine, Boston Children's Hospital, Harvard Medical School; Departments of ⁵Epidemiology and ⁶Nutrition, Harvard School of Public Health, Boston, Massachusetts; ⁷Ludwig Boltzmann-Institute for Applied Cancer Research, KFJ-Spital; ⁸Applied Cancer Research, Institute for Translational Research Vienna (ACR-ITR VIENNA), Vienna, Austria; ⁹University of Washington School of Nursing, Seattle, Washington; ¹⁰Department of Nutrition, College of Agriculture and Life Sciences and ¹¹Arizona Cancer Center, University of Arizona, Tucson, Arizona

Abstract

Folate deficiency induces DNA breaks and may alter cellular capacity for mutation and epigenetic methylation. Few studies have examined the influence of one-carbon nutrients on pancreatic cancer risk, although recent studies suggest a potential protective effect for one-carbon nutrients from food sources, but not from supplements. We conducted a prospective nested case-control study to examine plasma concentrations of folate, vitamin B₆ [whose main circulating form is pyridoxal-5'-phosphate (PLP)], vitamin B₁₂, and homocysteine in relationship to pancreatic cancer, using four large prospective cohorts. Multivariable adjusted odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using conditional logistic regression. All statistical tests were two sided. Among 208 cases and 623 controls, we observed no association between folate, PLP, vitamin B₁₂, or homocysteine and pancreatic cancer risk. Comparing the highest to lowest quartiles of plasma concentration, the ORs were 1.20 (95% CI, 0.76–1.91) for folate, 0.80 (95% CI, 0.51–1.25) for B₆, 0.91 (95% CI, 0.57–1.46) for B₁₂, and 1.43 (95% CI, 0.90–2.28) for homocysteine. In analyses restricted to nonusers of multivitamins, we observe a modest inverse trend between folate, PLP, and B₁₂ and pancreatic cancer risk. In contrast, no such inverse associations were observed among study subjects who reported multivitamin supplement use. Among all participants, plasma levels of folate, B₆, B₁₂, and homocysteine were not associated with a significant reduction in the risk of pancreatic cancer. Among participants who obtain these factors exclusively through dietary sources, there may be an inverse relation between circulating folate, B₆, and B₁₂ and risk. [Cancer Res 2007;67(11):5553–60]

Introduction

Pancreatic cancer is the fourth leading cause of cancer death, causing more than 30,000 deaths in the United States annually (1). Relatively little is known about the pathogenesis and epidemiology of this malignancy other than cigarette smoking is associated with an increased risk (2, 3). Recently, positive associations between obesity and pancreatic cancer have been observed in several large

prospective studies (4–8). However, the relationship between nutrient status and pancreatic cancer is less defined, particularly for nutrients which are known to play a role in DNA methylation.

DNA methylation and DNA synthesis are largely dependent on the availability of one-carbon, methyl-donating nutrients, and deficiencies in nutrients such as folate or vitamin B₆ and vitamin B₁₂ may increase the probability of gene mutations and DNA double strand breaks (9). A few prospective observational studies have examined the associations between one-carbon nutrients and risk of pancreatic cancer. In the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Study cohort of Finnish men, both plasma folate (10) and dietary folate (11) were inversely associated with pancreatic cancer risk, but not among those taking supplemental folic acid (11). However, because the ATBC cohort consisted of male smokers with a high proportion of participants with deficient folate status (i.e., <3 ng/mL; ref. 12), the generalizability of these findings to other populations has been questioned. In two large prospective cohort studies conducted in the United States, total folate intake was not associated with pancreatic cancer risk, although when confined to folate from food sources only, an inverse trend was seen in both cohorts; in contrast, multivitamin supplement users in these two cohorts seemed to have a nonsignificant, although somewhat higher, risk of pancreatic cancer (13). Most recent evidence from a population-based cohort study in Europe lends further support to the notion that an increased intake of folate from food sources, but not from supplements, may be associated with a lower risk of pancreatic cancer (14).

To further assess the influence of one-carbon nutrients on pancreatic cancer risk, we examined the relation of plasma folate, vitamin B₆, vitamin B₁₂, and homocysteine to pancreatic cancer in the largest study to date, combining four prospective cohort studies of women and men, participating in the Nurses' Health Study (NHS), the Health Professionals Follow-up Study (HPFS), Physicians' Health Study (PHS), and Women's Health Initiative (WHI). Pooling of samples from these four prospective cohorts allows for a more rigorous examination of plasma micronutrients while minimizing the potential biases that are inherent in retrospective studies of pancreatic cancer epidemiology.

Materials and Methods

Study subjects. The Nurses' Health Study (NHS) is an ongoing prospective study of 121,701 U.S. female registered nurses. Details of the design and follow-up of this cohort have previously been described (15). Briefly, at enrollment in 1976, the participants, who were 30 to 55 years old, completed a mailed questionnaire providing information on risk factors for

Requests for reprints: Eva S. Schernhammer, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115. Phone: 617-525-4648; Fax: 617-525-2008; E-mail: eva.schernhammer@channing.harvard.edu.

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cancer and cardiovascular disease. Biennially, updated exposure and disease information is collected by mail. From 1989 to 1990, blood samples were collected from 32,826 of the NHS participants.

The Health Professionals Follow-up Study (HPFS) began in 1986 when 51,529 U.S. male dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians, ages 40 to 75 years, responded to a mailed questionnaire (16). These men provided baseline information on age, marital status, height and weight, ancestry, medications, smoking history, medical history, physical activity, and diet. Exposure and medical history information is updated every 2 years. Blood samples were collected between 1993 and 1994 from 18,025 participants.

The Physicians' Health Study (PHS) was a randomized, clinical trial of aspirin and β -carotene among 22,071 predominantly Caucasian-American male physicians, 40 to 84 years of age. Blood samples were collected at baseline, in 1982, from 14,916 (68%) of the physicians. The men were subsequently followed for incident cancer through annual mailed questionnaires.

The Women's Health Initiative (WHI) Observational Study enrolled 93,676 postmenopausal women ages 50 to 79 at baseline. Recruitment was conducted from 1994 to 1998 and the health of these participants was followed for an average of 8 years (range, 8–12 years) via periodic health forms and a clinic visit 3 years after enrollment. Blood was collected at the first screening visit from >95% of women.

Identification of case and control participants. Among participants who provided a baseline blood sample, we requested medical records from all those who reported an incident diagnosis of pancreatic cancer through 2004 in any of the four cohorts. Histopathologic reports were reviewed by a study investigator to confirm self-reported diagnoses of pancreatic cancer. We excluded pancreatic cancer cases with a prior history of malignancy (other than nonmelanoma skin cancer). Eligible controls supplied a blood sample and had no cancer diagnosis at the time the matched case was diagnosed. We chose at random three controls matched to each case on year of birth, cohort membership (WHI, NHS, HPFS, or PHS), smoking status (current, past, or never), fasting status at time of blood draw, and month of blood draw.

Among eligible women in the NHS, we confirmed 51 cases of pancreatic cancer diagnosed after blood collection through June 1, 2004 and 153 women who were free from cancer at the time of case assessment. Subsequently, one control was identified as a case in the NHS so that the final NHS set included 51 cases and 152 controls. Within the HPFS, we identified 38 cases and 114 matched controls. In the PHS, we confirmed 54 cases of pancreatic cancer and 162 controls, and within WHI, we confirmed 104 cases of pancreatic cancer and 312 women free from diagnosed cancer at the time of case assessment as matched controls. Thus, a total of 247 case patients and 740 control subjects were included in this pooled analysis.

Laboratory assays. In each of the four cohorts, venous blood samples were drawn into EDTA tubes and shipped to our laboratories within 24 h on chill packs. On arrival, samples were separated into plasma, buffy coat, and RBC and stored in liquid nitrogen. Assays for total folate, PLP, vitamin B₁₂, and homocysteine were conducted by Dr. Nader Rifai (Children's Hospital, Boston, MA); PLP assays were conducted at ARUP laboratories (Salt Lake City, UT). Folate and vitamin B₁₂ were measured by a quantitative sandwich enzyme immunoassay technique on a 2010 Elecsys auto-immunoanalyzer (Roche Diagnostics). Because PLP is the principle biologically active form of vitamin B₆ (17), we used plasma PLP to determine vitamin B₆ levels. PLP concentrations were determined using the Vitamin B₆ radioenzymatic assay (American Laboratory Products Co. Ltd.), which measures pyridoxal-5-phosphate (PLP). The concentration of total homocysteine was determined by an enzymatic assay on a Hitachi 917 analyzer (Roche Diagnostics), using reagents and calibrators from Catch, Inc.

Blood samples for the cancer cases and controls were handled together, shipped together in the same batch, and assayed in random order in the same analytic run. To assess laboratory precision, each batch included masked replicate plasma samples that were labeled in a manner identical to that for the regular sample. All laboratory personnel were blinded with respect to case or control status. The mean coefficients of variation were

<3.9% for folate, 10.1% for PLP, 7.6% for vitamin B₁₂, and 5.3% for homocysteine.

Statistical analyses. A total of 247 case patients and 740 control subjects were included in this analysis. We identified statistical outliers based on the generalized extreme studentized deviate many-outlier detection approach (18); two participants with improbable PLP and vitamin B₁₂ concentrations as well as seven participants with improbable homocysteine levels were identified as outliers and excluded from analyses that included these analytes. To reduce possible reverse causality bias, our primary analyses excluded all cases diagnosed within the first 2 years after blood collection. Thus, a total of 208 cases and 623 controls were included in our final data set (NHS, 49 cases and 146 controls; HPFS, 32 cases and 96 controls; PHS, 53 cases and 159 controls; WHI, 74 cases and 222 controls). The total numbers of cases and controls for some biomarkers were slightly lower because of missing data resulting from low plasma volume or laboratory error (number of missing values for folate, $n = 11$; PLP, $n = 3$; vitamin B₁₂, $n = 3$; and homocysteine, $n = 2$).

To test for differences in vitamin levels between cases and controls, we used mixed-effects regression models for clustered data to adjust for possible confounding due to the matching factors and for any residual correlation between cases and controls within the matched set (19). We compared the geometric means of plasma biomarkers of cases and controls using paired t tests. For other continuous variables, we used the Wilcoxon signed-rank test to evaluate differences. For categorical variables, we used a χ^2 test to compare cases and controls. Quartiles of vitamin levels were defined cohort specific on the basis of plasma levels of all controls for the overall analyses. Partial Pearson correlations among control subjects, adjusted for age and cohort, were used to evaluate the associations between the plasma biomarkers and age and body mass index (BMI). To estimate the odds ratios (OR) and 95% confidence intervals (95% CI), we used conditional logistic regression models, adjusting for the matching factors. In multivariate analyses, we additionally adjusted for other pancreatic cancer risk factors as well as gender and cohort. Factors we included were physical activity (NHS and HPFS, metabolic equivalents per week in quartiles; WHI and PHS, number of episodes "exercise to sweat" per week in four categories: none, some, 2–3 times per week, 4+ times per week), BMI (kg/m²), aspirin use (yes/no), energy intake (kcal), and a history of diabetes (yes/no). In addition, current multivitamin intake at blood draw was assessed in all four cohorts (yes/no). We also examined whether the ORs changed after further adjustment for parity and intakes of calcium and vitamin D. In stratified analyses, unconditional logistic regression was used, and these models were also adjusted for age (in 5-year age groups: <50, 50–54, 55–59, 60–64, 65–69, 70–75, ≥ 75 years) and date of blood draw. We used the cohort-specific medians of the categories of the plasma biomarkers in the controls in models (continuous variable) to test for linear trend by calculating the Wald statistics. All P values are two sided. To test for heterogeneity between the four cohorts, we assessed Cochran's Q (20). All statistical analyses were done using the SAS 9.1 statistical package (SAS Institute, Cary, NC). $P < 0.05$ was considered statistically significant.

Results

To minimize reverse causation bias, we restricted our main analyses to cases diagnosed 2 or more years after blood collection. Table 1 shows baseline characteristics of these 208 cases and 623 controls. The median time between blood collection and diagnosis was 66 months (range, 24–250 months). Cases had a higher BMI and were slightly more likely to use multivitamins when compared with controls. Neither folate, PLP, vitamin B₁₂, nor homocysteine levels varied significantly between cases and controls in the combined cohorts. As expected, folate, PLP, and vitamin B₁₂ were positively correlated with each other (Pearson partial correlation coefficients between 0.33 and 0.43, all P values <0.001) given the commonality in food sources of these nutrients (particularly fortified cereal products), whereas they were inversely correlated

Table 1. Baseline characteristics and plasma concentration of one-carbon nutrients(A) Baseline characteristics and plasma concentrations of folate, vitamin B₆, vitamin B₁₂, and homocysteine by case or control status

	Case	Control	<i>P</i> _{difference} *
Characteristics [†]			
<i>N</i>	208	623	
Age (y)	62.2 (8.3)	61.8 (8.4)	Matched
Current smoker (%)	18.8	17.2	Matched
BMI (kg/m ²)	26.6 (4.8)	26.1 (5.3)	0.05
History of diabetes (%)	6.7	4.0	0.27
Parity [‡]	2.8 (1.4)	2.9 (1.4)	0.15
Multivitamin use (%)	42.8	36.8	0.12
Aspirin use (%)	37.0	38.5	0.70
Alcohol intake (g/d)	9.7 (13.9)	9.3 (12.4)	0.91
Total energy intake (kcal) [§]	1,675 (555)	1,739 (619)	0.35
Total folate intake (μg/d) [§]	586 (292)	551 (288)	0.18
Folate from food only (μg/d) [§]	290 (128)	289 (128)	0.95
Total B ₆ intake (mg/d) [§]	9.0 (24.8)	10.3 (37.6)	0.62
B ₆ from food only (mg/d) [§]	1.8 (0.8)	1.9 (0.8)	0.51
Total B ₁₂ intake (μg/d) [§]	14.8 (42.3)	16.4 (45.0)	0.92
B ₁₂ from food only (μg/d) [§]	6.5 (3.6)	7.1 (4.5)	0.20
Plasma concentrations			
Folate (ng/mL)	7.1 (6.6–7.7)	7.0 (6.7–7.3)	0.59
Vitamin B ₆ (pmol/mL)	12.7 (11.2–14.3)	12.7 (11.9–13.6)	0.95
Vitamin B ₁₂ (pg/mL)	520 (490–551)	523 (504–542)	0.87
Homocysteine (nmol/mL)	10.6 (10.2–11.1)	10.6 (10.3–10.8)	0.79
Plasma concentrations among nonusers of multivitamins			
Folate (ng/mL)	5.7 (5.2–6.1)	6.1 (5.8–6.4)	0.10
Vitamin B ₆ (pmol/mL)	9.4 (8.1–10.8)	10.6 (9.8–11.4)	0.13
Vitamin B ₁₂ (pg/mL)	467 (432–505)	488 (467–510)	0.33
Homocysteine (nmol/mL)	11.1 (10.6–11.7)	10.8 (10.5–11.2)	0.30

(B) Cohort-specific plasma concentrations of folate, vitamin B₆, vitamin B₁₂, and homocysteine (controls only)

Plasma concentrations*	NHS (<i>n</i> = 146)**	HPFS (<i>n</i> = 96)**	PHS (<i>n</i> = 171)**	WHI (<i>n</i> = 222)**
Folate (ng/mL)	7.8 (7.1–8.5)	6.5 (5.8–7.2)	4.5 (4.2–4.8)	9.1 (8.5–9.7)
Vitamin B ₆ (pmol/mL)	10.8 (9.3–12.6)	15.8 (13.5–18.7)	12.5 (11.2–13.9)	12.9 (11.5–14.5)
Vitamin B ₁₂ (pg/mL)	520 (480–564)	564 (516–617)	427 (401–455)	585 (551–621)
Homocysteine (nmol/mL)	11.6 (11.0–12.2)	13.3 (12.5–14.2)	10.7 (10.2–11.3)	8.9 (8.4–9.3)

*Data are presented as mean, with SD given in parentheses.

†*P* values for comparison of mean natural methyl biomarker plasma levels between cases and controls based on mixed-effects regression models with adjustment for the matching variables (age, smoking status) and cohort/gender.

‡NHS and WHI; number of term pregnancies among parous women only.

§Not available for PHS cohort.

||Data are presented as geometric mean (95% CI).

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**Numbers vary slightly by assay because of missing values.

with homocysteine. After the exclusion of multivitamin users, these correlations remained similar (data not shown).

Within each cohort individually, none of the evaluated nutrient biomarkers was associated with pancreatic cancer risk, and this lack of association was consistent across cohorts (*Q*-statistic test for heterogeneity: folate, *P* = 0.85; PLP, *P* = 0.62; vitamin B₁₂, *P* = 0.93; homocysteine, *P* = 0.25). We therefore combined the cohorts for all subsequent analyses.

Circulating levels of folate, PLP, vitamin B₁₂, and homocysteine were not associated with pancreatic cancer risk; moreover,

neither stepwise (data not shown) nor complete mutual adjustment for these vitamin levels as well as for known or suspected pancreatic cancer risk factors, including BMI and physical activity, altered these estimates (Table 2). The risks remained virtually unchanged after the exclusion of cases that occurred within 4 years after blood collection and changed only slightly when we restricted our analyses to the 148 cases that occurred before 1998. Specifically, simple ORs (top versus bottom category) modeling only cases that occurred before 1998 (i.e., before folate fortification) were 0.90 for B₆ (95% CI, 0.52–1.53);

0.80 for B₁₂ (95% CI, 0.44–1.47); 1.01 for folate (95% CI, 0.58–1.57); and 1.77 for homocysteine (95% CI, 0.99–3.15). This is consistent with no benefit for folate both overall and in prefortification cases but suggests a perhaps somewhat stronger effect of hyper-

homocysteinuria in the prefortification era. The multivariate OR for folate (top versus bottom category) was 1.04 (95% CI, 0.43–1.94) in the WHI cohort, and 1.41 (95% CI, 0.79–2.53) in the other three cohorts combined.

Table 2. OR of pancreatic cancer by plasma quartiles of folate, vitamin B₆, vitamin B₁₂, and homocysteine in four prospective cohorts combined

Plasma one-carbon nutrient	Q1	Q2	Q3	Q4	<i>P</i> _{trend}
<i>Main model</i>					
Folate (ng/mL)*					
Cases/controls †	53/165	47/168	47/124	59/157	
Simple model	1.0	0.90 (0.57–1.43)	1.23 (0.76–2.00)	1.20 (0.76–1.91)	0.30
Multivariable model	1.0	0.89 (0.56–1.42)	1.26 (0.77–2.07)	1.22 (0.77–1.95)	0.28
Mutual adjustment	1.0	0.85 (0.53–1.37)	1.20 (0.71–2.04)	1.34 (0.79–2.26)	0.11
Vitamin B ₆ (pmol/mL)*					
Cases/controls †	58/154	41/132	59/172	49/162	
Simple model	1.0	0.82 (0.51–1.31)	0.93 (0.61–1.42)	0.80 (0.51–1.25)	0.25
Multivariable model	1.0	0.88 (0.55–1.42)	1.01 (0.66–1.55)	0.87 (0.55–1.37)	0.37
Mutual adjustment	1.0	0.81 (0.49–1.33)	0.93 (0.58–1.47)	0.83 (0.49–1.39)	0.29
Vitamin B ₁₂ (pg/mL)*					
Cases/controls †	47/154	55/155	64/156	42/155	
Simple model	1.0	1.16 (0.74–1.81)	1.34 (0.87–2.07)	0.91 (0.57–1.46)	0.71
Multivariable model	1.0	1.17 (0.74–1.83)	1.42 (0.92–2.21)	0.93 (0.58–1.49)	0.79
Mutual adjustment	1.0	1.23 (0.76–1.99)	1.40 (0.87–2.28)	0.86 (0.50–1.46)	0.68
Homocysteine (μmol/L)*					
Cases/controls †	40/169	55/132	52/138	60/182	
Simple model	1.0	1.73 (1.09–2.74)	1.61 (1.00–2.61)	1.43 (0.90–2.28)	0.24
Multivariable model	1.0	1.63 (1.02–2.62)	1.54 (0.94–2.52)	1.37 (0.85–2.20)	0.35
Mutual adjustment	1.0	1.73 (1.05–2.84)	1.57 (0.94–2.61)	1.48 (0.89–2.48)	0.38
<i>Excluding multivitamin users ‡</i>					
Folate (ng/mL)*					
Cases/controls †	20/52	32/102	27/108	39/130	
Simple model	1.0	0.58 (0.26–1.29)	0.45 (0.19–1.03)	0.55 (0.24–1.25)	0.19
Multivariable model	1.0	0.56 (0.24–1.32)	0.46 (0.19–1.12)	0.54 (0.23–1.30)	0.21
Mutual adjustment	1.0	0.58 (0.24–1.43)	0.48 (0.18–1.22)	0.63 (0.24–1.62)	0.34
Vitamin B ₆ (pmol/mL)*					
Cases/controls †	36/82	28/94	31/114	23/104	
Simple model	1.0	0.58 (0.30–1.15)	0.59 (0.31–1.10)	0.47 (0.24–0.92)	0.08
Multivariable model	1.0	0.65 (0.32–1.32)	0.69 (0.36–1.32)	0.51 (0.25–1.02)	0.15
Mutual adjustment	1.0	0.78 (0.37–1.65)	0.74 (0.37–1.48)	0.60 (0.27–3.24)	0.43
Vitamin B ₁₂ (pg/mL)*					
Cases/controls †	31/97	37/96	27/99	24/102	
Simple model	1.0	1.47 (0.78–2.76)	1.00 (0.52–1.93)	0.80 (0.41–1.54)	0.27
Multivariable model	1.0	1.34 (0.69–2.60)	1.01 (0.51–2.02)	0.81 (0.41–1.59)	0.35
Mutual adjustment	1.0	1.58 (0.77–3.24)	1.23 (0.58–2.57)	1.04 (0.49–2.19)	0.64
Homocysteine (μmol/L)*					
Cases/controls †	16/67	29/105	32/97	42/126	
Simple model	1.0	1.24 (0.60–2.57)	1.58 (0.72–3.46)	1.42 (0.68–2.94)	0.47
Multivariable model	1.0	1.21 (0.57–2.57)	1.59 (0.71–3.55)	1.33 (0.63–2.82)	0.57
Mutual adjustment	1.0	1.20 (0.54–2.64)	1.62 (0.70–3.76)	1.32 (0.59–2.93)	0.84

NOTE: Quartiles/test for trend (created based on median per quartile) based on controls only; Q1 denotes lowest quartile.

*Analyses based on simple conditional logistic regression models adjusting for the matching factors [year of birth, smoking status (current, past, or never), fasting status, month of blood draw] and cohort (i.e., thus also for gender); multivariable models are additionally adjusted for BMI, physical activity, and a history of diabetes; models labeled “mutual adjustment” are further adjusted for the other three biochemical indicators of methyl-group availability.

†Numbers vary slightly by assay because of missing values and different quartile distributions.

‡Quartiles are based on controls without any multivitamin use only and calculated as cohort specific; thus, ranges of quartiles are not provided because they overlap in analyses combining all four cohorts.

Table 3. OR of pancreatic cancer (stratified by BMI and smoking) by plasma concentrations of folate, vitamin B₆, vitamin B₁₂, and homocysteine in quartiles among 208 cases and 623 controls (including multivitamin users)

Plasma one-carbon nutrient	All four cohorts combined									
	Q1	Q2	Q3	Q4	<i>P</i> _{trend}	Q1	Q2	Q3	Q4	<i>P</i> _{trend}
	<i>Lower BMI (below the median, <24.7)</i>					<i>Higher BMI (above the median, ≥24.7)</i>				
Folate										
Cases/controls	23/81	23/78	18/76	30/81		30/84	24/90	29/48	29/76	
Multivariable OR	1.0	1.12 (0.57–2.22)	0.83 (0.41–1.71)	1.36 (0.70–2.62)	0.36	1.0	0.81 (0.43–1.52)	1.96 (1.02–3.77)	1.06 (0.57–1.96)	0.60
Vitamin B₆										
Cases/controls	27/71	21/61	27/97	20/91		31/83	20/71	32/75	29/71	
Multivariable OR	1.0	0.89 (0.44–1.83)	0.73 (0.38–1.39)	0.56 (0.28–1.12)	0.03	1.0	0.78 (0.40–1.52)	1.16 (0.63–2.12)	1.13 (0.60–2.11)	0.50
Vitamin B₁₂										
Cases/controls	22/75	27/77	29/82	17/85		25/79	28/78	35/74	25/70	
Multivariable OR	1.0	1.25 (0.65–2.41)	1.27 (0.66–2.43)	0.66 (0.32–1.36)	0.25	1.0	1.17 (0.61–2.23)	1.59 (0.85–2.97)	1.17 (0.60–2.28)	0.60
Homocysteine										
Cases/controls	19/99	27/70	21/66	27/84		21/70	28/62	31/72	33/98	
Multivariable OR	1.0	2.00 (1.02–3.94)	1.71 (0.84–3.49)	1.61 (0.81–3.21)	0.29	1.0	1.57 (0.78–3.15)	1.63 (0.83–3.23)	1.07 (0.55–2.06)	0.89
Never smokers										
Folate										
Cases/controls	19/54	23/64	18/50	17/61		34/110	24/103	29/74	42/96	
Multivariable OR	1.0	1.06 (0.51–2.24)	1.06 (0.48–2.33)	0.88 (0.40–1.92)	0.64	1.0	0.78 (0.43–1.42)	1.42 (0.79–2.57)	1.56 (0.90–2.70)	0.05
Vitamin B₆										
Cases/controls	23/46	13/56	23/68	18/63		35/108	28/75	36/103	31/99	
Multivariable OR	1.0	0.51 (0.23–1.15)	0.71 (0.35–1.46)	0.66 (0.31–1.43)	0.46	1.0	1.15 (0.64–2.08)	1.15 (0.67–2.00)	1.01 (0.57–1.77)	0.64
Vitamin B₁₂										
Cases/controls	15/66	23/55	25/53	15/59		32/87	32/100	39/102	27/96	
Multivariable OR	1.0	1.95 (0.92–4.16)	2.26 (1.06–4.83)	1.20 (0.53–2.71)	0.73	1.0	0.86 (0.48–1.54)	1.05 (0.60–1.84)	0.75 (0.41–1.37)	0.42
Homocysteine										
Cases/controls	18/78	22/53	19/41	18/61		22/91	33/79	33/96	42/120	
Multivariable OR	1.0	1.81 (0.87–3.76)	2.07 (0.94–4.56)	1.18 (0.54–2.54)	0.61	1.0	1.81 (0.96–3.42)	1.42 (0.76–2.65)	1.40 (0.77–2.54)	0.57

NOTE: ORs were, in addition to matching variables [year of birth, cohort membership (NHS, HPFS, PHS, WHI), smoking status (current, past, or never), fasting status, and month of blood draw], further adjusted for gender, BMI, physical activity, and a history of diabetes mellitus.

We further repeated our analyses after excluding participants who reported multivitamin supplement use at baseline. In analyses restricted to non-multivitamin users, we observed a modest inverse trend between plasma folate, PLP, and B₁₂ and pancreatic cancer risk, which reached statistical significance for PLP (top versus bottom quartile; OR, 0.47; 95% CI, 0.24–0.92), but was somewhat attenuated after further adjustment for BMI, physical activity, and a history of diabetes (OR, 0.51; 95% CI, 0.25–1.02).

We also examined the association between plasma levels of one-carbon nutrients and pancreatic cancer according to other risk factors for pancreatic cancer (Table 3). Associations with the various plasma factors were not materially modified across categories of age, gender, cohort, energy intake, smoking status, alcohol consumption, or physical activity (data shown in Table 3 for smoking only). For the entire study population, only 4.7% reported a history of diabetes mellitus; when we restricted to nondiabetics, our findings were unchanged. However, among participants who were below the median BMI (<24.7 kg/m²), we did observe a significant inverse trend between plasma PLP and pancreatic cancer risk (OR, 0.56; 95% CI, 0.28–1.12, comparing

highest to lowest quartiles; *P*_{trend} = 0.03), whereas plasma PLP was not associated with risk among participants with BMI ≥ 24.7 kg/m².

In light of prior analyses suggesting that the inverse association of one-carbon nutrients with risk was restricted to non-supplement users (11, 13, 14), we further repeated our stratified analyses after excluding participants who reported multivitamin use at blood collection (Table 4). Among nonusers of multivitamin supplements who were below the median BMI (<24.7 kg/m²), elevated circulating PLP and vitamin B₁₂ seemed to confer a reduced risk of pancreatic cancer, whereas folate did not. Comparing the highest to lowest quartiles of plasma concentrations, the ORs were 0.19 (95% CI, 0.06–0.59; *P*_{trend} = 0.02) for PLP, 0.27 (95% CI, 0.09–0.80; *P*_{trend} = 0.01) for vitamin B₁₂, and 0.41 (95% CI, 0.14–1.17; *P*_{trend} = 0.16) for folate. Similar associations were not apparent among subjects above the median BMI. Moreover, when we restricted our analysis of non-multivitamin users to never smokers, plasma folate was associated with a nonsignificant reduction in the risk of pancreatic cancer (OR, 0.30; 95% CI, 0.08–1.04), whereas no association was seen among past or current smokers.

Table 4. OR of pancreatic cancer among nonusers of multivitamins (stratified by BMI and smoking) by plasma concentrations of folate, vitamin B₆, vitamin B₁₂, and homocysteine in quartiles

Plasma one-carbon nutrient	All four cohorts combined*									
	Q1	Q2	Q3	Q4	P _{trend}	Q1	Q2	Q3	Q4	P _{trend}
	Lower BMI (below the median, <24.7)					Higher BMI (above the median, ≥24.7)				
Folate										
Cases/controls	9/23	12/46	13/56	16/67		11/29	20/56	14/52	23/63	
Multivariable OR	1.0	0.56 (0.19–1.64)	0.48 (0.16–1.38)	0.41 (0.14–1.17)	0.16	1.0	0.92 (0.37–2.28)	0.66 (0.25–1.71)	0.96 (0.39–2.38)	0.50
Vitamin B₆										
Cases/controls	18/41	13/39	14/58	6/55		18/41	15/55	17/56	17/49	
Multivariable OR	1.0	0.68 (0.27–1.71)	0.42 (0.17–1.05)	0.19 (0.06–0.59)	0.02	1.0	0.59 (0.26–1.36)	0.73 (0.32–1.65)	0.89 (0.39–2.04)	0.88
Vitamin B₁₂										
Cases/controls	14/44	15/49	15/48	7/52		17/53	22/47	12/51	17/50	
Multivariable OR	1.0	0.96 (0.39–2.36)	0.73 (0.30–1.79)	0.27 (0.09–0.80)	0.01	1.0	1.38 (0.63–3.02)	0.72 (0.30–1.72)	1.12 (0.49–2.60)	0.98
Homocysteine										
Cases/controls	7/40	14/54	12/42	18/57		9/27	15/51	20/55	24/69	
Multivariable OR	1.0	1.38 (0.46–4.16)	1.59 (0.53–4.79)	1.93 (0.65–5.73)	0.37	1.0	1.01 (0.37–2.74)	1.49 (0.56–4.01)	1.03 (0.39–2.69)	0.98
Never smokers										
Past/current smokers										
Folate										
Cases/controls	8/14	13/43	14/35	11/55		12/37	19/59	13/73	28/75	
Multivariable OR	1.0	0.45 (0.14–1.49)	0.70 (0.21–2.35)	0.30 (0.08–1.04)	0.07	1.0	0.99 (0.41–2.39)	0.57 (0.22–1.43)	1.34 (0.58–3.10)	0.79
Vitamin B₆										
Cases/controls	13/27	14/35	10/43	8/43		23/55	14/58	21/71	15/61	
Multivariable OR	1.0	0.95 (0.36–2.52)	0.57 (0.20–1.59)	0.46 (0.16–1.36)	0.36	1.0	0.53 (0.24–1.19)	0.77 (0.37–1.58)	0.68 (0.31–1.51)	0.45
Vitamin B₁₂										
Cases/controls	12/44	15/37	10/32	9/36		19/53	22/59	17/66	15/66	
Multivariable OR	1.0	1.64 (0.63–4.25)	1.15 (0.42–3.14)	1.00 (0.36–2.78)	0.96	1.0	1.08 (0.51–2.29)	0.73 (0.34–1.58)	0.60 (0.27–1.33)	0.06
Homocysteine										
Cases/controls	9/33	12/44	10/28	15/44		7/34	17/61	22/68	27/82	
Multivariable OR	1.0	0.93 (0.33–2.64)	1.18 (0.39–3.59)	0.97 (0.34–2.72)	0.73	1.0	1.18 (0.42–3.30)	1.45 (0.53–3.93)	1.29 (0.48–3.46)	0.39

NOTE: ORs were, in addition to matching variables [year of birth, cohort membership (NHS, HPFS, PHS, WHI), smoking status (current, past, or never), fasting status, and month of blood draw], further adjusted for gender, BMI, physical activity, and a history of diabetes mellitus.

*Quartiles were based on controls without any multivitamin use only; Q1 denotes lowest quartile.

Finally, we examined the influence of multivitamin use on risk in our study population, independent of plasma nutrient levels. Participants who reported multivitamin use at baseline experienced an OR for pancreatic cancer risk of 1.43 (95% CI, 1.03–1.99). Of note, when we restricted our plasma analyses to participants who reported multivitamin use, the multivariate ORs were 2.39 (top versus bottom quartile; 95% CI, 0.67–8.46) for folate, 1.66 (95% CI, 0.49–5.62) for PLP, 1.00 (95% CI, 0.33–3.02) for vitamin B₁₂, and 1.66 (95% CI, 0.63–4.39) for homocysteine.

Discussion

In this nested case-control study pooling data from four large prospective cohorts, plasma levels of folate, PLP, B₁₂, and homocysteine were not associated with pancreatic cancer risk. However, among participants who did not use multivitamin supplements, there seemed to be an inverse relation between circulating folate, PLP, and B₁₂ and pancreatic cancer risk, particularly among subjects who maintained a normal BMI. We

have previously reported results on dietary intake of these nutrients and pancreatic cancer risk in our cohorts (13), and, therefore, we did not include analyses of dietary intake in the current analysis. However, that analysis of dietary intake was consistent with the current analysis of plasma folate, suggesting that any benefit from folate was limited to dietary sources rather than from supplements.

Evidence for a possible link between folate pathways and pancreatic cancer risk comes from several studies reporting significant associations between methylenetetrahydrofolate reductase genotypes and pancreatic cancer risk (21–23).

Few previous studies have examined the association between plasma levels of one-carbon nutrients and the risk of pancreatic cancer. In a nested case-control study of the ATBC cohort of 29,133 male Finnish smokers, 126 participants who developed pancreatic cancer were matched with 247 controls (10). Serum folate and PLP (vitamin B₆) concentrations showed statistically significant inverse dose-response relationships with pancreatic cancer risk, with the highest serum tertiles having approximately half the risk of the

lowest [folate: OR, 0.45; 95% CI, 0.24–0.82 ($P_{\text{trend}} = 0.009$) and PLP: OR, 0.48; 95% CI, 0.26–0.88 ($P_{\text{trend}} = 0.02$)]. A decreased risk was also noted for the highest tertile of serum homocysteine (OR, 0.65; 95% CI, 0.36–1.18; $P_{\text{trend}} = 0.14$). These results suggest that maintaining adequate folate and pyridoxine status may reduce the risk of pancreatic cancer. Nonetheless, because the cohort consisted exclusively of male smokers, it remains unclear whether the findings are generalizable to the broader population-at-large. In addition, 90% of the ATBC participants had less than adequate plasma folate levels (10), of which 25% were deficient. Supplement use is uncommon in Europe and only 12% of these Finnish men were supplement users. It is conceivable that a demonstrable influence of folate consumption may be restricted to populations that are relatively folate deficient, and our data provide some support for this hypothesis. Although nearly all our blood samples were collected before fortification of flour and cereals with folic acid became mandatory in the United States, starting in January 1998 (24), our participants still had markedly higher plasma folate, PLP, and vitamin B₁₂ levels than ATBC participants. Thus, it is possible that we did not find such a strong association in the present study because our plasma folate levels did not include those in a low enough range.

Prior studies of folate intake and pancreatic cancer risk suggest that foods high in folate have a protective effect whereas folic acid supplements are not protective. In the ATBC Study cohort, dietary folate was inversely associated with pancreatic cancer risk (top versus bottom quintile; RR, 0.52; 95% CI, 0.31–0.87), whereas folic acid supplement users seemed to have a higher risk of pancreatic cancer [risk ratio (RR), 1.60; 95% CI, 0.92–2.77; ref. 11]. Similarly, in the NHS and the HPFS, there was no association between total folate intake and pancreatic cancer risk (top versus bottom quartile; RR, 1.03; 95% CI, 0.74–1.43), yet folate from food sources only was suggestive of an inverse association (top versus bottom quartile; RR, 0.66; 95% CI, 0.42–1.03; $P_{\text{trend}} = 0.12$). Moreover, within these two cohorts, supplement users seemed to have a somewhat higher risk of pancreatic cancer (13). Consistent with these findings, a population-based cohort study in Sweden (14) reported an inverse association between total folate intake and pancreatic cancer risk (top versus bottom quintile; RR, 0.33; 95% CI, 0.15–0.72), whereas there was no association between folate from supplements and pancreatic cancer risk (RR, 1.02; 95% CI, 0.56–1.88). Among the reasons for an inverse association with one-carbon nutrients only among nonusers of multivitamins is that the association between one-carbon nutrients and pancreatic cancer risk may be nonlinear. For folate, it has been discussed that there is potential for disruption of transport mechanisms through folic acid or potential for fostering growth at very high levels as seen in supplement users (25); our finding of an increased pancreatic cancer risk among multivitamin users is in line with this hypothesis. Further, unlike our study which uses plasma folate levels, previous studies using dietary assessments of folate have not been able to take the greater bioavailability of folate from supplements into account and thus may have been hampered by substantial misclassification.

Elevated risks of pancreatic cancer with supplement use have been noted both in European and U.S. cohorts (11, 13, 14), with studies supporting a beneficial effect of one-carbon nutrients when derived from food sources, but not from supplements. Among the proposed hypotheses to explain these surprising findings is a suggestion that food folate levels may be more representative of long-term folate exposure, which might be more relevant to pancreatic tumorigenesis than is recent exposure from multi-

vitamins (13). An alternative dietary factor that is correlated with dietary folate may otherwise account for the inverse association with folate from food sources only. Consistent with previous studies, we, too, noted a suggestion of an increased risk of pancreatic cancer risk with higher folate and PLP levels among the multivitamin users in our cohorts. The suggestion of an increased risk for pancreatic cancer among the multivitamin users requires confirmation; additional studies should further assess the influence of long-term multivitamin supplement use on pancreatic cancer risk.

We found a stronger inverse association for folate, PLP, and vitamin B₁₂ among non-multivitamin users who maintained a healthy weight or were nonsmokers. Such findings are consistent with the inverse relation observed in the ATBC cohort of male smokers, which included few overweight or obese participants (10, 11). This may be related to the fact that body weight overrides methylation status and/or nutrient status in terms of the strength of its relationship to pancreatic cancer risk. However, we cannot rule out the role of chance in these subgroup findings, especially because their mechanism remains unclear.

Our analysis has several limitations of note. Because we used four distinct cohorts for our analyses, covariate assessment was not always done in a comparable fashion, which may have compromised our ability to fully adjust for potential confounding factors. On the other hand, pooling of four cohorts created one of the largest data sets of pancreatic cancer cases to date and allowed us to look at several questions, including stratified analyses, in a more powerful way than previous studies. Although measurement error in the laboratory assays cannot be fully excluded, the relatively low coefficients of variability of our plasma measurements suggest that they were relatively reliable. Moreover, they have successfully been linked to other disease (e.g., colon cancer), indicating that measurement error is not large enough to hide any real associations.

Folate levels may have changed after national folic acid fortification, which began in 1997 and was mandatory by January 1, 1998. All samples used for measuring the biomarkers in our study were drawn markedly before fortification (NHS: between 1989 and 1990; HPFS: between 1993 and 1994; PHS: 1982), with the exception of the WHI cohort, in which blood draws occurred between 1994 and 1998. However, given that full fortification levels would likely only have occurred at or after 1998, even the samples drawn from WHI participants were unlikely to represent “post-fortification values.” Thus, the samples drawn from all cohorts were almost exclusively reflective of pre-fortification folate. When we stratified our data by cohort, results were very similar within each cohort individually. In addition, because the development of pancreatic cancer likely requires some induction period before the onset of a clinically apparent tumor, it is unlikely that the post-fortification folate exposure (which was not assessed by our pre-fortification plasma specimens) would substantially influence pancreatic cancer risk through 2002. Of note, we also assessed plasma vitamin B₆, which would not have been influenced by fortification. Interestingly, the results for B₆ seem to parallel our findings for folate, with the greatest benefit among non-supplement users. Moreover, our findings for plasma folate are consistent with other large prospective studies of folate intake (11, 13, 14), suggesting that any benefit from folate is limited to dietary sources rather than from supplements. Further, as can be seen on Table 1, plasma levels were very comparable between the four cohorts despite the fact the blood samples were drawn at various time periods between 1982

and 1998. We believe that this relative consistency in plasma levels between cohorts reflects the fact that folate fortification after 1998 did not influence plasma levels in our cohorts.

Strengths of our analysis include the relatively large sample size and its prospective design and high follow-up rates, both of which reduce the possibility that bias influenced our results.

In summary, our study does not support a clear association between circulating levels of one-carbon nutrients and the risk of pancreatic cancer. Among participants who achieve their intake of these factors exclusively through dietary sources, there may be an inverse relation between circulating folate, B₆, and B₁₂ and risk, particularly among subjects who maintained a normal BMI; nonetheless, such subset analyses must be viewed cautiously due

to multiple comparisons and smaller sample size within exposure groups. Additional experimental and observational studies are needed to clarify and confirm or refute these associations.

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