American Journal of Epidemiology

Vol. 160, No. 3
Printed in U.S.A.
DOI: 10.1093/aje/kwh214

A Prospective Study of Folate Intake and the Risk of Pancreatic Cancer in Men and Women

Halcyon G. Skinner[1], Dominique S. Michaud[2], Edward L. Giovannucci[2,3,4], Eric B. Rimm[2,3,4], Meir J. Stampfer[2,3,4], Walter C. Willett[2,3,4], Graham A. Colditz[2,3,4], and Charles S. Fuchs[3,5]

[1] Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL.
[2] Department of Epidemiology, Harvard School of Public Health, Boston, MA.
[3] Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.
[4] Department of Nutrition, Harvard School of Public Health, Boston, MA.
[5] Department of Adult Oncology, Dana-Farber Cancer Institute, Boston, MA.

Received for publication May 16, 2003; accepted for publication March 2, 2004.

Laboratory and human studies suggest that folate intake may influence the risk of some cancers. However, prospective information about the relation between folate intake and the risk of exocrine pancreatic cancer is limited. The authors examined the relation of dietary folate intake to the risk of pancreatic cancer in two large prospective US cohorts. Folate intake was assessed by food frequency questionnaire in 1984 in women and in 1986 in men. Multivariate relative risks were adjusted for age, energy intake, cigarette smoking, body mass index, diabetes, and height. During 14 years' follow-up in each cohort, 326 incident cases of pancreatic cancer were identified. Compared with participants in the lowest category of folate intake, participants in increasing 100-μg categories of total energy-adjusted folate intake had pooled multivariate relative risks for pancreatic cancer of 1.08, 1.10, and 1.03 (95% confidence interval: 0.74, 1.43; p_{trend} = 0.99). For energy-adjusted folate from food, the pooled relative risks for increasing 100-μg categories of intake were 0.81, 0.89, and 0.66 (95% confidence interval: 0.42, 1.03; p_{trend} = 0.12). There was no statistical interaction between folate intake and methionine, alcohol, fat, or caffeine. The results from these two large prospective cohorts do not support a strong association between energy-adjusted folate intake and the risk of pancreatic cancer.

adult; cohort studies; folic acid; human; nutrition assessment; pancreatic neoplasms; prospective studies

Abbreviations: ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CI, confidence interval; SE, standard error.

With over 31,000 deaths anticipated in 2004, cancer of the exocrine pancreas ranks as the fourth leading cause of cancer-related mortality in the United States (1). Overall median survival following a diagnosis of pancreatic ductal adenocarcinoma is less than 5 months, and only about 4 percent of cases survive 5 years (1, 2). Although malignant neoplasms can arise from either the endocrine or exocrine portion of the pancreas, exocrine malignancies predominated by ductal adenocarcinoma comprise more than 95 percent of all pancreatic cancers (3). Throughout this article, "pancreatic cancer" refers to malignancies of the exocrine pancreas.

Prospective epidemiologic studies are hampered by the relatively low incidence rates for cancers of the pancreas. Furthermore, the rapid mortality and high case fatality rate of the disease limit opportunities for retrospective studies of risks factors. Thus, little is known about the etiology of pancreatic cancer. Cigarette smoking is the only consistently identified modifiable risk factor for pancreatic cancer. However, the relative risk for current cigarette smokers is approximately 2.5, and only about 25 percent of cases in the United States are attributable to smoking cigarettes (4). Therefore, much of the variability in the incidence of pancreatic cancer must be related to other factors.

Folate, or folic acid, is an important dietary methyl-group donor involved in both nucleotide synthesis and DNA methylation. Folate deficiency can lead to inadequate conversion of uracil to thymidine with subsequent misincorporation of

Correspondence to Dr. Halcyon G. Skinner, Department of Preventive Medicine, 680 North Lake Shore Drive, Suite 1102, Chicago, IL 60611 (e-mail: hskinner@northwestern.edu).

uracil into DNA leading to chromosomal instability (5–8). Additionally, methyl-group availability from folate may influence the risk of cancer through global hypomethylation of DNA, leading to genomic instability and increased mutation rates (9, 10), or through hypermethylation of tumor suppressor genes. Pancreatic tumors exhibit a number of molecular-genetic alterations (11–13) and aberrant patterns of gene methylation (14, 15). Thus, variability in the availability of folate-derived methyl groups may plausibly influence the risk of pancreatic cancer through altered cellular capacity for mutation or epigenetic methylation.

In previous epidemiologic studies, increased folate consumption has been associated with a decreased risk of colon cancer in men (16) and in women (17) and among women with a positive family history of colon cancer in particular (18). Furthermore, among women who consume 15 or more g of alcohol per day, breast cancer risk appears to be lower among those with higher folate consumption (19) and plasma folate levels (20). In a case-control analysis nested within a cohort of male Finnish smokers, compared with those in the bottom tertile of serum folate, participants in increasing tertiles of serum folate had odds ratios for pancreatic cancer of 0.74 and 0.45 (21). Moreover, in a longitudinal analysis of the same cohort, higher dietary folate intake was associated with a lower risk of pancreatic cancer (22). In other case-control studies, both an inverse association between folate intake and pancreatic cancer (23) and no association have been observed (24).

The authors examined the relation of dietary folate to the risk of pancreatic cancer in two large prospective cohorts of women and men with detailed dietary information and up to 14 years of follow-up, the Nurses' Health Study and the Health Professionals Follow-up Study.

MATERIALS AND METHODS

Description of the cohorts

The Nurses' Health Study is an ongoing cohort study established in 1976, with 121,701 responses to a mailed questionnaire from married registered nurses in the United States who were aged 30–55 years. Detailed information on individual characteristics and behaviors was obtained from questionnaires at baseline and biennially thereafter. Dietary information was first assessed in the Nurses' Health Study in 1980 (25, 26). However, to maximize consistency with the Health Professionals Follow-up Study cohort, these analyses use the more detailed 1984 food frequency questionnaire, the baseline dietary measure. After exclusions for cancer prior to 1984 (except nonmelanoma skin cancer) and missing dietary information, 77,640 women were eligible for analysis at the 1984 baseline.

The Health Professionals Follow-up Study began in 1986 with 51,529 responses from male health professionals to a mailed questionnaire. The participants are US dentists, veterinarians, pharmacists, optometrists, osteopathic physicians, and podiatrists who were between the ages of 40 and 75 years at the beginning of the study. Detailed information on individual characteristics and behaviors was obtained from the questionnaires at baseline and biennially thereafter. After exclusions for cancer prior to 1986 (except nonmelanoma skin cancer) and missing dietary information, 47,840 men were eligible for analysis in 1986.

Dietary assessment

Baseline diet was assessed in 1984 for the Nurses' Health Study and in 1986 for the Health Professionals Follow-up Study using a 131-item semiquantitative food frequency questionnaire, described in detail elsewhere (27, 28). Participants were presented with a list of foods, each with a commonly used portion or serving size. Participants were asked how often, on average, they had consumed the specified amount of each food, with nine categories from which to choose. The questionnaire also asked for information on the brand of multivitamin typically used, as well as the brand and type of breakfast cereal used. Participants who were current multivitamin users were asked to state how many years they had been taking multivitamins. Nutrient intakes were computed by multiplying the consumption frequency of each unit of every food by the nutrient content of the portion specified. Values for the nutrient amounts in foods were obtained from the Harvard University Food Composition Database, derived from US Department of Agriculture sources (29). All nutrient values were adjusted for total energy intake by the residuals method (30). The validity of the nutrient consumption measured by the food frequency questionnaires was evaluated in subsamples of cohort participants residing in the Boston, Massachusetts, area who completed detailed 1-week diet records (27, 31). The Pearson correlation coefficient between the questionnaire estimates and the dietary record estimates of total energy-adjusted folate was $r = 0.73$. Furthermore, in the Health Professionals Follow-up Study of diet record validation, the correlation between folate calculated from the semiquantitative food frequency questionnaire and red cell folate level was 0.56 (32). Among women in the 1986 Nurses' Health Study of diet validation, the correlation between folate calculated from the semiquantitative food frequency questionnaire and red cell folate level was 0.55 (33). The mean erythrocyte folate levels of the mean by quintile of total folate intake from lowest to highest quintile were 301 (standard error (SE), 15), 341 (SE, 10), 355 (SE, 11), 355 (SE, 11), and 406 (SE, 21) ng/ml, respectively. The reproducibility and validity of self-reported alcohol consumption were assessed in both cohorts by comparing the responses of four 1-week dietary records with the answers provided on the semiquantitative food frequency questionnaire (34). The correlation between the two measures was high (Spearman's $r = 0.90$ in women and 0.86 in men).

Pancreatic cancer case and death ascertainment

In both cohorts, participants were asked to report specific medical conditions including cancers that had been diagnosed in the 2-year period prior to each follow-up questionnaire. Whenever a participant (or next of kin for decedents) reported a diagnosis of pancreatic cancer, permission was sought to obtain related medical records, including pathology reports. If permission to obtain records was

denied, an attempt was made to confirm the self-reported cancer with an additional letter or telephone call to the participant. If the primary cause of death listed on a death certificate was a previously unreported pancreatic cancer case, a family member was contacted (subject to state regulations) to obtain permission to retrieve medical records or at least to confirm the diagnosis of pancreatic cancer. Most deaths in these cohorts were reported by family members or by the postal service in response to the follow-up questionnaires. Additionally, searches of the National Death Index for nonrespondents were conducted, resulting in a sensitivity of about 98 percent in identifying decedents (35). We were able to obtain pathology reports confirming the diagnosis of pancreatic cancer for greater than 90 percent of cases. For the remaining cases, we obtained confirmation of the self-reported cancer from a secondary source (e.g., death certificate, physician, or telephone interview of a family member). All medical records had complete information on cytohistology (hospitals were recontacted if the original information sent was incomplete). All associations were initially examined both including and excluding cases with missing records. Because no material differences were observed between these two types of cases, we included those cases without medical records in the final analyses. Following the exclusion of participants with prior cancers or missing dietary information, 139 confirmed incident pancreatic cancer cases were diagnosed between 1984 and 1998 among the women of the Nurses' Health Study, and 187 cases were diagnosed between 1986 and 2000 among the men of the Health Professionals Follow-up Study.

Statistical analyses

We computed person-time of follow-up for each participant from the return date of the baseline questionnaire to the date of pancreatic cancer diagnosis, death from any cause, or the end of follow-up, whichever came first. Incidence rates of pancreatic cancer were computed by dividing the number of incident cases by the number of person-years in each category of exposure. We computed the relative risk for each of the upper exposure categories by dividing the incidence rate in each category by the rate in the lowest category.

Relative risks adjusted for potential confounders were approximated by Cox proportional hazards regression (36). SAS/STAT PROC PHREG software (SAS Institute, Inc., Cary, North Carolina) was used for proportional hazards regression analysis, and the Anderson-Gill data structure was used to adjust for time-varying covariates efficiently (37). A new data record is created for every questionnaire cycle at which a participant was at risk, with covariates set to their values at the time that the questionnaire was returned. To control for confounding by age, calendar time, and any possible two-way interactions between these two time scales, we stratified the analysis jointly by age in 5-year categories at the start of follow-up and by calendar year of the current questionnaire cycle. For multivariate analyses, height was categorized into quintiles. Cigarette smoking status was categorized as current, former, or never smokers and updated biennially. In multivariable models, we controlled for the presence or absence of a history of diabetes, updating biennially (38, 39). On the basis of previous analyses of these cohorts (40), participants were categorized into five groups of baseline body mass index using whole number cutpoints including widely used definitions of overweight and obesity (38, 41). Body mass index was not updated in the analysis because pancreatic cancer is frequently associated with profound weight loss, and previous findings in these cohorts showed the strongest associations for baseline body mass index (40). Information on physical activity was first assessed in detail in 1986 in both cohorts. On the basis of previous analyses, total vigorous and nonvigorous activity was divided into categories of metabolic equivalent tasks (40). Glycemic load, glycemic index, and physical activity were excluded from multivariate models because they were not confounders in these analyses. Moreover, the intakes of total fats, beta-carotene, and caffeine did not confound or modify the relation between folate intake and pancreatic cancer. We present multivariate models adjusted for age and the covariates previously identified to have the strongest associations with pancreatic cancer in these cohorts: body mass index, height, cigarette smoking, and diabetes.

We used questionnaire responses to determine the duration of use of multivitamin supplements at baseline in both cohorts. For the Nurses' Health Study, the duration of multivitamin use was asked in the prebaseline 1980 questionnaire. Therefore, to compute a 1984 baseline value of multivitamin supplement use, we used the value given on the 1980 questionnaire and added 4 years of duration for women who reported current use in both 1980 and 1984 or carried the 1980 value forward for women who reported no current multivitamin supplement use in 1984. For the Health Professionals Follow-up Study, the response to the 1986 baseline question on the duration of multivitamin use was used. Before 1973, the maximum dose allowed in supplements by the US Food and Drug Administration was 100 μg, and many supplement formulations did not contain folic acid (42). Thus, the authors considered 1973 (when doses of 400 μg were first allowed) to be the earliest possible starting point.

Statistical interaction was assessed by likelihood ratio tests, comparing full models, including interaction terms, with reduced models without interaction terms. Tests for linear trend were performed using the median value of the independent variable for each category. We pooled the data from the two cohorts using a random-effects model for the log of the relative risks (43). Tests of heterogeneity using the Q statistic were performed before pooling (43). The proportionality of hazards was tested by likelihood ratio tests comparing saturated models having age-by-variable interactions with constrained models without interaction terms. The models presented all satisfy the proportionality of hazards assumption. All statistical procedures were performed using SAS version 8.2 software (SAS Institute, Inc.). All p values are based on two-sided tests.

RESULTS

Baseline characteristics of women in the Nurses' Health Study and of men in the Health Professionals Follow-up

TABLE 1. Age-standardized* characteristics of women in the Nurses' Health Study in 1984 and men in the Health Professionals Follow-up Study in 1986 by daily energy-adjusted total folate intake

Characteristic	Energy-adjusted total daily folate intake (μg/day)			
	<300	300–399	400–499	≥500
Men in the Health Professionals Follow-up Study				
No. of individuals (%)	11,745 (24.6)	13,563 (28.4)	7,421 (15.5)	15,111 (31.6)
Age (years) (SD†)	53.1 (9.8)	54.2 (9.9)	54.8 (9.9)	55.1 (10.1)
Folate supplement use (%)	35.2	48.4	57.2	89.7
Height (m)	1.8	1.8	1.8	1.8
Body mass index (kg/m^2)	25.3	25.1	24.7	24.7
Smoking history (%)				
Current	14.4	8.7	7.0	7.9
Former	41.6	41.8	41.4	42.0
Never	40.2	45.6	47.6	46.1
Diabetic (%)	2.7	3.3	3.3	3.3
Daily alcohol (g)	13.0	11.3	10.1	10.7
Daily methionine (g)	2.1	2.0	2.2	2.2
	Energy-adjusted total daily folate intake (μg/day)			
	<200	200–299	300–399	≥400
Women in the Nurses' Health Study				
No. of individuals (%)	11,067 (14.2)	27,402 (35.3)	14,468 (18.6)	24,703 (31.8)
Age (years) (SD)	49.1 (7.1)	50.4 (7.1)	51.7 (7.1)	51.6 (7.1)
Folate supplement use (%)	37.1	54.8	64.6	92.8
Height (m)	1.6	1.6	1.6	1.6
Body mass index (kg/m^2)	24.0	24.1	24.0	23.6
No. of births	3.0	3.0	2.9	2.9
Smoking history (%)				
Current	35.4	25.1	20.1	20.9
Former	26.4	31.1	33.4	34.5
Never	38.0	43.7	46.4	44.4
Diabetic (%)	2.1	3.0	3.3	3.2
Daily alcohol (g)	7.5	7.2	6.4	6.6
Daily methionine (g)	1.6	1.7	1.7	1.7

* All values except "number of individuals" and "age" are age-standardized to the distribution of eligible participants within each cohort at baseline.
† SD, standard deviation.

Study categorized by total energy-adjusted folate intake are shown in table 1. The relations between folate intake and age-standardized covariates were similar in men and women. Those with the lowest folate intake tended to be younger, to smoke cigarettes, and to have higher alcohol consumption than those with higher folate consumption. Height, body mass index, history of diabetes, and methionine intake were not appreciably different across categories of folate intake.

Total energy-adjusted folate intake was not associated with the risk of pancreatic cancer in either men or women (table 2). Compared with the risk for men who consumed less than 300 μg of folate per day, the multivariate-adjusted relative risks of pancreatic cancer for men having increasing 100-μg categories of total energy-adjusted folate intake were 1.07, 1.14, and 0.98 (95 percent confidence interval (CI): 0.65, 1.46; $p_{trend} = 0.73$). Among women, compared with the risk for those who had a daily intake of less than 200 μg of total folate, the relative risks of pancreatic cancer for increasing 100-μg categories of total energy-adjusted folate were 1.10, 1.03, and 1.15 (95 percent CI: 0.65, 2.04; $p_{trend} = 0.65$). Furthermore, in pooled results from both cohorts, there was no discernable trend in risk of pancreatic cancer with increasing consumption of total energy-adjusted folate. Compared with the risk of the lowest category, multivariate-adjusted relative risks for increasing categories of total folate intake were 1.08, 1.10, and 1.03 (95 percent CI: 0.74, 1.43; $p_{trend} = 0.99$).

Because multivitamin supplement use contributed 25 percent of total folate intake in these cohorts, we examined the relation of folate intake from supplements (i.e., nonfood

TABLE 2. Relative risk* and 95% confidence intervals of pancreatic cancer by categories of baseline daily energy-adjusted total folate and methionine intake in the Health Professionals Follow-up Study (1986–2000) and in the Nurses' Health Study (1984–1998)

	No. of cases	Person-years	Age-adjusted		Multivariate	
			Relative risk	95% confidence interval	Relative risk	95% confidence interval
Men in the Health Professionals Follow-up Study						
Total folate (food and supplements)						
<300 μg	42	152,346	1.00	Referent	1.00	Referent
300–399 μg	54	176,025	1.01	0.67, 1.51	1.07	0.71, 1.60
400–499 μg	33	96,565	1.07	0.68, 1.69	1.14	0.72, 1.81
≥500 μg	58	195,620	0.91	0.61, 1.36	0.98	0.65, 1.46
p_{trend}			0.54		0.73	
Folate from food						
<300 μg	69	250,765	1.00	Referent	1.00	Referent
300–399 μg	67	235,526	0.77	0.55, 1.08	0.81	0.58, 1.14
400–499 μg	37	117,833	0.81	0.54, 1.20	0.87	0.58, 1.30
≥500 μg	14	58,433	0.60	0.34, 1.07	0.66	0.37, 1.18
p_{trend}			0.08		0.17	
Folate from supplements						
<300 μg	144	499,078	1.00	Referent	1.00	Referent
300–399 μg	12	40,145	0.96	0.53, 1.73	0.95	0.53, 1.72
400–499 μg	16	36,615	1.30	0.78, 2.18	1.31	0.78, 2.21
≥500 μg	15	77,719	1.07	0.63, 1.81	1.08	0.63, 1.84
p_{trend}			0.56		0.52	
Methionine						
Quintile 1	43	123,319	1.00	Referent	1.00	Referent
Quintile 2	24	125,268	0.56	0.34, 0.92	0.57	0.34, 0.93
Quintile 3	35	127,078	0.79	0.51, 1.24	0.81	0.52, 1.26
Quintile 4	35	121,212	0.80	0.51, 1.26	0.83	0.53, 1.29
Quintile 5	50	123,671	1.05	0.70, 1.57	1.05	0.70, 1.59
p_{trend}			0.37		0.37	
Women in the Nurses' Health Study						
Total folate (food and supplements)						
<200 μg	16	149,115	1.00	Referent	1.00	Referent
200–299 μg	48	369,043	1.06	0.60, 1.87	1.10	0.62, 1.94
300–399 μg	26	194,751	0.96	0.52, 1.80	1.03	0.55, 1.93
≥400 μg	49	331,683	1.07	0.61, 1.88	1.15	0.65, 2.04
p_{trend}			0.85		0.65	
Folate from food						
<200 μg	27	201,205	1.00	Referent	1.00	Referent
200–299 μg	63	511,165	0.79	0.50, 1.23	0.82	0.52, 1.29
300–399 μg	39	247,383	0.87	0.53, 1.43	0.93	0.57, 1.53
≥400 μg	10	84,839	0.60	0.29, 1.25	0.65	0.31, 1.35
p_{trend}			0.32		0.45	

Table continues

TABLE 2. Continued

	No. of cases	Person-years	Age-adjusted		Multivariate	
			Relative risk	95% confidence interval	Relative risk	95% confidence interval
Folate from supplements						
<200 μg	98	788,745	1.00	Referent	1.00	Referent
200–299 μg	9	59,259	1.28	0.64, 2.52	1.32	0.67, 2.62
300–399 μg	14	91,151	1.13	0.65, 1.98	1.18	0.67, 2.06
≥400 μg	18	105,437	1.22	0.74, 2.02	1.26	0.76, 2.08
p_{trend}			0.34		0.26	
Methionine						
Quintile 1	29	170,313	1.00	Referent	1.00	Referent
Quintile 2	31	214,791	0.89	0.54, 1.48	0.89	0.54, 1.48
Quintile 3	21	249,468	0.51	0.29, 0.90	0.51	0.29, 0.89
Quintile 4	27	195,539	0.82	0.49, 1.38	0.80	0.47, 1.36
Quintile 5	31	214,481	0.83	0.50, 1.38	0.80	0.48, 1.33
p_{trend}			0.58		0.48	
Pooled cohorts						
Total folate (food and supplements)						
Category 1	58	301,461	1.00	Referent	1.00	Referent
Category 2	102	545,068	1.03	0.74, 1.42	1.08	0.77, 1.50
Category 3	59	291,316	1.03	0.72, 1.49	1.10	0.76, 1.60
Category 4	107	527,303	0.96	0.69, 1.33	1.03	0.74, 1.43
p_{trend}			0.72		0.99	
Folate from food						
Category 1	96	451,970	1.00	Referent	1.00	Referent
Category 2	130	746,691	0.77	0.59, 1.01	0.81	0.62, 1.07
Category 3	76	365,216	0.83	0.61, 1.34	0.89	0.65, 1.22
Category 4	24	143,272	0.60	0.38, 0.95	0.66	0.42, 1.03
p_{trend}			0.04		0.12	
Folate from supplements						
Category 1	242	1,287,823	1.00	Referent	1.00	Referent
Category 2	21	99,404	1.08	0.69, 1.69	1.10	0.70, 1.71
Category 3	30	127,766	1.22	0.83, 1.78	1.25	0.85, 1.83
Category 4	33	183,156	1.14	0.79, 1.65	1.17	0.81, 1.69
p_{trend}			0.27		0.20	
Methionine						
Quintile 1	72	293,632	1.00	Referent	1.00	Referent
Quintile 2	55	340,059	0.70	0.45, 1.11	0.70	0.45, 1.11
Quintile 3	56	376,546	0.66	0.43, 1.01	0.66	0.43, 1.01
Quintile 4	62	316,751	0.81	0.58, 1.14	0.80	0.57. 1.13
Quintile 5	81	338,152	0.96	0.70, 1.31	0.94	0.68, 1.29
p_{trend}			0.65		0.79	

* All relative risks were adjusted for age (5-year categories), time period (calendar year), and energy intake. Multivariate relative risks were additionally adjusted for cigarette smoking (current, former, never), diabetes (yes/no), body mass index (cutpoints: 23.0, 25.0, 27.0, 30.0), and height (quintiles).

TABLE 3. Age-standardized* characteristics of women in the Nurses' Health Study in 1984 and men in the Health Professionals Follow-up Study in 1986 by multivitamin supplement use

Characteristic	Multivitamin supplement use		
	Never	Former	Current
Men in the Health Professionals Follow-up Study			
No. of individuals (%)	17,575 (37.8)	9,178 (19.7)	19,799 (42.5)
Age (years) (SD†)	54.3 (9.8)	52.7 (9.9)	54.9 (10.0)
Folate supplement use (%)	0.3	1.1	79.8
Height (m)	1.8	1.8	1.8
Body mass index (kg/m^2)	25.2	25.0	24.7
Smoking history (%)			
Current	10.2	8.6	9.4
Former	40.0	43.9	42.4
Never	45.6	43.5	44.4
Diabetic (%)	3.0	3.2	3.1
Daily alcohol (g)	11.2	10.7	11.7
Daily methionine (g)	2.2	2.2	2.2
Women in the Nurses' Health Study			
No. of individuals (%)	40,362 (52.7)	7,753 (10.1)	28,731 (37.3)
Age (years) (SD)	50.4 (7.1)	51.7 (7.1)	51.7 (7.1)
Folate supplement use (%)	37.1	54.8	64.6
Height (m)	1.6	1.6	1.6
Body mass index (kg/m^2)	24.1	23.9	23.6
No. of births	3.0	2.9	2.9
Smoking history (%)			
Current	25.7	23.8	22.3
Former	30.5	32.9	33.7
Never	43.6	43.3	43.7
Diabetic (%)	2.1	3.0	3.3
Daily alcohol (g)	6.7	6.8	7.0
Daily methionine (%)	1.7	1.7	1.7

* All values except "number of individuals" and "age" are age-standardized to the distribution of eligible participants within each cohort at baseline.

† SD, standard deviation.

folate), multivitamin use, and pancreatic cancer. The baseline characteristics of women in the Nurses' Health Study and of men in the Health Professionals Follow-up Study categorized by multivitamin supplement use are shown in table 3. In pooled analyses, compared with never users, past and current users of multivitamins had multivariate relative risks of 1.47 (95 percent CI: 0.98, 2.21) and 1.31 (95 percent CI: 1.02, 1.67), respectively (table 4). There was no clear trend with increasing duration of use. Compared with the risk of the lowest category of intake, pooled multivariate relative risks for increasing categories of energy-adjusted supplemental folate were 1.10, 1.25, and 1.17 (95 percent CI: 0.81, 1.69; $p_{trend} = 0.20$).

When confining the analyses to folate from food (i.e., folate not from multivitamins or other supplements), we observed a suggestion of an inverse relation with pancreatic cancer in both cohorts (table 2). Among men in the Health Professionals Follow-up Study, those in the top category of food folate intake (≥500 μg) had an adjusted relative risk of pancreatic cancer of 0.66 (95 percent CI: 0.37, 1.18) compared with those in the category of less than 300 μg of folate from food ($p_{trend} = 0.17$). We observed a similar pattern among women in the Nurses' Health Study, with a relative risk of pancreatic cancer of 0.65 (95 percent CI: 0.31, 1.35) comparing those who consumed 400 μg or more of folate from food daily with those who consumed less than 200 μg daily ($p_{trend} = 0.45$). When cohorts were pooled, the relative risk was 0.66 (95 percent CI: 0.42, 1.03; $p_{trend} = 0.12$) in a comparison of risk in the highest food-folate category with that in the lowest category.

In a previous analysis of these cohorts, alcohol intake was not associated with pancreatic cancer risk (40). However, because alcohol can impair folate status as well as antagonize methylation pathways, alcohol consumption could modify the relation of folate intake to cancer risk (44). We therefore assessed the influence of folate intake according to

TABLE 4. Relative risk* and 95% confidence intervals of pancreatic cancer by categories of baseline multivitamin use, duration of use, and quantity of use in the Health Professionals Follow-up Study (1986–2000) and in the Nurses' Health Study (1984–1998)

	No. of cases	Person-years	Age-adjusted		Multivariate	
			Relative risk	95% confidence interval	Relative risk	95% confidence interval
Men in the Health Professionals Follow-up Study						
Multivitamin use						
Never	59	230,193	1.00	Referent	1.00	Referent
Past	33	120,459	1.22	0.80, 1.86	1.22	0.80, 1.87
Current	88	258,276	1.26	0.91, 1.75	1.27	0.91, 1.77
Duration of multivitamin use						
Never	59	230,193	1.00	Referent	1.00	Referent
<2 years	4	12,955	1.40	0.56, 3.49	1.39	0.56, 3.48
2–5 years	15	38,432	1.74	1.01, 2.98	1.74	1.02, 2.99
6–9 years	11	50,479	1.06	0.59, 1.89	1.07	0.60, 1.91
≥10 years	32	93,554	1.13	0.74, 1.72	1.14	0.75, 1.74
Women in the Nurses' Health Study						
Multivitamin use						
Never	60	546,708	1.00	Referent	1.00	Referent
Past	20	105,067	1.85	1.11, 3.07	1.85	1.12, 3.07
Current	58	385,776	1.31	0.91, 1.88	1.35	0.94, 1.94
Duration of multivitamin use						
Never	60	546,708	1.00	Referent	1.00	Referent
<2 years	11	70,622	1.53	0.81, 2.92	1.54	0.81, 2.92
2–5 years	40	235,453	1.54	1.03, 2.30	1.58	1.06, 2.35
6–9 years	12	89,911	1.18	0.63, 2.19	1.20	0.65, 2.24
≥10 years	16	98,059	1.31	0.76, 2.28	1.36	0.78, 2.37
Pooled cohorts						
Multivitamin use						
Never	119	776,901	1.00	Referent	1.00	Referent
Past	53	225,526	1.46	0.98, 2.20	1.47	0.98, 2.21
Current	146	644,052	1.28	1.00, 1.64	1.31	1.02, 1.67
Duration of multivitamin use						
Never	119	776,901	1.00	Referent	1.00	Referent
<2 years	15	83,577	1.49	0.88, 2.52	1.49	0.88, 2.52
2–5 years	55	273,885	1.61	1.16, 2.21	1.63	1.18, 2.25
6–9 years	23	140,390	1.11	0.73, 1.70	1.13	0.74, 1.73
≥10 years	48	191,613	1.19	0.85, 1.67	1.22	0.87, 1.70

* All relative risks were adjusted for age (5-year categories), time period (calendar year), and energy intake. Multivariate relative risks were additionally adjusted for cigarette smoking (current, former, never), diabetes (yes/no), body mass index (cutpoints: 23.0, 25.0, 27.0, 30.0), and height (quintiles).

alcohol consumption. Because statistical power was limited, alcohol intake was categorized as greater than or equal to or as less than 10 g per day for men and as greater than or equal to or as less than 5 g per day for women. There was no significant inverse association between folate intake and pancreatic cancer risk within either stratum of alcohol consumption. Moreover, tests for statistical interaction between folate and alcohol intake were not significant in either cohort.

As an important methyl-group donor, methionine may modify the effect of folate consumption on the risk of pancreatic cancer (45). In particular, low levels of methionine may increase the need for folate-supplied methyl groups (33). The pooled multivariate relative risks for

increasing quintiles of methionine intake compared with the risk in the lowest quintile of intake were 0.70, 0.66, 0.80, and 0.94 (p_{trend} = 0.79) (table 2). Furthermore, after stratification according to low (quintiles 1 and 2) or high (quintiles 3–5) methionine intake, total folate intake was not associated with pancreatic cancer, and tests for statistical interaction between folate and methionine intake were not significant in either cohort.

Cigarette smoking impairs folate metabolism (46) and may interact with folate in association with pancreatic cancer risk. We therefore repeated analyses after stratifying the cohort according to cigarette smoking status. There was no significant heterogeneity of associations between folate intake and pancreatic cancer risk in either cohort when stratified by categories of cigarette smoking. In a comparison with results from the Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) cohort, those from analyses restricted to currently smoking males yielded 33 cases in 60,775 person-years of follow-up. Among currently smoking men, compared with risk for less than 300 μg of intake, the age-adjusted relative risks for increasing 100-μg categories of food-folate intake were 0.81, 0.71, and 0.83 (95 percent CI: 0.19, 3.62). Relative risks for analogously increasing 100-μg categories of folate from supplements were 4.03, 1.90, and 1.73 (95 percent CI: 0.51, 5.83). Data for smokers were notably sparse, and estimates of associations between folate intake and pancreatic cancer were imprecise.

DISCUSSION

In this prospective analysis of two large cohorts, total energy-adjusted folate intake was not associated with the risk of pancreatic cancer. Results were similar for men and women, and the findings remained unchanged after stratification by alcohol or methionine intake or by smoking status.

Few studies have examined the relation between folate intake and the risk of pancreatic cancer, and the results have been conflicting (21–24). In 27,101 male Finnish smokers, a significant inverse relation between dietary folate and pancreatic cancer risk (22) was seen. However, in a nested substudy of plasma folate in this cohort, 90 percent of participants had less than adequate levels, and 25 percent would be considered deficient (21). When examining the relative risks across quintiles of folate intake in the Finnish cohort, we found that the principal result was an elevated risk within the lowest quintile of intake rather than a monotonic relation with increasing intake (relative risks for each quintile = 1.00, 0.67, 0.59, 0.89, 0.52). Thus, a demonstrable influence of folate consumption may be restricted to populations that are relatively folate deficient.

Although we found no influence of supplemental folate or total folate (from food and supplements combined), we did observe a nonsignificant inverse trend for folate from food sources in both cohorts. For comparison, fewer than 6 percent of participants in the Finnish cohort used folate-containing supplements, and the apparent effect of folate in those studies was reflective of food sources only and therefore most similar to our analysis of food-folate intake. Moreover, participants in the Finnish cohort who reported supplement use actually experienced a nonsignificant increased risk of pancreatic cancer (relative risk = 1.60, 95 percent CI: 0.92, 2.77), a result similar in direction to the relative risk for current or past multivitamin users in our cohorts.

The reasons for the different apparent effect for dietary folate compared with supplemental folate observed in our cohorts as well as in the Finnish ATBC cohort are unclear. Supplemental folate is substantially more bioavailable than folate from food sources and therefore would be expected to offer greater potency. Within our cohorts, total folate and dietary folate assessed by questionnaire were correlated similarly with plasma folate levels (r = 0.63 for total folate; r = 0.61 for folate excluding supplements) (47). This suggests that another dietary item that is correlated with dietary folate may have accounted for the suggestion of an inverse association between folate from food sources and pancreatic cancer risk. Alternatively, if the latency between developing and detecting a pancreatic cancer exceeded the follow-up period of this study, then the baseline folate assessment may misclassify the exposure temporally. Moreover, if dietary folate consumption patterns are stable over time, then food folate levels may represent folate exposure in the distant past that are more relevant to tumorigenesis than is recent exposure from multivitamin supplements. Finally, given the modestly increased risk observed with multivitamin supplement use, some component of multivitamin supplements that increases risk for pancreatic cancer may counterbalance an inverse association with total folate consumption.

The strengths of this study include its prospective design; validation of the relation between the questionnaire's measurement of folate intake and serum concentrations with a biochemical marker; detailed data on many potential confounders; high follow-up response rate, and data from two completely separate, large cohorts. The prospective design precluded recall bias and the need for next-of-kin respondents. We cannot exclude measurement error as an explanation for the lack of a significant association within either cohort in this study. Misclassification of folate intake as measured by the food frequency questionnaire may have attenuated the results to some degree; however, this is an unlikely explanation for the lack of any association over extreme levels of intake, since it is improbable that participants were misclassified from one extreme category to the other. Moreover, other analyses in these same cohorts have reported significant inverse associations between total folate intake and breast (44) and colon (16, 33, 48) cancers.

We cannot exclude that these findings may not be generalizable to populations of smokers or to those who are relatively folate deficient, such as the Finnish ATBC cohort. Cigarette smoking is associated with low folate status, interferes with methyl metabolism, and may modify any effect of folate intake on pancreatic cancer. In contrast, the majority of participants in the cohorts of this study were either never or former smokers. Furthermore, the distribution of alcohol consumption in this study's cohorts limited our ability to examine the influence of folate intake at high levels of alcohol consumption.

In summary, we observed no clear relation between folate intake and the risk of pancreatic cancer in two large prospective cohort studies. Further studies may reveal whether folate from food sources or some factor associated with food folate intake decreases the risk of pancreatic cancer. Although we cannot exclude the possibility that very low folate intake increases the risk of pancreatic cancer, among relatively folate-replete women and men in the United States, greater folate intake is unlikely to substantially influence the risk of pancreatic cancer.

ACKNOWLEDGMENTS

Supported by grants CA55075, CA86102, CA9001, and CA87969, the main Nurses' Health Study grants. The Nurses' Health Study is supported for other specific projects by the following grants from the National Institutes of Health: CA75016, HL63841, AG/CA14742, CA46475, DAMD170010165, DK52866, CA82838, HL57871, EY09611, CA67883, AG15424, HL34594, CA49449, CA70817, DK58845, CA78293, DK54900, HL64108, HL03804, CA86271, CA65725, CA89393, ES08074, CA93683, DK59583, HL65582, DE12102, and AR02074.

In addition, for activities related to the Nurses' Health Study, the authors have received modest additional resources at various times and for varying periods since January 1, 1993, from the Alcoholic Beverage Medical Research Foundation, the American Cancer Society, Amgen, the California Prune Board, the Centers for Disease Control and Prevention, the Ellison Medical Foundation, the Florida Citrus Growers, the Glaucoma Medical Research Foundation, Hoffman-La Roche, Kellogg's, Lederle, the Massachusetts Department of Public Health, Mission Pharmacal, the National Dairy Council, Rhone Poulenc Rorer, the Robert Wood Johnson Foundation, Roche, Sandoz, the US Department of Defense, the US Department of Agriculture, the Wallace Genetics Fund, Wyeth-Ayerst, Merck, and private contributors.

REFERENCES

1. American Cancer Society. Cancer facts and figures—2004. Atlanta, GA: American Cancer Society, 2004.
2. Greenlee RT, Hill-Harmon MB, Murray T, et al. Cancer statistics, 2001. CA Cancer J Clin 2001;51:15–36.
3. Cubilla AL, Fitzgerald PJ. Cancer of the exocrine pancreas: the pathologic aspects. CA Cancer J Clin 1985;35:2–18.
4. Fuchs C, Colditz G, Stampfer M, et al. A prospective study of cigarette smoking and the risk of pancreatic cancer. Arch Intern Med 1996;156:2255–60.
5. Wickramasinghe S, Fisa S. Bone marrow cells from vitamin B_{12}- and folate-deficient patients misincorporate uracil into DNA. Blood 1994;83:1656–61.
6. Blount BC, Ames BN. Analysis of uracil in DNA by gas chromatography-mass spectrometry. Anal Biochem 1994;219:195–200.
7. Blount BC, Ames BN. DNA damage in folate deficiency. Baillieres Clin Haematol 1995;8:461–78.
8. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A 1997;94:3290–5.
9. Chen J, Giovannucci E, Hankinson SE, et al. A prospective study of methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms, and risk of colorectal adenoma. Carcinogenesis 1998;19:2129–32.
10. Xu GL, Bestor TH, Bourc'his D, et al. Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. Nature 1999;402:187–91.
11. Hruban RH, Goggins M, Parsons J, et al. Progression model for pancreatic cancer. Clin Cancer Res 2000;6:2969–72.
12. Argani P, Rosty C, Reiter RE, et al. Discovery of new markers of cancer through serial analysis of gene expression: prostate stem cell antigen is overexpressed in pancreatic adenocarcinoma. Cancer Res 2001;61:4320–4.
13. Tascilar M, Skinner HG, Rosty C, et al. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. Clin Cancer Res 2001;7:4115–21.
14. Ueki T, Walter KM, Skinner H, et al. Aberrant CpG island methylation in cancer cell lines arises in the primary cancers from which they were derived. Oncogene 2002;21:2114–17.
15. Ueki T, Toyota M, Skinner H, et al. Identification and characterization of differentially methylated CpG islands in pancreatic carcinoma. Cancer Res 2001;61:8540–6.
16. Giovannucci E, Rimm EB, Ascherio A, et al. Alcohol, low-methionine–low-folate diets, and risk of colon cancer in men. J Natl Cancer Inst 1995;87:265–73.
17. Giovannucci E, Stampfer MJ, Colditz GA, et al. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. Ann Intern Med 1998;129:517–24.
18. Fuchs CS, Willett WC, Colditz GA, et al. The influence of folate and multivitamin use on the familial risk of colon cancer in women. Cancer Epidemiol Biomarkers Prev 2002;11:227–34.
19. Zhang S, Hunter DJ, Hankinson SE, et al. A prospective study of folate intake and the risk of breast cancer. JAMA 1999;281:1632–7.
20. Zhang SM, Willett WC, Selhub J, et al. Plasma folate, vitamin B_6, vitamin B_{12}, homocysteine, and risk of breast cancer. J Natl Cancer Inst 2003;95:373–80.
21. Stolzenberg-Solomon RZ, Albanes D, Nieto FJ, et al. Pancreatic cancer risk and nutrition-related methyl-group availability indicators in male smokers. J Natl Cancer Inst 1999;91:535–41.
22. Stolzenberg-Solomon RZ, Pietinen P, Barrett MJ, et al. Dietary and other methyl-group availability factors and pancreatic cancer risk in a cohort of male smokers. Am J Epidemiol 2001;153:680–7.
23. Baghurst PA, McMichael AJ, Slavotinek AH, et al. A case-control study of diet and cancer of the pancreas. Am J Epidemiol 1991;134:167–79.
24. Silverman DT, Swanson CA, Gridley G, et al. Dietary and nutritional factors and pancreatic cancer: a case-control study based on direct interviews. J Natl Cancer Inst 1998;90:1710–19.
25. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
26. Colditz GA, Willett WC, Stampfer MJ, et al. The influence of age, relative weight, smoking, and alcohol intake on the reproducibility of a dietary questionnaire. Int J Epidemiol 1987;16:392–8.
27. Rimm EB, Giovannucci EL, Stampfer MJ, et al. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health

professionals. Am J Epidemiol 1992;135:1114–26.
28. Willett WC. Nutritional epidemiology. New York, NY: Oxford University Press, 1990.
29. US Department of Agriculture. Composition of foods—raw, processed, and prepared, 1963–1992. (Agricultural handbook no. 8 series). Washington, DC: US Department of Agriculture, 1993.
30. Willett WC, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
31. Willett WC, Sampson L, Browne ML, et al. The use of a self-administered questionnaire to assess diet four years in the past. Am J Epidemiol 1988;127:188–99.
32. Rimm EB, Willett WC, Hu FB, et al. Folate and vitamin B_6 from diet and supplements in relation to risk of coronary heart disease among women. JAMA 1998;279:359–64.
33. Giovannucci E, Stampfer MJ, Colditz GA, et al. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. Ann Intern Med 1998;129:517–24.
34. Giovannucci E, Colditz G, Stampfer MJ, et al. The assessment of alcohol consumption by a simple self-administered questionnaire. Am J Epidemiol 1991;133:810–17.
35. Rich-Edwards J, Corsano K, Stampfer M. Test of the National Death Index and Equifax Nationwide Death Search. Am J Epidemiol 1994;140:1016–19.
36. Cox D, Oakes D. Analysis of survival data. London, United Kingdom: Chapman and Hall, 1984.
37. Therneau TM. Extending the Cox model. In: Lin DY, Fleming TR, eds. First Seattle Symposium in Biostatistics: Survival Analysis. Seattle, WA: Springer Verlag, 1997:51–84.
38. Everhart J, Wright D. Diabetes mellitus as a risk factor for pancreatic cancer: a meta-analysis. JAMA 1995;273:1605–9.
39. Silverman DT, Schiffman M, Everhart J, et al. Diabetes mellitus, other medical conditions and familial history of cancer as risk factors for pancreatic cancer. Br J Cancer 1999;80:1830–7.
40. Michaud DS, Giovannucci E, Willett WC, et al. Physical activity, obesity, height, and the risk of pancreatic cancer. JAMA 2001;286:921–9.
41. Baik I, Ascherio A, Rimm EB, et al. Adiposity and mortality in men. Am J Epidemiol 2000;152:264–71.
42. US Food and Drug Administration. Statement of general policy or interpretation: subchapter B—food and food products, part 121—food additives. Federal Register 1973;38:20725–6.
43. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
44. Zhang S, Hunter DJ, Hankinson SE, et al. A prospective study of folate intake and the risk of breast cancer. JAMA 1999;281:1632–7.
45. Mason JB, Levesque T. Folate: effects on carcinogenesis and the potential for cancer chemoprevention. Oncology (Huntingt) 1996;10:1727–36, 1742–3.
46. Bailey LB. Folate status assessment. J Nutr 1990;120(suppl 11):1508–11.
47. Jacques PF, Sulsky SI, Sadowski JA, et al. Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. Am J Clin Nutr 1993;57:182–9.
48. Fuchs CS, Willett WC, Colditz GA, et al. The influence of folate and multivitamin use on the familial risk of colon cancer in women. Cancer Epidemiol Biomarkers Prev 2002;11:227–34.