Folate Intake and Pancreatic Cancer Incidence: A Prospective Study of Swedish Women and Men

Susanna C. Larsson, Niclas Håkansson, Edward Giovannucci, Alicja Wolk

**Background:** Epidemiologic evidence supports an association between high folate intake and reduced risk of some cancers, in particular colorectal cancer. However, epidemiologic data concerning the relationship between folate and pancreatic cancer risk are sparse. We examined the association between folate intake and risk of pancreatic cancer in a population-based prospective study of Swedish women and men. **Methods:** We prospectively followed 81 922 women and men in the Swedish Mammography Cohort and the Cohort of Swedish Men who were cancer-free and completed a 96-item food-frequency questionnaire in 1997. Cox proportional hazards models were used to estimate multivariable rate ratios (RRs) with 95% confidence intervals (CIs). All statistical tests were two-sided. **Results:** A total of 135 incident pancreatic cancer cases were diagnosed during a mean follow-up of 6.8 years. In multivariable analyses controlling for age, smoking, fruit and vegetable consumption, and other potential confounders, dietary and total folate intakes were statistically significantly inversely associated with risk of pancreatic cancer. The multivariable rate ratios of pancreatic cancer for those in the highest category of folate intake (>350 μg/day) compared with the lowest category of intake (<200 μg/day) were 0.25 (95% CI = 0.11 to 0.59; P trend = .002) for dietary folate and 0.33 (95% CI = 0.15 to 0.72; P trend = .01) for total folate (combining dietary and supplemental sources). Folic acid from supplements was not associated with pancreatic cancer (for ≥300 μg/day compared with 0 μg/day of supplemental folic acid, multivariable RR = 1.02; 95% CI = 0.56 to 1.88). The sex- and age-standardized incidence rates of pancreatic cancer per 100 000 person-years were 41 for the lowest and 18 for the highest category of dietary folate intake. **Conclusion:** Our results suggest that increased intake of folate from food sources, but not from supplements, may be associated with a reduced risk of pancreatic cancer. [J Natl Cancer Inst 2006;98:407–13]

The role of folate in carcinogenesis has attracted increasing attention. Folate is an important factor for DNA methylation and for the biosynthesis of nucleotides needed for DNA synthesis and repair (1,2). Mounting evidence from experimental and human studies indicates that folate deficiency is associated with aberrant DNA methylation, DNA strand breaks, decreased DNA repair activities, and increased mutation rates and that folate supplementation can correct some of these defects (1). Thus, deficient folate intake may predispose individuals to cancer as a consequence of disruption of DNA methylation, synthesis, and/or repair.

Several epidemiologic studies have suggested a protective role of folate against colorectal, breast, and other cancers (3–14), especially among individuals with high alcohol consumption. Only a few studies, however, have considered the possible influence of folate on pancreatic cancer risk, and the results have been inconsistent. In a cohort of male Finnish smokers, dietary folate intake and serum folate levels were inversely associated with risk of pancreatic cancer (15,16). A reduction in pancreatic cancer risk associated with a high dietary folate intake was also observed in a prospective study of U.S. nurses and health professionals, but the findings were not statistically significant (17). There are no prospective data on the association of folate with risk of pancreatic cancer in a general population. We therefore examined folate intake in relation to the incidence of pancreatic cancer in a large population-based prospective study of Swedish women and men. Because alcohol consumption and cigarette smoking may impair folate metabolism (18–21), we also investigated whether the association of folate intake with risk of pancreatic cancer was modified by these variables.

**Subjects and Methods**

**Study Population**

Data for this analysis were derived from two population-based prospective cohort studies: the Swedish Mammography Cohort (SMC) and the Cohort of Swedish Men (COSM). The SMC was established between 1987 and 1990, when all women born between 1914 and 1948 and residing in two counties in central Sweden (Västmanland and Uppsala counties) received a mailed questionnaire that sought information on diet, weight, height, and education; 66 651 women (74% of the source population) returned a completed questionnaire. In the autumn of 1997, an expanded questionnaire with about 350 items concerning diet, other lifestyle factors, and medical history was mailed to all women who were still alive and residing in the study area; 39 227 women (70% of eligible) completed the questionnaire. The COSM began in the autumn of 1997, when 48 850 men (49% of the source population) born between 1918 and 1952 and residing in two counties in central Sweden (Västmanland and Örebro counties) answered a mailed questionnaire that was identical (except for some sex-specific questions) to the SMC questionnaire from 1997.

Eligible participants for the current study were women and men who completed the 1997 questionnaire. We excluded participants with erroneous or missing national registration number.
those with implausibly low or high total energy intake (i.e., three standard deviations from the mean value for log$_e$-transformed energy in women and men separately), and those with cancer (except nonmelanoma skin cancer) diagnosed before baseline. The final baseline population consisted of 81,922 participants (36,616 women and 45,306 men) aged 45 to 83 years in 1997. The Regional Ethical Review Board in Stockholm approved this study.

**Dietary Assessment**

To assess dietary intake, the 1997 questionnaire included a 96-item food-frequency questionnaire. Participants were asked to indicate the average frequency of consumption of each food item during the previous year. There were eight mutually exclusive predefined response categories, ranging from never to three or more times per day. Intakes of nutrients were calculated by multiplying the average frequency of consumption of each food by the nutrient content of age- and sex-specific portion sizes. Values for the amounts of nutrients in the foods were obtained from the Swedish Food Administration Database (22). The questionnaire also asked for information on the use of dietary supplements, including specific folic acid supplements (i.e., supplements containing only folic acid), B-vitamin supplements, and multivitamins. Total folate intake was calculated by summing intake of folate from foods and dietary supplements. The validity of the food-frequency questionnaire has been reported previously (23); the Spearman correlation coefficient for total folate intake between the dietary questionnaire and the average of fourteen 24-hour recall interviews was 0.50.

**Case Ascertainment and Follow-Up**

We ascertained incident cases of pancreatic cancer by computerized record linkage with the National Swedish Cancer Register and the Regional Cancer Register covering the study area, both of which are estimated to be almost 100% complete (24). Cases of pancreatic cancer were defined as primary malignant neoplasm of the exocrine pancreas [International Classification of Diseases, Ninth Revision (ICD-9) code = 157]. We excluded islet-cell carcinomas (ICD-9 code = 157.4) because the etiology of these tumors may be different from that of tumors of the exocrine pancreas. Through linkage to the Swedish Death and Population registers at Statistics Sweden, we obtained information on dates of death and dates of migration.

**Statistical Analysis**

Each participant accrued follow-up time from January 1, 1998, to the date of pancreatic cancer diagnosis, death from any cause, migration, or December 31, 2004, whichever occurred first. We categorized dietary and total (i.e., dietary plus supplemental) folate intake into five groups corresponding to approximately quintiles rounded to the nearest 50 μg (<200, 200–249, 250–299, 300–349, and ≥350 μg/day) and calculated incidence rates of pancreatic cancer by dividing the number of incident cases by the number of person-years in each category. The rate ratios (RRs) were computed by dividing the incidence rate of pancreatic cancer among women and men in a specific category of folate intake by the incidence rate among those in the lowest category.

We used Cox proportional hazards models (25) to estimate the rate ratio with 95% confidence intervals (CIs). The proportional hazards assumption was tested for each of the variable in the model using the likelihood ratio test. None of the tests were statistically significant, indicating that the hazard ratio for the variables was reasonably constant over time. Separate models for women and men showed similar associations. We therefore present results for both sexes combined, controlling for sex as a stratification variable in the Cox model to allow for different baseline hazard rates. All Cox models were additionally stratified by age in months at baseline. In multivariable analyses, in addition to age and sex, we adjusted for education less than high school, high school graduate, or more than high school, smoking status and pack-years of smoking (never, past <20 pack-years, past ≥20 pack-years, current <20 pack-years, current 20–39 pack-years, or current ≥40 pack-years), body mass index (<23.0, 23.0–24.9, 25.0–29.9, or ≥30 kg/m$^2$), exercise (≤1 hour/week, 2–3 hours/week, 4–5 hours/week, or >5 hours/week), history of diabetes (yes/no), and intakes of total energy (continuous), alcohol (quartiles), and carbohydrate (quartiles). In a second multivariable model, we further controlled for consumption of fruits and vegetables. Intakes of folate and carbohydrate were adjusted for total energy intake by using the residual method (26). Tests of linear trend across categories of folate intake were conducted by assigning the median folate intake to each intake category and then treating these values as a continuous variable. We also used restricted cubic spline regression to model dietary folate intake as a continuous variable in relation to risk of pancreatic cancer (27,28). Four knot positions were specified for dietary folate, at 180, 250, 310, and 480 μg/day, corresponding to approximately the 5th, 35th, 65th, and 95th percentiles of the observations.

We examined the relation between dietary folate intake and pancreatic cancer according to levels of alcohol consumption (<5 and ≥5 g/day, with the cut point representing the median intake in the study population) and smoking status (never, past, and current) and tested the statistical significance of the interactions with the likelihood ratio test. We used SAS, version 9.1 (SAS Institute Inc., Cary, NC) for all analyses. All reported $P$ values are two-sided; $P$ values of less than .05 were considered statistically significant.

**Results**

Baseline characteristics of women and men according to categories of dietary folate intake are shown in Table 1. Compared with women and men with a low dietary folate intake, those with higher folate intakes were more likely to have a postsecondary education, to have a history of diabetes, and to use multivitamins and folate (folic acid) supplements but were less likely to smoke. The dose–response relationship of increasing diabetes history with increasing folate intake probably reflects diet changes following diagnosis of diabetes, rather than a causal association between folate intake and diabetes. Women and men with greater dietary folate intake also tended to exercise more and to consume more fruits, vegetables, and carbohydrates than those with a low folate intake. Higher dietary folate intake was associated with lower age among women and with lower consumption of alcohol among men.

A total of 135 incident cases of exocrine pancreatic cancer (61 women and 74 men) were diagnosed during 553,530 person-years.
### Table 1. Baseline characteristics of the study population according to sex and categories of dietary folate intake*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Dietary folate intake (μg/day) among women† (n = 36,616)</th>
<th>Dietary folate intake (μg/day) among men† (n = 45,306)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of individuals</td>
<td>1439</td>
<td>4716</td>
</tr>
<tr>
<td>Age, y</td>
<td>63.6</td>
<td>62.3</td>
</tr>
<tr>
<td>Postsecondary education,</td>
<td>7.9</td>
<td>11.4</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker, %</td>
<td>44.0</td>
<td>50.2</td>
</tr>
<tr>
<td>Past smoker, %</td>
<td>23.6</td>
<td>24.1</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>32.4</td>
<td>25.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>24.9</td>
<td>24.9</td>
</tr>
<tr>
<td>200–249</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250–299</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300–349</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥350</td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of diabetes, %</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Regular multivitamin use,</td>
<td>13.7</td>
<td>16.6</td>
</tr>
<tr>
<td>%§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid supplement use,</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>%§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean daily intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits (servings)</td>
<td>0.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Vegetables (servings)</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>239</td>
<td>240</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>3.6</td>
<td>3.7</td>
</tr>
</tbody>
</table>

*All variables (except age) are age-standardized. BMI = body mass index.
†Nutrient intakes are energy-adjusted (to 2000 kcal/day) via the residual method (26).
‡Most multivitamin supplements contain folic acid (200–400 μg per tablet).
§Supplements containing only folic acid (300–400 μg per tablet).
(mean = 6.8 years) of follow-up from January 1, 1998, through December 31, 2004. Current smoking, obesity (body mass index ≥30 kg/m²), a history of diabetes, and postsecondary education were statistically significantly more common among pancreatic cancer patients than among nonpatients (29).

The associations between intake of dietary folate and of total folate (i.e., combining dietary and supplemental sources) with pancreatic cancer risk are shown in Table 2. Dietary folate intake was statistically significantly inversely associated with pancreatic cancer. After adjustment for age, sex, and other potential risk factors (model 1), the multivariable rate ratio of pancreatic cancer for the highest compared with the lowest quintile of dietary folate intake was 0.37 (95% CI = 0.19 to 0.74; P_trend = .008). Further adjustment for intakes of fruit and vegetables (multivariable model 2) yielded a corresponding association (RR = 0.25, 95% CI = 0.11 to 0.59; P_trend = .002).

### Table 2. Rate ratios (RRs) of pancreatic cancer, with 95% confidence intervals (CIs), according to categories of intake of dietary folate and total folate (from foods and supplements)

<table>
<thead>
<tr>
<th>Categories of folate intake, μg/day</th>
<th>&lt;200</th>
<th>200–249</th>
<th>250–299</th>
<th>300–349</th>
<th>≥350</th>
<th>P_trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dietary folate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>20</td>
<td>28</td>
<td>47</td>
<td>19</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Person-years</td>
<td>50134</td>
<td>130763</td>
<td>153002</td>
<td>106391</td>
<td>113240</td>
<td></td>
</tr>
<tr>
<td>Age- and sex-adjusted RR (95% CI)</td>
<td>1.00</td>
<td>0.55 (0.31 to 0.97)</td>
<td>0.76 (0.44 to 1.29)</td>
<td>0.43 (0.22 to 0.82)</td>
<td>0.42 (0.22 to 0.82)</td>
<td>.02</td>
</tr>
<tr>
<td>Multivariable RR 1 (95% CI)*</td>
<td>1.00</td>
<td>0.53 (0.30 to 0.96)</td>
<td>0.76 (0.44 to 1.32)</td>
<td>0.40 (0.21 to 0.79)</td>
<td>0.37 (0.19 to 0.74)</td>
<td>.008</td>
</tr>
<tr>
<td>Multivariable RR 2 (95% CI)†</td>
<td>1.00</td>
<td>0.51 (0.28 to 0.93)</td>
<td>0.66 (0.35 to 1.24)</td>
<td>0.32 (0.15 to 0.69)</td>
<td>0.25 (0.11 to 0.59)</td>
<td>.059</td>
</tr>
<tr>
<td><strong>Total folate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>19</td>
<td>26</td>
<td>43</td>
<td>17</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Person-years</td>
<td>47138</td>
<td>121311</td>
<td>139882</td>
<td>94956</td>
<td>150243</td>
<td></td>
</tr>
<tr>
<td>Age- and sex-adjusted RR (95% CI)</td>
<td>1.00</td>
<td>0.54 (0.30 to 0.98)</td>
<td>0.74 (0.43 to 1.29)</td>
<td>0.43 (0.22 to 0.85)</td>
<td>0.46 (0.25 to 0.84)</td>
<td>.03</td>
</tr>
<tr>
<td>Multivariable RR 1 (95% CI)*</td>
<td>1.00</td>
<td>0.53 (0.29 to 0.97)</td>
<td>0.75 (0.43 to 1.32)</td>
<td>0.42 (0.21 to 0.84)</td>
<td>0.42 (0.22 to 0.78)</td>
<td>.02</td>
</tr>
<tr>
<td>Multivariable RR 2 (95% CI)†</td>
<td>1.00</td>
<td>0.52 (0.28 to 0.96)</td>
<td>0.69 (0.37 to 1.30)</td>
<td>0.36 (0.16 to 0.80)</td>
<td>0.33 (0.15 to 0.72)</td>
<td>.01</td>
</tr>
</tbody>
</table>

*Multivariable model 1 was stratified by age at baseline (in months) and sex and were adjusted for education (less than high school, high school graduate, or more than high school), smoking status and pack-years of smoking (never, past <20 pack-years, current 20–39 pack-years, or current ≥40 pack-years), body mass index (≤23.0, 23.0–24.9, 25.0–29.9, or ≥30 kg/m²), history of diabetes (yes/no), exercise (≤1 hour/week, 2–3 hours/week, 4–5 hours/week, or >5 hours/week), and intakes of total energy (continuous), alcohol (quartiles), and carbohydrate (quartiles).
†Multivariable model 2 was adjusted for the same covariates as multivariable model 1 and additionally for intakes of fruits (quartiles) and vegetables (quartiles).
Dietary folate were 1.00 (refferent), 0.65 (95% CI = 0.30 to 1.44), 0.65 (95% CI = 0.28 to 1.56), 0.33 (95% CI = 0.12 to 0.94), and 0.20 (95% CI = 0.05 to 0.75). Total folate intake was inversely associated with pancreatic cancer risk (Table 2), but the association was weaker than that observed for dietary folate intake. The spline curve (Fig. 1) showed a dose–response relationship between dietary folate intake and pancreatic cancer risk, and a test for linearity did not indicate departure from linearity within the intake range in this population (P = .46; null hypothesis, the association is linear).

There was no association between folic acid from supplements and pancreatic cancer risk. The multivariable rate ratio (model 2, including dietary folate) for those with a supplemental folic acid intake (mainly from multivitamins) of 300 μg/day or more compared with 0 μg/day of supplemental folic acid was 1.02 (95% CI = 0.56 to 1.88). Multivitamin supplement use was not associated with risk of pancreatic cancer (for multivitamin users compared with nonusers, multivariable RR [model 2] = 1.11; 95% CI = 0.70 to 1.77).

The sex- and age-standardized incidence rates of pancreatic cancer per 100,000 person-years were 41 among participants whose dietary consumption of folate was less than 200 μg/day, 23 among those whose consumption was 200–249 μg/day, 33 among those whose consumption was 250–299 μg/day, 18 among those whose consumption was 300–350 μg/day, and 18 among those whose consumption was ≥350 μg/day. For total folate intake, the incidence rates per 100,000 person-years were 33 among those whose consumption was <200 μg/day, 23 among those whose consumption was 200–249 μg/day, 33 among those whose consumption was 250–299 μg/day, 18 among those whose consumption was 300–350 μg/day, and 19 among those whose consumption was ≥350 μg/day.

To examine whether the association between dietary folate intake and pancreatic cancer was modified by alcohol consumption and smoking, we cross-classified participants according to dietary folate intake and alcohol consumption (above or below the median) or smoking (never, past, or current). There was no evidence of an interaction between dietary folate intake and alcohol consumption in relation to pancreatic cancer risk (Pinteraction = .66). Likewise, a test for interaction between dietary folate intake and smoking was not statistically significant (Pinteraction = .33; Fig. 2).

**Discussion**

In this large, prospective population-based study of Swedish women and men, we observed an inverse association between intake of folate from foods, but not from supplements, and the risk of pancreatic cancer. The association with dietary folate was independent of other known and potential risk factors for pancreatic cancer, including age, smoking, obesity, physical activity, and history of diabetes, and it persisted after controlling for consumption of fruits and vegetables, many of which (i.e., oranges and orange juice, green leafy vegetables, and cruciferous vegetables) are naturally high in folate.

Our findings for dietary folate are consistent with those from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort of 27,101 male Finnish smokers in which a relative risk of 0.52 (95% CI = 0.31 to 0.87) was reported for men in the top quintile of dietary folate intake (16). Furthermore, in a case-control analysis nested within that cohort, a statistically significant 55% reduction in pancreatic cancer risk was found for men who consumed the highest levels of dietary folate. However, the authors of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study noted that some of the protective effect of dietary folate might be due to the statistical association between dietary folate intake and cigarette smoking (16). In our cohort, there was an inverse association between dietary folate intake and cigarette smoking status. Multivariable models were stratified by age at baseline (in months) and sex and adjusted for education (less than high school, high school graduate, or more than high school), smoking status and pack-years of smoking (never, past <20 pack-years, past ≥20 pack-years, current <20 pack-years, current 20–39 pack-years, or current ≥40 pack-years), body mass index (<23.0, 23.0–24.9, 25.0–29.9, or ≥30 kg/m2), history of diabetes (yes/no), exercise (≤1 hour/week, 2–3 hours/week, 4–5 hours/week, or >5 hours/week), and intakes of total energy (continuous), alcohol (quartiles), carbohydrate (quartiles), fruits (quartiles), and vegetables (quartiles). Solid line = adjusted rate ratios of pancreatic cancer; dotted lines = 95% confidence intervals.
in the highest tertile of serum folate levels compared with those in the lowest tertile (15). A recent report combining data from the Nurses’ Health Study and the Health Professionals Follow-up Study (17) also suggested an inverse association for dietary folate (RR = 0.66; 95% CI = 0.42 to 1.03, for the highest versus the lowest category). A high dietary folate intake was associated with a more than halving in risk of pancreatic cancer in a case–control study conducted in Australia (30), but no association was observed in a U.S. case–control study (31).

In agreement with our findings, the Finnish (16) and U.S. (17) cohorts did not show an inverse association between supplemental folic acid intake and pancreatic cancer risk. In the U.S. cohorts (17), use of multivitamin supplements was even associated with an elevated risk (RR = 1.31; 95% CI = 1.02 to 1.67, for current versus never use), and the Finnish cohort (16) showed nonstatistically significant positive associations with the use of supplemental folic acid, vitamin B₆, and vitamin B₁₂.

The reason for the divergent findings for dietary folate compared with supplemental folic acid in the current Swedish cohorts, as well as in the Finnish (16) and U.S. cohorts (17), is not clear. One possibility is that folate from food sources better represents long-term folate intake than folic acid from (recent and possibly irregular) use of supplements, and long-term regular exposure to folate may be more relevant to carcinogenesis. Data from studies conducted in animal models of colorectal cancer have suggested that exceptionally large doses of folic acid and folic acid supplementation provided after microscopic neoplasia are established may promote rather than suppress carcinogenesis (32). In this regard, it is noteworthy that folate antagonists are used in cancer treatments (33). It is, therefore, theoretically possible that high intakes of supplemental folic acid, which is more bioavailable than folate from foods, may promote the progression of pancreatic cancer in individuals with already existing, undiagnosed cancer. Hence, based on available data, we do not advocate folic acid chemoprevention of pancreatic cancer. In the U.S., all flour and uncooked cereal-grain products have been fortified with folic acid since January 1998 (34). Future studies conducted in the U.S. may provide insight into the role of folate naturally found in foods versus folic acid from fortified foods and supplements.

Alcohol consumption has been shown to modify the association between folate intake and risk of colon (12), breast (7,35), and ovarian cancer (4). In the present study we did not find an interaction between alcohol consumption and folate intake in relation to pancreatic cancer risk. However, consumption of alcohol was low in our study population, and we cannot preclude an interaction with heavy alcohol consumption.

The mechanisms whereby folate may affect pancreatic carcinogenesis remain speculative. Folate is an important dietary methyl group donor involved in DNA synthesis and repair and in DNA methylation; aberrations in any of these pathways may contribute to cancer. Diminished folate levels may cause misincorporation of uracil for thymine in DNA, leading to DNA strand breaks and impaired DNA repair activities (36–38). Alternatively, folate deficiency may reduce the availability of S-adenosylmethionine, the universal methyl donor for methylation of DNA, RNA, and protein. Disruption of DNA methylation may result in genomic instability, increased mutation rates, and altered expression of proto-oncogenes and tumor suppressor genes (1,37–39). DNA hypomethylation is a frequent epigenetic event in pancreatic cancer and is associated with the overexpression of affected genes (40). In addition, hypermethylation at promoter CpG islands of tumor suppressor genes is associated with the transcriptional silencing in pancreatic cancers (41,42). However, although it is theoretically plausible that folate deficiency specifically induces aberrant DNA methylation in the pancreas, there is currently no evidence that this actually occurs.

Experimental animal studies suggest that the pancreas has a requirement for high levels of folate and methyl donors. The pancreas contains high levels of folate (second only to the liver) (43) and glycine N-methyltransferase, which regulates the ratio of S-adenosylmethionine to S-adenosylhomocysteine (44). Folate-deficient animals display statistically significantly decreased ratios of S-adenosylmethionine to S-adenosylhomocysteine (43) and less incorporation of thymidine into pancreatic DNA (45). Studies in animal also have shown that diets deficient in methyl group donors (choline and methionine) cause abnormal pancreatic acinar cell differentiation (46,47) and impaired exocrine pancreatic function (48,49). Moreover, animals treated with ethionine, an inhibitor of cellular methylation, develop acute pancreatitis (50,51), and chronic pancreatitis has been associated with increased risk of pancreatic cancer (52–54).

The methylenetetrahydrofolate reductase (MTHFR) enzyme acts as a critical juncture in folate metabolism by catalyzing the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, thereby directing folate metabolites toward cellular methylation. A common variant of the MTHFR gene has been identified with a change of a cytosine to a thymine at nucleotide 677, and this variant is associated with reduced enzyme activity (55). In two recent case–control studies, the MTHFR 677TT (i.e., variant) genotype was associated with a statistically significant two- to fivefold increased risk of pancreatic cancer compared with the MTHFR CC (wild-type) genotype (56,57). The MTHFR C677T polymorphism was not associated with the risk of pancreatic cancer in another case–control study (58). However, separate analyses in the same study (58) showed that pancreatic cancers with defective MTHFR genotypes had more DNA hypomethylation and more chromosomal deletions, especially at folate-sensitive fragile sites and at tumor suppressor gene loci. The results from these studies on MTHFR thus provide further support for a potential role of DNA hypomethylation and thus possibly of a role for folate deficiency in pancreatic carcinogenesis.

The strengths of our study include a prospective population-based design and the availability of detailed data on diet as well as potential risk factors for pancreatic cancer. The prospective design eliminated recall bias, and the practically complete follow-up of our study population through linkages to various population-based registers minimizes the concern that our findings were affected by differential loss to follow-up.

With regard to limitations, misclassification of folate intake is likely to have occurred to some extent because of the use of a self-administered food-frequency questionnaire to assess diet and because of changes in diet during follow-up. Nevertheless, such misclassification is expected to be nondifferential, and random nondifferential misclassification would tend to attenuate rather than exaggerate any true relationship. Imprecise measurement of covariates may be of concern. However, because the age- and sex-adjusted and the multivariable results were similar, the influence of measurement error in covariates should be minimal. Despite controlling for many potential confounders in our multivariable analyses, we cannot rule out the possibility that the
observed association was attributable to unrecognized confounding factors. Although we were unable to control for chronic and hereditary pancreatitis and family history of pancreatic cancer, it is unlikely that lack of adjustment for these factors contributed to the observed association because pancreatitis and family history of pancreatic cancer contribute to only a small percentage of the total number of cases (59). Also, individuals’ diets are unlikely to differ appreciably according to family history of cancer.

In summary, in this population-based prospective study of Swedish women and men, we found a strong inverse association between dietary folate intake and risk of pancreatic cancer. However, we did not observe a relationship with folic acid from supplements. Although our results suggest that increased consumption of foods naturally rich in folate may be beneficial, they do not encourage increased use of supplements for the prevention of pancreatic cancer.

REFERENCES


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