Dietary Folate and Folate Vitamers and the Risk of Pancreatic Cancer in the Netherlands Cohort Study

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Abstract

An association between high intake of folate and reduced risk of cancer has been suggested by previous research. However, epidemiologic data from cohort studies regarding the relationship between dietary folate and pancreatic cancer are sparse and inconsistent. We examined the association between dietary folate intake and risk of pancreatic cancer within the Netherlands Cohort Study on diet and cancer. Men and women (120,852), ages 55 to 69 years, were recruited. Information on diet was collected at baseline by means of food frequency questionnaires, and the cohort was followed for 13.3 years. Total folate and vitamer intake were calculated using folate contents of food items derived from a validated liquid chromatography trienzyme method. Cases (n = 363) were identified by record linkage with regional cancer registries and the Dutch National Database of Pathology Reports. A case-cohort approach was used using the follow-up data of a random subcohort (n = 5,000) identified at the onset of the cohort. Multivariable hazard ratios with 95% confidence intervals were estimated using Cox proportional hazards model. After adjusting for age, gender, smoking status, number of years smoked, number of cigarettes smoked per day, and intake of added sugar multivariate hazard ratio comparing the highest and lowest quintiles of folate intake for pancreatic cancer risk was 1.37 (confidence interval, 0.97-1.94; P_{trend} = 0.07). When folate vitamers were analyzed separately, results did not show a difference in association. Our results do not support a protective association of total dietary folate or individual folate vitamers on the risk of pancreatic cancer. (Cancer Epidemiol Biomarkers Prev 2009;18(6):1785–91)

Introduction

Although pancreatic cancer does not rank among the most common cancers in the Western world, it is the sixth most common cause of cancer death in Europe (1) and fourth in the United States (2). Its prognosis is one of the most dismal of all cancers and in both Europe (3) and the United States (4), 5-year survival rates of only 4% to 5% for all tumors, and <1% for nonresectable tumors (5) have been reported. To date, no effective means of early detection, prevention, or treatment are available.

Epidemiologic studies have suggested associations of pancreatic cancer with chronic pancreatitis, type II diabetes mellitus, body mass index (BMI), and several dietary factors, including positive associations with cholesterol, carbohydrate, and meat intake and inverse relationship with fruits, vegetables, and dietary fibers (6, 7). The most consistently documented risk factor is cigarette smoking, which doubles the risk of pancreatic cancer (7, 8).

Folate is a naturally occurring one-carbon unit carrier (e.g., methyl and formyl groups), which is important in the process of DNA methylation and the biosynthesis of nucleotides (9, 10). Folate deficiency is thought to influence cancer risk through disturbances in DNA methylation and integrity, and disruption of DNA synthesis and repair (10).

A substantial body of evidence supports the role of folate deficiency in colorectal carcinogenesis (9), and previous research suggests a possible protective effect of folate in breast, gastric, esophageal, and ovarian cancer (11-13). The report of the World Cancer Research Fund concludes that foods containing folate probably protect against pancreatic cancer (14). There are, however, only few epidemiologic studies that have addressed the relationship between dietary folate and pancreatic cancer. Most of these studies were conducted with selected populations, and their results were inconsistent. Case-control studies have shown inverse associations, as well as lack of association between dietary folate and pancreatic cancer (15, 16). In a Finnish cohort study of male smokers, and in a Swedish cohort of men and women, risk of pancreatic cancer was associated with a low dietary intake of folate (17, 18). A statistically nonsignificant inverse association of pancreatic cancer risk and dietary folate intake was found in a prospective study of U.S. nurses and health professionals (19).

In a nested case-control study from 2007, plasma concentrations of folate in samples from four cohort studies were examined (20). No statistically significant association between folate plasma levels and pancreatic cancer risk was observed. In the analyses restricted to nonusers of multivitamins, a modest inverse trend was observed between folate levels and pancreatic cancer risk.

It has been shown that cigarette smoking is associated with lower levels of folate in plasma, RBC, and buccal

Received 12/19/08; revised 3/22/09; accepted 4/8/09; published online 6/8/09.

Grant support: Dutch Cancer Society.

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doi:10.1158/1055-9965.EPI-08-1220

mucosa (21), and chronic alcohol consumption impairs intestinal folate absorption, decreases hepatic folate uptake, and increases renal folate excretion (22). Smoking and alcohol intake may thus modify the relationship between folate intake and risk of pancreatic cancer. Such interaction between alcohol and folate intake has been suggested in breast cancer (23, 24) and colon cancer (25), and between smoking and folate intake in colorectal cancer (26). Studies to date, however, have not shown a modifying effect of smoking or alcohol ingestion on pancreatic cancer (17-19).

In the present study, we investigate the incidence of pancreatic cancer in relation to dietary folate and folate vitamer intake, and the modifying effects of alcohol consumption and cigarette smoking, in the Netherlands Cohort Study on Diet and Cancer (NLCS).

Materials and Methods

Population. The NLCS is a large-scale prospective cohort study initiated in 1986 that recruited 58,279 men and 62,573 women ages 55 to 69 years (27). Ethical approval to conduct the study was obtained from the University Hospital Maastricht and the Netherlands Organization for Applied Scientific Research (TNO). The sample originated from 204 municipal population registries throughout the Netherlands by gender-stratified random sampling of the 55- to 69-years age group. All participants completed a self-administered questionnaire on habitual dietary intake, dietary supplement use, selected medical conditions, life-style, smoking habits, occupational history, socioeconomic status, family history of cancer, reproductive history, drug use, obesity, and physical activity. A random subcohort (n = 5,000) was selected immediately after identification of cohort members and followed biennially for migration and vital status to estimate the accumulated person-years of the whole cohort. The choice for the size of the subcohort was based on relative efficiency comparisons of risk ratios that would be obtained from a full cohort study versus a case-cohort design (27).

Follow-up. Incident cases of pancreatic cancer were identified during 13.3 years of follow-up by an annual computerized record linkage with regional cancer registries in the Netherlands and the Dutch National Database of Pathology Reports (PALGA). The completeness of cancer registries is estimated to be higher than 95% (28). Cases for this study (n = 363) were defined as malignant neoplasm of the pancreas [ICD-O-3 code C25, excluding C25.4 (islet cell carcinoma)]. The diagnosis was made with microscopic confirmation or by the clinician providing care, based on clinical symptoms, physical examination, and imaging results, and abstracted and recorded by a trained tumor registrar (29).

Eligible participants were individuals who completed the initial questionnaire in 1986 and did not have cancer at baseline (other than skin cancer). Individuals were excluded if they had an unacceptably incomplete food frequency questionnaire (FFQ), defined as >60 blank items (of 150 items) and fewer than 35 items eaten at least once a month; or 1 or more item blocks (i.e., groups of items) left blank; or an error index score of 10 or higher. The error index was calculated as the sum of scores of 15 variables indicating the presence of a specific response error (30).

Assessment of Determinants. Nutrient intake of study participants were derived from a 150-item semiquantitative FFQ completed after recruitment of the cohort. The FFQ was validated against a thrice 3-days period diet record (30). Pearson correlation coefficients for nutrient intake evaluated by questionnaire and diet records ranged from 0.6 to 0.8 for most nutrients. The Spearman correlation coefficients for vegetables, fruits, meat products, bread, and eggs were 0.38, 0.60, 0.54, 0.80, and 0.61, respectively. The stability of dietary habits in the NLCS cohort was evaluated in a 5-year reproducibility study of the FFQ (31). The test-retest Pearson correlation coefficients ranged from 0.42 for selenium to 0.9 for alcohol intake, and only a minor decrease in the capacity of the baseline questionnaire to rank individuals within the distribution of future nutrient intake was detected.

Folate intake was calculated using data from a validated liquid chromatography trienzyme method used to quantify folate content of 125 Dutch food items that contribute ~90% of total folate intake (32). Intakes of tetrahydrofolate, 5-methyltetrahydrofolate, 5-formyltetrahydrofolate, 10-formylfolic acid, 10-formyldihydrofolate, and folic acid were calculated, and a separate analysis was done to investigate the possible effect of specific folate vitamers. Intake of other nutrients was calculated using the computerized Dutch food composition table.

Statistical Analysis. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) for pancreatic cancer were estimated in age (year) and gender-adjusted and multivariable Cox proportional hazards model analysis (33). Quintiles of folate intake, based on the distribution in the subcohort, were used in the analysis separately for men and women, because of sex differences in folate intake. SEs were estimated using a robust covariance matrix estimator to account for increased variance due to sampling from the cohort (34). The proportional hazards assumption was tested using the scaled Schoenfeld residuals (35). Dose-response trends in pancreatic cancer were tested by fitting ordinal exposure variables as continuous terms. We used Wald statistics to test for interactions and considered a two-sided P value of <0.05 as statistically significant. STATA was used to carry out the analysis (release 9; Stata Corporation).

We considered BMI (kg/m^2), smoking status (currently smoking or not smoking), number of cigarettes smoked, number of years smoked, daily intake of vegetables (grams per day), fruits (grams per day), alcohol (grams per day), fibers (grams per day), added sugar in the diet (grams per day), β-carotene (micrograms per day), vitamin B1 (milligrams per day), methionine (milligrams per day), total carbohydrate (grams per day), total energy intake (kJoules per day), consumption of sweets (grams per day), iron intake (milligrams per day), and vitamin B6 intake (milligrams per day) as possible confounders in our analysis. These potential confounding variables were added to the multivariable-adjusted model if they (*a*) were associated with the disease and with folate intake and (*b*) changed the risk estimate of the model containing folate intake, age, and sex by at least 10%, resulting in a multivariable-adjusted model including age at baseline, sex, current smoking (yes/no), number of cigarettes smoked per day, number of years smoked, and intake of added sugar. We did not use vegetable intake in the multivariable model to avoid possible over adjustment.

Separate models for men and women showed similar results and there was no statistically significant interaction by sex ($P = 0.71$). We therefore present analyses combined for men and women. Interaction of folate intake with alcohol consumption and smoking status was tested using cross-product terms in the regression models and by examining stratum specific HR estimates. Folate intake was analyzed in tertiles, and categories of alcohol intake below and above median (4.5 grams) were used for this analysis.

Intakes of food and nutrients were adjusted for total energy intake in the regression models using the residual method for adjustment (36).

Secondary analysis was done when only histologically confirmed cases were included in the analysis and when only cases occurring >2 years after inception of the cohort were analyzed.

Results

Table 1 presents the baseline characteristics of subcohort members and cases, separately for men and women. Both male and female cases were older than subcohort members. Nutrient intakes, as well as BMI, were comparable between cases and subcohort members. Alcohol consumption was higher in cases. The prevalence of diabetes was substantially higher in male cases compared with subcohort members (10% versus 3%), and the proportion of current smokers were higher within cases than in the subcohort (43% versus 34%, and 27% versus 21% for men and women, respectively). The number of years smoked (24.6 ± 18.3 years versus 20.3 ± 18.1 years) and the number of cigarettes smoked (10.9 ± 10.8 per day versus 9.6 ± 10.9 per day) were also higher in cases than in subcohort members. The baseline characteristics were also compared between histologically confirmed ($n = 241$) and nonconfirmed cases ($n = 122$; data not shown). Among men, confirmed cases had a higher BMI (25.6 kg/m^2 versus 24.6 kg/m^2; $P = 0.039$) and a higher prevalence of diabetes (14% versus 8%), but the difference between both case groups was not statistically significant. Histologically confirmed cases among women were older than nonconfirmed cases (63.8 years versus 61.5 years; $P < 0.001$). Table 2 presents total folate and folate vitamer intake for the study population. There were no substantial differences between subcohort members and cases.

We did Cox regression models with possible confounding variables and found that age, gender, smoking status, number of years smoked, number of cigarettes smoked per day, intake of vegetables, and added sugar were confounders in our analysis. We used these variables, except vegetable intake to avoid over adjustment, in subsequent regression models to adjust for confounding effect. The association between dietary folate intake and pancreatic cancer risk represented by HRs for folate intake quintiles are presented in Table 3. A statistically significant positive association was seen only for the highest quintile of folate intake in the model adjusted for age and sex (HR, 1.44; 95% CI, 1.02-2.03). A linear trend in the increase of risk was also seen in this model ($P_{trend} = 0.035$). In the multivariate model, the strength of association was lower and not statistically significant ($P_{trend} = 0.07$). The

Table 1. Baseline characteristics of the study population in the NLCS, 1986-1999

	Men		Women	
	Subcohort (n = 1,963)	Cases (n = 188)	Subcohort (n = 2,068)	Cases (n = 175)
Age (y)	61.2 ± 4.2	62.0 ± 4.1	61.3 ± 4.3	62.3 ± 4.2
Smoking status % (n)				
Never smoked	13% (267)	7% (13)	59% (1,224)	51% (90)
Former smoker	53% (1,034)	50% (95)	20% (420)	22% (38)
Current smoker	34% (662)	43% (80)	21% (424)	27% (47)
BMI (kg/m^2)	24.9 ± 2.6	25.3 ± 3.0	25.0 ± 3.5	25.5 ± 3.6
History of diabetes % (n)	3% (62)	10% (18)	4% (74)	5% (8)
Physical activity outside profession % (n)				
<30 min/d	18% (341)	15% (28)	23% (481)	24% (42)
30-60 min/d	31% (598)	34% (64)	32% (651)	30% (52)
60-90 min/d	19% (382)	24% (45)	23% (467)	25% (44)
>90 min/d	32% (624)	27% (51)	22% (446)	21% (36)
Highest level of education				
Primary school	23% (450)	27% (52)	33% (667)	38% (66)
Lower vocational	20% (395)	15% (28)	23% (477)	18% (31)
High school	37% (721)	37% (69)	35% (722)	37% (64)
Higher vocational/university	20% (387)	21% (39)	9% (192)	7% (13)
Mean intake of				
Total folate (μg/d)	225 ± 66	228 ± 71	199 ± 61	206 ± 61
Vitamin B1 (mg/d)	1.2 ± 0.2	1.2 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
Vitamin B2 (mg/d)	1.6 ± 0.4	1.6 ± 0.4	1.4 ± 0.3	1.5 ± 0.4
Vitamin B6 (mg/d)	1.5 ± 0.3	1.6 ± 0.3	1.3 ± 0.2	1.4 ± 0.2
Methionine (mg/d)	1,713 ± 292	1,731 ± 309	1,493 ± 276	1,496 ± 304
β-carotene (μg/d)	3,012 ± 1,501	3,041 ± 1,371	2,946 ± 1,510	3,017 ± 1,475
Fiber (grams/d)	29.0 ± 7.3	28.7 ± 7.3	25.4 ± 5.8	25.9 ± 5.4
Energy (kJ/d)	9,108 ± 2,120	9,044 ± 1,937	7,094 ± 1,632	7,186 ± 1,693
Fruits (grams/d)	157 ± 114	153 ± 120	197 ± 118	196 ± 106
Vegetables (grams/d)	193 ± 83	197 ± 91	197 ± 80	212 ± 80
Carbohydrate (grams/d)	227 ± 38	223 ± 37	179 ± 27	177 ± 27
Added sugar(grams/d)	28.8 ± 29.1	25.1 ± 29.1	11.1 ± 19.2	9.2 ± 17.9
Alcohol (grams/d)	14.9 ± 16.6	17.4 ± 17.3	5.9 ± 9.5	7.4 ± 11.0

NOTE: Data presented as mean ± SD or percentage, as appropriate. Nutrient and food intakes are energy adjusted by the residual method (36).

Table 2. Adjusted intakes of total folate and folate vitamers (μg/d) in the NLCS, 1986-1999

	Men		Women	
	Subcohort (n = 1,963)	Cases (n = 188)	Subcohort (n = 2,068)	Cases (n = 175)
Total folate	225.1 ± 66.2	228.3 ± 70.9	199.1 ± 61.0	206.4 ± 61.1
Monoglutamates	70.6 ± 41.5	72.4 ± 38.1	55.9 ± 32.3	56.1 ± 26.6
Polyglutamates	130.2 ± 38.4	132.3 ± 45.8	119.7 ± 36.6	127.7 ± 40.3
Tetrahydrofolate	18.5 ± 28.9	18.1 ± 24.7	14.4 ± 22.2	14.4 ± 17.6
5-Methyl-tetrahydrofolate	128.7 ± 41.9	130.8 ± 49.0	119.9 ± 40.7	127.6 ± 45.2
5-Formyl-tetrahydrofolate	26.3 ± 9.7	26.7 ± 9.2	22.6 ± 7.0	23.6 ± 7.3
Folic acid	7.2 ± 6.1	8.3 ± 7.8	5.7 ± 5.2	5.2 ± 3.4
10-Formyl-dihydrofolate	10.1 ± 5.1	10.8 ± 4.8	8.5 ± 4.3	8.5 ± 4.5
10-Formyl-folate	20.9 ± 8.6	21.4 ± 8.5	15.0 ± 4.6	15.1 ± 4.6

NOTE: Data presented as mean ± SD. Energy adjusted by the residual method (36).

rate estimates suggested a positive association between folate intake and pancreatic cancer.

To exclude the possibility of bias by including falsely diagnosed cases, we did an analysis with cases whose diagnosis was confirmed histologically. The results of regression analysis did not differ substantially from the analysis including all cases. The HR for the highest quintile of folate intake in the age and sex adjusted model was 1.38 (95% CI, 0.91-2.09), and for the multivariate model, 1.34 (95% CI, 0.88-2.04). In separate analysis, we excluded cases diagnosed in the first 2 years of follow-up (n = 27), to eliminate the possible effect of preclinical symptoms on dietary habit. The results were similar to our main analysis. HR for the highest quintile of folate intake in the multivariate model was 1.38 (95% CI, 0.97-1.98) and for histologically confirmed cases, 1.31 (95% CI, 0.86-2.02).

To examine the association of separate folate vitamers and risk of pancreatic cancer, we carried out age- and sex-adjusted, as well as multivariate analysis for polyglutamate and monoglutamates and separately for specific folate vitamers. Results of the multivariate analysis, which were similar to the age- and sex-adjusted results, are presented in Table 4. HRs were, in general, close to 1, with lowest values for the 3rd quintile for tetrahydrofolate (HR, 0.72; 95%CI, 0.50-1.04). Positive associations for 10-formyl-dihydrofolate were observed in all quintiles, although none were statistically significant. Restricting the analyses to histologically confirmed cases did not substantially change the results (data not shown).

We tested and found no evidence of interaction between dietary folate intake and alcohol consumption in relation to risk of pancreatic cancer using above and below median levels of alcohol consumption ($P_{interaction}$ = 0.82). Examination of interaction with smoking status is presented in Table 5. The test for interaction was not statistically significant (P = 0.34). The HRs were higher than one in all categories and they were statistically significant in the highest tertile of folate intake in never- and current-smokers.

Discussion

In this prospective study of a Dutch cohort, energy-adjusted dietary folate intake did not show an inverse relationship with the incidence of pancreatic cancer. Risk estimates suggested a slight increase in risk, but results were not statistically significant when controlling for possible other confounding effects. Studies investigating the relationship between dietary folate intake and risk of pancreatic cancer have shown diverse results. In a prospective study of U.S. nurses and health professionals, no statistically significant association was shown (19). In a Finnish cohort of male smokers, risk of pancreatic cancer was associated with a low intake of folate (>373 μg/day versus ≤280 μg/day; HR, 0.52; 95% CI, 0.31-0.87; ref. 17). The same investigators also showed inverse relationship of pancreatic cancer and serum folate levels, a biomarker of folate effect, which may better reflect biologically active dose than intake levels, in a nested case-control study of 126 cases and 247 matched controls (odds ratio, 0.45; 95% CI, 0.24-0.82; P_{trend} = 0.009; ref. 37). A nested case-control

Table 3. HR of pancreatic cancer, with 95% CIs, according to quintiles of energy-adjusted folate intake; NLCS, 1986-1999

Quintiles of folate intake, μg/d	Cases	Person years in subcohort	Age and sex adjusted		Multivariate*	
			HR	95% CI	HR	95% CI
1 male (<176.3) Female (<154.1)	61	9,599.7	1.00	Reference	1.00	Reference
2 male (176.6-200.3) Female (154.2-176.5)	62	9,682.1	1.00	0.70-1.45	1.00	0.69-1.45
3 male (200.4-224.8) Female (176.6-199.9)	83	9,865.3	1.30	0.92-1.84	1.27	0.89-1.80
4 male (224.9-259.1) female (200-233.1)	69	9,882.7	1.10	0.77-1.58	1.07	0.75-1.54
5 male (>259.1) female (>233.1)	88	9,950.6	1.44	1.02-2.03	1.37	0.97-1.94
P value for linear trend			0.035		0.07	

*Multivariate model was adjusted for age (y), sex, current smoking (yes/no), number of cigarettes smoked per day, number of years smoked and intake of added sugar (grams/d).

Table 4. HRs of pancreatic cancer, with 95% CIs, according to quintiles of energy-adjusted folate vitamer intake; NLCS, 1986-1999

Vitamer, μg/d	Quintiles of folate vitamer intake, HR (95% CI)					*P* for linear trend
	Q1	Q2	Q3	Q4	Q5	
Monoglutamates	1.00	0.90 (0.62-1.30)	1.05 (0.73-1.51)	1.07 (0.74-1.55)	1.09 (0.76-1.58)	0.46
Polyglutamates	1.00	0.82 (0.56-1.20)	1.06 (0.74-1.53)	1.04 (0.72-1.51)	1.13 (0.78-1.64)	0.28
Tetrahydrofolate	1.00	0.94 (0.66-1.34)	0.72 (0.50-1.04)	0.87 (0.61-1.25)	1.08 (0.76-1.54)	0.77
5-Methyl-tetrahydrofolate	1.00	0.86 (0.59-1.24)	0.96 (0.67-1.38)	0.92 (0.63-1.35)	1.07 (0.72-1.58)	0.56
5-Formyl-tetrahydrofolate	1.00	0.82 (0.56-1.20)	0.90 (0.61-1.32)	1.05 (0.72-1.55)	0.99 (0.66-1.49)	0.45
Folic acid	1.00	0.84 (0.59-1.20)	0.80 (0.55-1.16)	0.84 (0.57-1.24)	1.05 (0.73-1.52)	0.70
10-Formyl-dihydrofolate	1.00	1.41 (0.97-2.04)	1.19 (0.80-1.78)	1.34 (0.90-1.99)	1.40 (0.92-2.13)	0.29
10-Formyl-folate	1.00	1.11 (0.76-1.61)	1.27 (0.87-1.85)	1.24 (0.84-1.83)	0.99 (0.66-1.49)	0.94

NOTE: Multivariate models were adjusted for age (y), sex, current smoking (yes/no), number of cigarettes smoked per day, number of years smoked and intake of added sugar (grams/day).

study from four American cohorts did not show statistically significant association between plasma folate levels and risk of pancreatic cancer (20). However, a limitation of this study is the possible effect of mandatory folic acid fortification of grains in the United States. In a prospective cohort of Swedish men and women, pancreas cancer incidence was found to be inversely related to folate intake from food sources (rate ratio, 0.25; 95% CI, 0.11-0.59; ref. 18). In case-control studies, inverse association, as well as lack of association between dietary folate and pancreatic cancer, has been reported (15, 16). Relatively lower levels, and less variation in folate intake in our study population compared with previous studies [≤280 μg/day versus >373 μg/day (17); <200 μg/day versus ≥350 μg/day (18); <300 μg/day versus ≥500 μg/day (19)] could possibly explain the dissimilar results in different studies, although association has been reported in a relatively small range and lack of association in relatively wider range of folate intake as well (18, 19). A possible explanation of the different range in folate intake in our study is the lack of folate supplementation of foods and vitamin supplements in the Netherlands.

Possible mechanisms involved in the effect of folate on pancreatic cancer include disturbances in DNA methylation, DNA synthesis, and repair (10). Low folate levels may lead to DNA strand breaks and may impair DNA repair (38). Folate deficiency may also reduce S-adenosylmethionine levels, which is the universal methyl donor for methylation of nucleic acids. DNA hypomethylation and hypermethylation has been detected in pancreatic cancer (39). Hypomethylation has been associated with overexpression, whereas hypermethylation of suppressor gene promoters has been associated with transcriptional silencing in pancreatic cancer. It is a plausible theory that folate deficiency induces abnormal DNA methylation in the pancreas, but it has not been proven.

Overall, research regarding the effect of folate on carcinogenesis in general and risk of pancreatic cancer in particular suggests a protective effect of folate. However, some animal studies support the possibility that folate deficiency may reduce carcinogenesis (40, 41), and some epidemiologic studies suggest that high folate levels increase cancer risk (42). A dual effect of folate on carcinogenesis has been suggested by recent research (43, 44), which may explain some of the inconsistencies regarding the effect of folate. One of the possible mechanism by which folate may promote the progression of precursor lesions is by providing nucleotide precursors to rapidly replicating neoplastic cells. Folate may also contribute to gene inactivation of tumor suppressor genes by promoting *de novo* methylation of promoter CpG islands (43). We did an analysis excluding cases that occurred in the first 2 years of follow-up. The outcome of this analysis was not substantially different from the primary analysis. This also suggests that the possible effects of preclinical symptoms of undiagnosed pancreatic cancer on dietary habits have not influenced our results.

To our knowledge, this is the first study to assess the association between individual folate vitamers in diet and the risk of pancreatic cancer. We did not find an association with polyglutamates or monoglutamates nor with specific vitamers. It has been reported that different

Table 5. HR of pancreatic cancer, with 95% CIs, according to tertiles of energy-adjusted folate intake for never-, ex- and current-smokers in NLCS, 1986-1999

Tertiles of folate intake, μg/d	Never-smoker				Ex-smoker				Smoker			
	Cases	Person years in subcohort	HR	95% CI	Cases	Person years in subcohort	HR	95% CI	Cases	Person years in subcohort	HR	95% CI
1 male (<193.2) female (<170.6)	23	6,044	1.00	(Reference)	39	5,668	1.47	0.78-2.78	42	4,526	1.94	0.90-4.17
2 male (193.2-236.3) Female (170.6-209)	38	6,653	1.43	0.84-2.44	51	5,863	1.79	0.91-3.51	38	3,959	1.88	0.88-4.03
3 male (>236.3) female (>209)	42	6,064	1.83	1.08-3.12	43	5,988	1.48	0.78-2.83	47	4,216	2.25	1.06-4.78
P value for linear trend	0.12				0.34				0.57			

NOTE: Models were adjusted for age (y), sex, number of cigarettes smoked per day, number of years smoked and intake of added sugar (grams/d). $P_{interaction} = 0.34$.

folate vitamers show varied bioavailability (45). Thus, it is conceivable that folate vitamers are different in terms of their association with cancer risk. In another analysis of folate vitamers within the NLCS, 5-formyltetrahydrofolate intake showed an inverse association with colon carcinoma risk (46).

In contrast to dietary folate, association of pancreatic cancer risk with folic acid supplement in multivitamins was not shown in previous cohort studies (17-19). We did not test for such an association, however, because in the Netherlands, folic acid in vitamin supplements was not allowed until the middle of the nineties, therefore the effect of folic acid supplementation in this cohort is negligible.

We did not find an indication of interaction between alcohol or smoking status and folate intake in relation to the occurrence of pancreatic cancer. This result is consistent with previous findings (18), although interaction has been shown for breast and colon cancers (23-26). Studies with larger sample size will be needed to assess interaction with smoking and alcohol consumption.

Strengths of this study include its prospective design, the availability of information on numerous potential confounders, the nearly complete follow-up of the study population through linkage to cancer registries, and the use of a compiled folate database, with food folate values, which was established using a validated trienzyme high-performance liquid chromatography method. The prospective design eliminated recall bias, and the extent of follow-up minimized the possibility that differential loss to follow-up would have affected our results.

We have reduced possible bias introduced by misclassification of disease through a secondary analysis of only microscopically confirmed cases. This analysis did not indicate difference compared with the primary analysis, suggesting that disease misclassification is probably not a major issue in our study. In previous research looking at the association of anthropometric factors and risk of pancreatic cancer, BMI was associated to pancreatic cancer only in microscopically confirmed cases, emphasizing the importance of careful evaluation of disease status (47).

The possibility of measurement error due to the use of food-frequency questionnaire is a limitation of our study. Misclassification of exposure could have attenuated true association. However, it is unlikely to have happened across extreme levels of folate intake. We also cannot entirely exclude the possibility of residual confounding by unmeasured variables, such as for example vitamin B12, which might have masked a true association.

In summary, we have observed a nonsignificant positive association between dietary folate intake and the incidence of pancreatic cancer in a population-based prospective study of Dutch individuals. Future studies will have to address whether dietary folate or some other factor associated with it is related to the risk of pancreatic cancer, as studies thus far show inconsistent results, and larger studies are needed for assessing interaction with cigarette smoking and alcohol consumption. Our results also add to the international debate on the issue of the benefits of fortification of food products with folate.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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We thank the participants of this study and further wish to thank the cancer registries (IKA, IKL, IKMN, IKN, IKO, IKR, IKST, IKW, IKZ, and VIKC), and the Netherlands nationwide registry of pathology (PALGA); L. Schouten for methodologic advice and NLCS database support; Dr. A. Volovics and Dr. A. Kester for statistical advice; S. van de Crommert, H. Brants, J. Nelissen, C. de Zwart, W. van Dijk, M. Jansen, and A. Pisters for assistance; and H. van Montfort, T. van Moergastel, L. van den Bosch, J. Berben for programming assistance.

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