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Intake of Methyl-Related Nutrients and Risk of Pancreatic Cancer in a Population-Based Case-Control Study in Minnesota

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Abstract

Background—Folate, vitamin B_6, vitamin B_{12}, and methionine are involved in DNA synthesis and methylation and thus may modulate pancreatic cancer risk. We investigated these associations in a population-based case-control study conducted in 1994–1998.

Methods—Cases (n=150) were identified from all hospitals in the metropolitan areas of the Twin Cities and the Mayo Clinic, Minnesota. Controls (n=459) were selected randomly from the general population and were frequency matched to cases by age, sex, and race. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for risk of pancreatic cancer in relation to intake of nutrients considered.

Results—Dietary intake of folate was associated with a reduced pancreatic cancer risk [OR (95% CI) for quartile (Q) 4 vs. Q1: 0.31 (0.12–0.78)]. A composite score (range from 2 to 8), reflecting combined dietary intake of folate and vitamin B_6, was also inversely associated with pancreatic cancer risk [OR (95% CI) for Q4 vs. Q1: 0.24 (0.08–0.70)]. Null associations were found for intake of vitamin B_{12} and methionine.

Conclusions—Dietary folate intake was associated with a reduced pancreatic cancer risk, and this association became stronger when dietary intake of folate and vitamin B_6 was combined in analysis.

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Introduction

Pancreatic cancer is one of the deadliest cancers primarily because it lacks an effective screening test and is usually diagnosed at an advanced stage (1, 2). The 5-year survival rate is only 8.2% in the U.S. (3). In 2017, there were an estimated 53,670 cases of pancreatic cancer and 43,090 deaths from this disease in the U.S. (4). Despite advances in medical treatment, pancreatic cancer mortality rates in the U.S. have continued to climb in recent years (5). Therefore, primary prevention remains a top priority for reducing the burden of pancreatic cancer. To achieve this goal, it is necessary to elucidate its largely unknown etiology.

Diet may play a role in the etiology of pancreatic cancer (6). It is estimated that 30–50% of pancreatic cancer cases are attributable to dietary factors or practices (7). Of potential significance to pancreatic cancer risk are dietary nutrients involved in methyl-group metabolism, including folate, vitamin B_6 (pyridoxine), vitamin B_{12} (cobalamin), and methionine. Folate participates in the conversion of homocysteine to methionine, a biochemical reaction catalyzed by methionine synthase that has vitamin B_{12} as a cofactor (8, 9). As an essential amino acid in the human diet, methionine serves as a methyl-group donor in the form of S-adenosylmethionine. Vitamin B_6 is a cofactor for multiple critical enzymes in the methyl-group metabolism pathway (10). Given that all these nutrients are required in DNA synthesis and methylation (11), it is possible that they are implicated in the etiology of pancreatic cancer. This hypothesis has gained support from experimental studies that revealed frequently aberrant DNA methylation in some pancreatic tumors and cancer cell lines (12, 13).

Despite the biological plausibility, the associations between intake of nutrients involved in methyl-group availability and the risk of pancreatic cancer have been inconsistent across previous studies, with both inverse and null associations reported (14–18). The present analysis was thus conducted to investigate the associations between dietary and supplementary intake of folate, vitamin B_6, vitamin B_{12}, and methionine and the risk of pancreatic cancer in a population-based case-control study in Minnesota.

Materials and Methods

Study Population

A population-based case-control study of pancreatic cancer was conducted in Minnesota from April 1994 to September 1998, and its design and methodology have been described in detail elsewhere (19, 20). Briefly, this study was based in the Upper Midwestern United States and the cases were recruited from all hospitals in the Minneapolis and St. Paul metropolitan areas in Minnesota and the Mayo Clinic, Rochester, Minnesota. Cases enrolled from the Mayo Clinic were restricted to residents in the Upper Midwest. Cases were patients with a recent diagnosis of pathologically-confirmed pancreatic cancer (*International Classification of Disease for Oncology*, 3rd edition, code C25). To be eligible, the patients had to be at least 20 years of age, proficient in English, and mentally competent. As many pancreatic cancer patients die quickly after diagnosis, a rapid case recruitment procedure was employed to recruit cases, resulting in a median number of 13 days between diagnosis

and first contact for enrolled cases. A total of 460 identified cases met the eligibility criteria. Of these, 85 did not participate due to death prior to being contacted or interviewed, 79 refused participation, 31 were disallowed by their physician, and 7 could not be reached or contacted. After these exclusions (n = 202 in total), 258 cases participated in the study, yielding a response rate of 56%.

Controls were selected randomly from the same metropolitan areas of Minneapolis and St. Paul, Minnesota. Controls between 20 and 64 years of age were identified from a database of drivers' licenses and state identity cards. Controls of at least 65 years of age were found in the database of the Centers for Medicare and Medicaid Services. Controls had the same inclusion criteria as cases, disallowing pancreatic cancer diagnosis. Frequency matching was used to match controls to cases by age (within 5 years), sex, and race. Of 1,141 eligible controls ascertained, 676 chose to participate in the study, resulting in a response rate of 59%.

Data on diet and alcohol consumption were not available from 108 cases and 217 controls primarily due to the frailty of cases to endure the interview process or controls declining to complete the food frequency questionnaire (FFQ). In total, 150 cases and 459 controls provided the data for the present analysis.

Data Collection

The University of Minnesota and the Mayo Clinic institutional review boards approved the study protocol, and written informed consent was collected from all study participants before the interview. A general questionnaire was used to solicit data on demographic characteristics (e.g. age, sex, and race), socioeconomic factors (e.g. education), and lifestyle factors (e.g. status, amount, and duration of cigarette smoking, intensity and duration of physical activity) as well as personal history of disease (e.g. diabetes). The usual diet of study subjects was assessed with a slightly modified version of the Willett FFQ (21). Validation studies have shown that the Willett FFQ offers reasonable levels of reproducibility and validity (against dietary records) for intakes of nutrients and individual foods (21, 22). Both the general questionnaire and FFQ were administered by trained research staff during in-person interviews.

The FFQ used in this case-control study is composed of 153 individual foods or food groups (including alcohol consumption) commonly consumed in the U.S. During the interview, subjects recalled how frequently they consumed each of the food items listed in the FFQ in the year preceding pancreatic cancer diagnosis for cases or in the previous year for controls. Energy and nutrient intake was calculated by multiplying the pre-specified portion size amount in each food item by the recalled frequency of consumption and summed over all food items. The Minnesota Colon Cancer Prevention Research Unit Studies database was employed to estimate the amounts of energy and nutrients contained in portion sizes of all food items included in the FFQ used. In the present study, the nutrients evaluated in relation to pancreatic cancer risk are folate, vitamin B_6, vitamin B_{12}, and methionine. Data on both dietary and supplemental sources of all these nutrients (except methionine) were available for analysis.

Statistical Analysis

Cases and controls were compared for differences in age, sex, race, education, cigarette smoking, alcohol intake, physical activity, diabetes history, and insulin use. *Chi*-square and t-tests were used to examine differences in categorical and continuous variables, respectively. Differences in dietary and total intake of selected nutrients between cases and controls were evaluated with t-tests.

Logistic regression analysis was performed to estimate pancreatic cancer risk in relation to the nutrients of interest. Dietary and total intake of folate, vitamin B_6, and vitamin B_{12} as well as dietary intake of methionine were each divided into quartiles using cutoff-points based on the respective intake data of the controls. Subjects in the lowest (first) quartile for each of these dietary variables were treated as the reference group to calculate the odds ratio (OR) and 95% confidence interval (CI) for those in the three upper quartiles. Three regression models were constructed for each of the nutrients considered. Model 1 was built to estimate the crude associations between intake of each of selected nutrients and the risk of pancreatic cancer. Adjusted ORs and 95% CIs were calculated for those associations in model 2 and model 3. Age (continuous), sex, race (white, black, and other), education (three levels), cigarette smoking (never, former, and current), alcohol consumption (serving/week), and physical activity (light, moderate, and heavy) were controlled for in model 2. Model 3 additionally adjusted for intake of energy, fat, fiber, fruits, and vegetables. The aforementioned covariates were introduced into models 2 and 3 as established or suspected confounders to assess the independent associations between methyl-group availability factors and pancreatic cancer risk. The statistical significance of the linear trend across quartiles of each of the nutrients examined was tested by assigning a median intake value to each quartile and then treating these as values of a continuous variable.

A composite score was created to evaluate the effect of combined dietary intake of folate and vitamin B_6 on pancreatic cancer due to the shared roles of these two nutrients in DNA methylation through the regulation of circulating homocysteine concentrations (23). Specifically, 1, 2, 3, and 4 were assigned to subjects in quartiles 1, 2, 3, and 4 of dietary folate, respectively. The same method was applied to dietary intake of vitamin B_6. The composite score for each subject was then calculated by summing a subject's values assigned to those two nutrients; scores ranged from 2 (lowest) to 8 (highest). All statistical analyses were performed by using SAS (version 9.4; SAS Institute Inc., Cary, NC). A p-value of <0.05 (2-sided) was considered statistically significant.

Results

Study subjects were predominantly white, with a mean age of 65.8 years for cases and 66.5 years for controls. Cases, versus controls, were more likely to be current smokers and diagnosed with type-2 diabetes; cases also reported lower levels of education and physical activity (Table 1). Cases appeared to have a lower dietary intake of both folate and vitamin B_6 than controls (folate: 320 vs. 351 μg/day, p=0.041; vitamin B_6: 6.17 vs. 9.53 mg/day, p=0.084) (Table 2).

After adjustment for all covariates included in model 3, dietary intake of folate was associated with a reduced risk of pancreatic cancer (Table 3). Compared with subjects in the first quartile of dietary intake of folate, ORs (95% CIs) for those in the second, third, and fourth quartiles were 0.63 (0.35–1.14), 0.77 (0.40–1.49), and 0.31 (0.12–0.78) (p-trend = 0.036), respectively. A similar inverse association was observed for total intake of folate (i.e. folate from both dietary and supplemental sources) [OR (95% CI) for the third vs. the first quartile: 0.47 (0.23–0.90)], but this inverse association was not observed when the fourth and the first quartiles of total intake of folate were compared. It appeared that total intake of vitamin B_6 was inversely, and total intake of vitamin B_{12} was positively, associated with pancreatic cancer risk, but these associations were not statistically significant. There are no apparent associations between dietary intake of methionine and risk of pancreatic cancer. Compared with subjects in the first quartile of the composite score, those in the fourth quartile of the score exhibited a 76% reduced risk of pancreatic cancer [OR (95% CI): 0.24 (0.08–0.70)] (p-trend = 0.024) (Table 4).

As a dietary source of folate and vitamin B_6, fruits and vegetables were removed from the multivariable regression models to avoid potential overadjustment. The risk estimates observed remained materially unchanged after exclusion of these food items. As the number of non-whites was very small, the risk estimates obtained were virtually the same when non-whites were excluded from the analysis.

Discussion

The primary findings of the present analysis were that dietary intake of folate was associated with a reduced risk of pancreatic cancer and that a composite score (combined dietary intake of folate and vitamin B_6) was also inversely associated with pancreatic cancer risk. The tests for trend in the adjusted models were statistically significant for dietary folate and the composite score.

Our results suggest a potential protective effect of dietary folate intake on pancreatic cancer risk and are consistent with results from an analysis of two Swedish cohorts (15) and the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study (16). The magnitude of the inverse association in our study (OR = 0.31 for 500 vs. 195 μg/day) was similar to the Swedish study (RR = 0.25 for >350 vs. <200 μg/day) but somewhat stronger than the ATBC study (RR = 0.52 for >373 vs. <280 μg/day). Of note, the validity of the dietary folate results observed in the ATBC study were strengthened when analyses were conducted with serum folate concentrations from study participants (9).

An inverse association between folate intake and pancreatic cancer risk was not found in the Health Professionals Follow-up Study (HPFS), the Nurses' Health Study (NHS), or a combined analysis of 14 cohort studies (17, 24). It should be pointed out that 10 of the 14 cohorts were conducted among U.S. residents with a high prevalence of multivitamin use (e.g. 43% in HPFS and NHS, and 56% in the California Teachers Study). It may be that the beneficial effect of folate intake is primarily confined to subjects with a relatively low intake of this nutrient, e.g. residents of European countries where the percentage of multivitamin use is not high (25–27).

In the present study, dietary intake of folate was associated with a reduced risk of pancreatic cancer, but this inverse association was not observed for total intake of folate. A similar difference in the effects of folate from dietary and supplemental sources was reported in the ATBC study (16) and the Swedish cohorts (15), although it was not confirmed in a case-control study in the San Francisco Bay Area (14). The reasons for these discrepant results are unclear. One possible explanation is that dietary intake of folate is a better measure of long-term exposure to folate rather than recent or irregular use of supplemental folic acid and thus is more relevant to the etiology of pancreatic cancer (15).

We found an inverse, though not statistically significant, association of both dietary intake and total intake of vitamin B_6 with the risk of pancreatic cancer [(OR (95% CI) comparing the fourth with the first quartiles: 0.47 (0.19–1.17) and 0.66 (0.34–1.24), respectively). Previous studies have yielded mixed results on the effect of vitamin B_6 on pancreatic cancer (10). Intake of vitamin B_6 or levels of its circulating biomarker (pyridoxal-5′-phosphate) were associated with a reduced risk of pancreatic cancer in some (9, 18, 28), but not all (14, 16, 29), studies. A meta-analysis showed that dietary intake of vitamin B_6 was significantly associated with the risk of pancreatic cancer as well as esophageal, gastric, and colorectal cancers [(OR (95% CI) comparing the extreme categories: 0.57 (0.47–0.69) for gastrointestinal tract cancers] (10). The specific role of vitamin B_6 in pancreatic carcinogenesis warrants further investigation. As dietary intakes of both folate and vitamin B_6 were inversely associated with pancreatic cancer risk in our study, we examined their combined effect for the reasons mentioned previously. Our analysis revealed that the composite score, derived from intake of these two nutrients, was more strongly associated with the risk of this disease than either nutrient alone. This analytic approach has not been used in previous studies.

Several lines of experimental evidence provide biochemical mechanisms supporting a role for folate and vitamin B_6 in pancreatic cancer. The pancreas contains the highest concentrations of folate after the liver (30). Folate and vitamin B_6 are key nutrients required for adequate DNA methylation (11). Abnormal DNA methylation may alter expression of proto-oncogenes and tumor suppressor genes (31, 32). The hypermethylation and hypomethylation of several dozens of genes have been detected in pancreatic tumor and cancer cell lines (12). In addition, folate is also critical for DNA synthesis. Human studies have revealed that folate deficiency resulted in misincorporation of uracil into DNA and chromosome breakage, and folate supplementation could effectively reduce the occurrence of these DNA lesions (33, 34).

Relatively few epidemiological studies have evaluated the associations between intake of vitamin B_{12} and methionine and the risk of pancreatic cancer. Our study did not show any significant associations between these two nutrients and the risk of this malignancy, despite elevated ORs. Null results for vitamin B_{12} have been also reported in several other studies (16, 18, 28, 35). However, a significantly increased risk associated with vitamin B_{12} intake was found among subjects in the case-control study in the San Francisco Bay Area (14) and among people who smoked 20 cigarettes or less per day in the ATBC study (9). An inverse association between plasma levels of vitamin B_{12} and the risk of pancreatic cancer was found among Finnish smokers who were non-users of multivitamins and had a median body

mass index of <24.7 (ref. 8), but the possibility of chance finding in this Finnish study could not be ruled out due to multiple comparisons.

No significant association between methionine intake and pancreatic cancer risk existed in the present study, which was consistent with the results of one case-control study (14) and three cohort studies (16, 18, 28). A significant inverse association between methionine intake and pancreatic cancer risk was observed in a cohort study of Swedish men and women (29), but this potential beneficial effect was not replicated in the European Prospective Investigation into Cancer and Nutrition in which plasma levels of methionine were measured as a biomarker of dietary intake (28).

Our study has several strengths. All cases were identified through a rapid case-ascertainment system to avoid proxy interviews that are prone to recall bias. Proxy interviews have been used in some case-control studies of pancreatic cancer due to its rapid fatality (36, 37). In our study, in-person interviews were performed by trained research staff. To help study subjects accurately estimate serving sizes for foods they consumed, food models were used.

Our study has some weaknesses. A response rate of less than 60% was obtained for both cases and controls. Such rates have been reported in other case-control studies of pancreatic cancer (35, 38–40). Nevertheless, subjects who agreed to participate in the study might be different from those who refused with regard to demographic, socioeconomic, and lifestyle factors, limiting the generalization of our obtained results. Recall bias is always a concern in case-control studies. Recall of dietary habits among cases might have been affected by dietary changes in response to clinical symptoms and/or medical treatments of the disease. Therefore, reverse causality could not be entirely ruled out for our observed significant inverse associations shown in Table 3 and Table 4. The Willett FFQ has been validated against dietary record for vitamin B_6 (r=0.58, p<0.05) (ref. 41) and against plasma folate levels for folate (deattenuated r = 0.54; 95% CI: 0.46–0.61) (ref. 42) but not for vitamin B_{12}. Therefore, our reported results would be strengthened if intake of the three vitamins examined were validated against their corresponding biomarkers or if these biomarkers were evaluated in relation to pancreatic cancer risk. Dietary assessment error derived from the FFQ used might have resulted in misclassification of subjects with regard to their dietary intake of the nutrients evaluated in the present study. Such misclassification error, if non-differential, tends to bias risk estimates toward the null. Betaine and choline are also nutrients involved in methyl-group metabolism, but were not evaluated in this study due to lack of data. Biomarkers, i.e., plasma concentrations of folate, vitamin B_6, and vitamin B_{12}, were not available in our study. Although body mass index has been associated with pancreatic cancer (43), we did not adjust for it in our analysis as subject height and weight were not measured due to an oversight. However, the risk estimates reported were controlled for both energy intake and physical activity, the two main factors that determine the development of overweight and obesity.

In this population-based case-control study, we found that dietary intake of folate and a composite score reflective of dietary intake of folate and vitamin B_6 were associated with a reduced risk of pancreatic cancer. The present study provides additional evidence for the role of methyl-related nutrients in the etiology of pancreatic cancer. Our findings need to be

confirmed by future studies that not only assess intake of these nutrients, but also measure their reliable biochemical indicators among populations with various dietary habits. Research in this area is expected to offer novel avenues for the primary prevention and control of pancreatic cancer.

Acknowledgments

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Table 1

Characteristics of cases and controls in a population-based case-control study of pancreatic cancer in Minnesota, 1994–1998a

Characteristics	Cases (n=150)	Controls (n=459)	*p*-value
Age (year)	65.8 (10.9)	66.5 (12.1)	0.13
Sex			
Male	89 (59.3%)	261 (56.9%)	
Female	59 (39.3%)	198 (43.1%)	
Missing	2 (1.3%)	0 (0%)	0.48
Race			
White	137 (91.3%)	450 (98.0%)	
Black	7 (4.7%)	3 (0.7%)	
Other	5 (3.3%)	6 (1.3%)	
Missing	1 (0.7%)	0 (0%)	0.0003
Education			
Some high school or less	25 (16.7%)	56 (12.2%)	
High school graduate	56 (37.3%)	116 (25.3%)	
Some college or more	67 (44.7%)	287 (62.5%)	
Missing	2 (1.3%)	0 (0%)	0.0019
Cigarette Smoking			
Never smoked	57 (38.0%)	215 (46.8%)	
Former smoker	63 (42.0%)	196 (42.7%)	
Current smoker	23 (15.3%)	48 (10.5%)	
Missing	7 (4.7%)	0 (0%)	0.062
Alcohol intake (serving/week)	3.4 (6.9)	4.7 (8.5)	0.065
Physical activity (hour/week)			
Light	23.0 (16.9)	27.1 (16.2)	0.013
Moderate	15.1 (13.1)	18.1 (12.7)	0.022
Heavy	5.1 (11.8)	3.9 (5.5)	0.27
Total	43.3 (27.7)	49.1 (25.4)	0.025
Diabetes history			
Yes	31 (20.7%)	33 (7.2%)	
No	101 (67.3%)	426 (92.8%)	
Unknown/missing	18 (12.0%)	0 (0%)	<0.0001
Insulin use			
Yes	13 (8.7%)	13 (2.8%)	
No	12 (8.0%)	20 (4.4%)	
Unknown/missing	125 (83.3%)	426 (92.8%)	0.019

[a]Data shown are mean (SD) for continuous variables or n (%) for categorical variables.

Table 2

Intake of methyl-related nutrients in a population-based case-control study of pancreatic cancer in Minnesota, 1994–1998

Nutrients	Cases (n=150)	Controls (n=459)	*p*-value
	Mean (SD)	**Mean (SD)**	
Dietary folate (μg/day)	320 (170)	351 (155)	0.041
Total folate (μg/day)	470 (299)	497 (277)	0.31
Dietary vitamin B_6 (mg/day)	2.24 (1.26)	2.43 (1.09)	0.11
Total vitamin B_6 (mg/day)	6.17 (18.5)	9.53 (26.0)	0.084
Dietary vitamin B_{12} (μg/day)	7.2 (4.9)	7.0 (6.7)	0.77
Total vitamin B_{12} (μg/day)	12.3 (15.8)	11.0 (13.5)	0.36
Methionine (g/day)	1.88 (0.8)	1.94 (0.9)	0.46

Table 3

Risk of pancreatic cancer in relation to intake of folate, vitamin B$_6$, vitamin B$_{12}$, and methionine in a population-based, case-control study of pancreatic cancer in Minnesota, 1994–1998

Nutrients		Quartile			*p*-trend
	First	Second	Third	Fourth	
Dietary folate					
Median (μg/day)	195	288	375	500	
Cases/controls	54/115	34/115	38/115	24/114	
Crude OR (95% CI)	1.00	0.63 (0.38–1.04)	0.70 (0.43–1.14)	**0.49 (0.26–0.77)**	0.007
Adjusted OR1 (95% CI)[a]	1.00	0.66 (0.38–1.15)	0.89 (0.52–1.52)	**0.42 (0.21–0.79)**	0.026
Adjusted OR2 (95% CI)[b]	1.00	0.63 (0.35–1.14)	0.77 (0.40–1.49)	**0.31 (0.12–0.78)**	0.036
Total folate					
Median (μg/day)	230	357	529	790	
Cases/controls	52/115	34/115	25/115	39/114	
Crude OR (95% CI)	1.00	0.65 (0.39–1.08)	**0.48 (0.28–0.82)**	0.76 (0.46–1.23)	0.28
Adjusted OR1 (95% CI)[a]	1.00	0.67 (0.39–1.16)	**0.45 (0.24–0.84)**	0.71 (0.41–1.24)	0.21
Adjusted OR2 (95% CI)[b]	1.00	0.69 (0.38–1.22)	**0.47 (0.23–0.90)**	0.73 (0.38–1.37)	0.37
Dietary vitamin B$_6$					
Median (mg/day)	1.38	2.04	2.53	3.36	
Cases/controls	52/115	34/115	34/115	30/114	
Crude OR (95% CI)	1.00	0.65 (0.39–1.08)	0.65 (0.39–1.08)	**0.58 (0.34–0.97)**	0.043
Adjusted OR1 (95% CI)[a]	1.00	0.58 (0.33–1.02)	0.64 (0.36–1.11)	0.57 (0.32–1.02)	0.066
Adjusted OR2 (95% CI)[b]	1.00	0.57 (0.31–1.03)	0.61 (0.31–1.19)	0.47 (0.19–1.17)	0.10
Total vitamin B$_6$					
Median (mg/day)	1.62	2.65	3.94	7.20	
Cases/controls	52/116	33/116	30/113	35/114	
Crude OR (95% CI)	1.00	0.64 (0.38–1.05)	**0.59 (0.35–0.99)**	0.69 (0.41–1.13)	0.26
Adjusted OR1 (95% CI)[a]	1.00	0.67 (0.38–1.15)	0.57 (0.32–1.01)	0.66 (0.37–1.17)	0.21
Adjusted OR2 (95% CI)[b]	1.00	0.68 (0.37–1.22)	0.56 (0.29–1.10)	0.66 (0.34–1.24)	0.32

Nutrients	Quartile				
	First	Second	Third	Fourth	*p*-trend
Dietary vitamin B$_{12}$					
Median (µg/day)	2.7	4.4	6.6	12.2	
Cases/controls	32/115	39/116	33/115	46/113	
Crude OR (95% CI)	1.00	1.21 (0.71–2.07)	1.03 (0.59–1.79)	1.46 (0.87–2.48)	0.17
OR2 (95% CI)[a]	1.00	1.26 (0.69–2.33)	1.32 (0.72–2.47)	1.42 (0.78–2.60)	0.32
OR3 (95% CI)[b]	1.00	1.38 (0.74–2.59)	1.62 (0.83–3.19)	2.03 (0.98–4.26)	0.080
Total vitamin B$_{12}$					
Median (µg/day)	3.6	6.2	10.0	17.8	
Cases/controls	37/115	32/115	28/115	53/114	
Crude OR (95% CI)	1.00	0.87 (0.50–1.48)	0.76 (0.43–1.32)	1.45 (0.89–2.38)	0.058
Adjusted OR1 (95% CI)[a]	1.00	1.15 (0.64–2.08)	0.78 (0.41–1.46)	1.38 (0.79–2.44)	0.30
Adjusted OR2 (95% CI)[b]	1.00	1.18 (0.65–2.16)	0.90 (0.46–1.73)	1.66 (0.89–3.15)	0.10
Methionine					
Median (g/day)	1.10	1.55	2.05	2.78	
Cases/control	42/118	36/113	37/114	35/114	
Crude OR (95% CI)	1.00	0.90 (0.53–1.50)	0.91 (0.55–1.52)	0.86 (0.51–1.45)	0.61
Adjusted OR1 (95% CI)[a]	1.00	1.05 (0.60–1.85)	1.01 (0.57–1.77)	0.82 (0.45–1.48)	0.48
Adjusted OR2 (95% CI)[b]	1.00	1.12 (0.61–2.05)	1.14 (0.58–2.26)	0.91 (0.37–2.27)	0.87

OR, odds ratio; CI, confidence interval.

[a] Adjusted for age, sex, race, education, cigarette smoking, alcohol consumption, and total physical activity.

[b] Additionally adjusted for intake of energy, total fat, fiber, vegetables, and fruits.

Table 4

Composite score for combined dietary intake of folate and vitamin B_6 in a population-based, case-control study of pancreatic cancer in Minnesota, 1994–1998

| | Quartiles of Composite Score | | | | |
	First (reference)	Second	Third	Fourth	p-trend
Cases/controls	60/133	38/129	35/115	17/82	
Crude OR (95% CI)	1.00	0.65 (0.41–1.04)	0.68 (0.41–1.09)	**0.46 (0.25–0.83)**	0.011
Adjusted OR1 (95% CI)[a]	1.00	**0.57 (0.33–0.91)**	0.76 (0.45–1.29)	**0.41 (0.19–0.81)**	0.026
Adjusted OR2 (95% CI)[b]	1.00	**0.52 (0.29–0.92)**	0.60 (0.30–1.21)	**0.24 (0.08–0.70)**	0.024

OR = odds ratio; CI = confidence interval.

[a] Adjusted for age, sex, race, education, cigarette smoking, and alcohol consumption.

[b] Additionally adjusted for intake of energy, total fat, fiber, vegetables, and fruits.