

**Dietary folate intake and pancreatic cancer risk:
results from the European Prospective Investigation into Cancer and Nutrition**

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Novelty and impact statement: This large investigation with 865 incident pancreatic cancer cases from the EPIC study showed no significant association between dietary folate intake and pancreatic cancer risk. Dietary folate intake was ascertained using the standardised folate dataset compiled for EPIC which provided comparable folate data across the participating countries with minimum influence of folic acid fortification or supplementation.

Abstract

Pancreatic cancer (PC) has an exceptionally low survival rate and primary prevention strategies are limited. Folate plays an important role in one-carbon metabolism and has been associated with the risk of several cancers, but not consistently with PC risk. We aimed to investigate the association between dietary folate intake and PC risk, using the standardised folate database across 10 European countries. A total of 477,206 participants were followed up for 11 years, during which 865 incident primary PC cases were recorded. Folate intake was energy-adjusted using the residual method. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional hazards models. In multivariable analyses stratified by age, sex, study centre and adjusted for energy intake, smoking status, BMI, educational level, diabetes status, supplement use and dietary fibre intake, we found no significant association between folate intake and PC risk: the HR of PC risk for those in the highest quartile of folate intake (≥ 353 $\mu\text{g/d}$) compared with the lowest (< 241 $\mu\text{g/d}$) was 0.81 (95% CI: 0.51, 1.31; $P_{\text{trend}} = 0.38$). In current smokers, a positive trend was observed in PC risk across folate quartiles (HR=4.42 (95% CI: 1.05, 18.62) for ≥ 353 $\mu\text{g/d}$ vs. < 241 $\mu\text{g/d}$, $P_{\text{trend}} = 0.01$). Nonetheless, there was no significant interaction between smoking and dietary folate intake ($P_{\text{interaction}} = 0.99$). We found no association between dietary folate intake and PC risk in this large European study.

19 Introduction

20 The diagnosis of pancreatic cancer (PC) is rarely made at an early stage and it has an
21 exceptionally low survival rate due to a late diagnosis and limited treatment options, making
22 it the seventh leading cause of cancer death worldwide with an almost equal number of new
23 cases diagnosed each year¹. The burden of this cancer is increasing with an aging population,
24 and is particularly high in more developed countries. The incidence of pancreatic cancer
25 varies greatly across regions, which suggests a role of environmental, dietary or lifestyle
26 factors ^{2,3}. Consistently identified risk factors for pancreatic cancer include tobacco smoking,
27 body fatness, conditions characterized by high insulin secretion, chronic pancreatitis, heavy
28 alcohol intake and family history of the disease⁴⁻⁶. Nonetheless, the aetiology of the cancer is
29 largely unknown and prevention strategies are limited.

30 Folate, naturally available in a wide variety of foods including fruits and vegetables, is a
31 water-soluble vitamin B that plays an important role in the synthesis and methylation of DNA
32 as a crucial cofactor in one-carbon metabolism together with other B vitamins such as
33 vitamin B2, vitamin B6 and vitamin B12⁷. Inadequate folate status may contribute to
34 carcinogenesis through aberrations in DNA methylation and uracil misincorporation, leading
35 to DNA instability ⁷⁻⁹.

36 Previous epidemiological studies have shown inconsistent results for associations
37 between folate status and pancreatic cancer risk suggesting a weak inverse association with
38 dietary folate intake from natural sources, but not from supplements ¹⁰⁻¹⁴. A recent meta-
39 analysis supported this observation¹⁵ whereas a large pooled analysis of 14 prospective cohort
40 studies found no association ¹⁶. In the former meta-analysis, the overall estimates were from
41 both case-control and prospective cohort studies, and a significant heterogeneity was found
42 across those studies. In the latter pooled analyses, folate data might not be comparable across
43 studies and countries due to the use of various databases and analytical methods, thus

attenuating potential relationships with pancreatic cancer. A recent meta-analysis of randomised trials of folic acid supplementation with an average duration of 5 years of treatment found no significant effect on overall or site-specific cancer incidence including pancreatic cancer¹⁷. The results from plasma measurements of folate intake in association with pancreatic cancer were also inconclusive¹⁸⁻²⁰. In a more recent nested case-control study in the EPIC cohort, including 463 incident pancreatic cancer case, a weak U-shaped association was observed between plasma folate and pancreatic cancer risk¹⁸.

The aim of this study was to investigate the association between dietary folate intake and pancreatic cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC) study, benefitting from a large number of cases with an extended follow-up time and standardised dietary folate intake data from a comprehensive EPIC Nutrient DataBase (ENDB) where folate information was harmonized using common procedures and guidelines, with support from the local national compilers in 10 countries participating in EPIC²¹.

Subjects and Methods

Study population

The EPIC study is a multicentre prospective cohort study designed to investigate the associations between diet and various lifestyle, environmental risk factors and the incidence of different cancers and other chronic diseases. The full rationale and methods of the study were reported elsewhere^{22, 23}. Briefly, the EPIC cohort consists of 23 study centres in 10 European countries (Denmark, France, Greece, Germany, Italy, Netherlands, Norway, Spain, Sweden, and the UK), with over 521,330 participants. EPIC participants were mostly recruited from the general population residing within defined geographical areas between

1992 and 2000, with some exceptions: women members of a health insurance for school employees (France); women attending breast cancer screening (Utrecht, the Netherlands, Florence, and Italy); blood donors (centres in Italy and Spain) and a cohort with a large proportion (approximately 50%) of vegetarians ('health conscious' cohort in Oxford, UK). The participants completed dietary and lifestyle questionnaires and had their anthropometric measurements recorded by trained health professionals (self-reported in France, Norway and Oxford). All participants gave their written informed consent, and the study was approved by the local ethics committee in the participating countries and the Ethics Committee of the Internal Agency for Research on Cancer, Lyon, France.

Our study is based on data from 477,206 participants (142,228 men and 334,978 women) after a priori exclusion of individuals with prevalent cancer at recruitment, missing diagnosis or censoring date, missing dietary or lifestyle information, and implausible extreme values in the top and bottom one percent of the distribution of the ratio of reported total energy intake to estimated energy requirement (estimated from age, sex, and body weight and height).

Diet and lifestyle data

Diet including folate and other B vitamins over the previous 12 months was measured by country/centre-specific validated dietary assessment methods, mostly food frequency questionnaire designed to capture local dietary habits and to allow high compliance. The relative validity and reproducibility of the questionnaires has previously been published²⁴. The questionnaire, validated within each country, was self-administered in all centres, except in Greece, two Italian centres, and Spain, where it was administered by interviewers.

Dietary folate intake was estimated using the updated EPIC Nutrient DataBase (ENDB)²¹. The ENDB project was initiated and nutrient databases were harmonized using common

procedures and guidelines, with support from the local national compilers in 10 countries in EPIC²⁵. The ENDB was first completed for 26 priority components to provide a standardised reference instrument for calibrating the EPIC dietary measurements at the nutrient level²⁶. This work has been extended to cover other nutrients including folate and other B vitamins²¹. Although the ENDB values were obtained from country-specific food composition tables, they were standardized as much as possible across the EPIC countries by matching of EPIC foods to the national databases according to the recommendation given in a recent review²¹. In particular, a microbiological assay was chosen as the reference analytical method for folate values in the ENDB. Folate values of unavailable foods were derived by recipe calculation or borrowed from similar foods²¹. During the ENDB compilation for folate, to address the issue of voluntary fortification of breakfast cereals particularly in the UK and France where cereal consumption was substantially higher, aggregation was re-done taking into account the brand names and folic acid fortification levels of cereals²¹.

In the Scandinavian countries and in the Netherlands, folate fortification was not allowed at the time of data collection. In other EPIC countries, breakfast cereal consumption was very low and the information on folic acid-fortified foods was not always available²¹. It was therefore, decided not to adopt the dietary folate equivalent (DFE) conversion which considers lower bioavailability of naturally occurring folate compared to synthetic folic acid. Information on dietary intakes of other nutrients including other B vitamins was also estimated using the ENDB.

Self-reported data on lifestyle factors, including total physical activity, educational level, smoking history, diabetes status and ever use of vitamin or mineral supplements considered in the analysis were collected at baseline through standardised questionnaires and clinical examinations, and have been described elsewhere^{23, 27-30}.

Endpoints

Incident pancreatic cancer cases were identified through population cancer registries (Denmark, Italy, Netherlands, Norway, Spain, Sweden, and the UK) or by active follow-up (France, Germany, Naples, and Greece). The active follow-up procedure used a combination of different strategies, including health insurance records, cancer and pathology registries, and contacts with participants and their next of kin ²³. Participants were followed up from study entry until cancer diagnosis (except non-melanoma skin cancer), death, emigration or until the end of the follow-up period, whichever occurred first. Forty-five cases were censored because the tumours were neuroendocrine (n = 42), benign (n = 1), carcinoma in situ (n = 1), or with uncertain primary origin (n = 1). After a mean follow-up of approximately 11 years, 865 first incident pancreatic cancers were available for analysis and were classified corresponding to the International Classification of Diseases 10th revision as C25 (C25.0–C25.3 and C25.7–C25.9).

Statistical analysis

Multivariable Cox proportional hazard models were fitted to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs). Disease models were fitted with intake of folate and other B vitamins as continuous variables and categorisation of the variables in quartiles based on the distribution of the whole study population. Dietary folate intake and other nutrients were energy adjusted using the residual method ³¹. To preserve the geographical specificity in the dietary assessment in EPIC, centre-specific residuals for total dietary folate were computed. Centre-specific mean values were then added to residuals to recuperate the original scale and ease interpretability.

The following potential confounders were considered based on the literature review: total energy intake (kcal/d), BMI (kg/m²), physical activity (<moderately inactive/≥moderately

active), smoking status (never/former/current), education (<secondary school/ \geq secondary school), ever use of vitamin or mineral supplements (no/yes), history of diabetes (no/yes) and intake of other dietary factors (g/day) including dietary fibre, carbohydrate, and alcohol. In the multivariable models, the variables that changed the unadjusted risk estimate by at least ~10% were considered as confounders and adjusted for. These include smoking status, BMI, educational level, history of diabetes, supplement use and dietary fibre intake. Energy intake was further included in the model for complete energy adjustment³¹ even though it did not alter the unadjusted risk substantially.

Quartiles of dietary folate intake were determined on the basis of the whole cohort, with the lowest quartile as the reference. Disease models were stratified by age at recruitment, sex, and study centre (Model 1) and adjusted for smoking status, total energy intake and BMI, education, diabetes status, supplement use and dietary fibre intake (Model 2). A test for trend was made by modelling a score variable using quartile-specific medians as a continuous variable. In addition, the association between dietary folate and the risk of pancreatic cancer was examined using four-knot restricted cubic splines³² with the median of the fifth decile of folate intake as the reference category.

Alcohol intake was not considered as a covariate in the models as it did not change the unadjusted risk estimates. However, alcohol has a role as a folate antagonist and has been shown to have suppressive effects on methyl group metabolism³³, and we investigated the association according to tertiles of alcohol consumption. Likewise, we further explored the association between dietary folate intake and pancreatic cancer risk stratified by smoking status as smoking has been the most consistently known risk factor for pancreatic cancer and current smoking status was related to lower dietary folate intake³⁴. Models with main effects and cross-product terms were fitted to test for interactions.

The effect of very high (≥ 500 $\mu\text{g/d}$) or very low folate intake (< 150 $\mu\text{g/d}$) in relation to pancreatic cancer risk was additionally explored, with the reference category set at 200-300 $\mu\text{g/d}$, which was observed to be the average intake range in a previous EPIC study comparing standardised dietary folate intake across ten participating countries³⁴.

A model including the combined effects of folate tertiles and three smoking categories in relation to overall pancreatic cancer risk was developed and the joint effects was presented in comparison with never smokers in the highest folate tertile as a reference.

Sensitivity analyses were performed 1) excluding any dietary supplement users to examine a possible impact of supplement use on the association between dietary folate and pancreatic cancer risk 2) excluding the cases diagnosed within the first 2 years of follow-up to assess possible influence of preclinical factors that might cause a change in diet among participants and 3) excluding the microscopically non-confirmed cases ($n = 257$) to minimize possible misclassification of tumours.

All statistical tests were two-sided and analyses were performed using STATA (version 13, Stata Corporation, College Station, Texas).

Results

A total of 477,206 participants without any history of cancer and with complete dietary folate information were included in the analysis among which 397 men and 468 women developed a first primary pancreatic cancer during an average of 11 years of follow-up. **Table 1** shows baseline characteristics of the participants according to quartiles of energy-adjusted dietary folate intake. Participants in the highest category of folate intake tended to be more educated, were less likely to report being a current smoker and more likely to be physically active or

190 dietary supplement users; and consumed more dietary fibre, fruit and vegetables compared
 191 with those with lower dietary folate intake.

192 When we investigated energy-adjusted folate intake as a continuous variable in
 193 association with pancreatic cancer in the fully adjusted model, we found an HR of 1.03 (95%
 194 CI: 0.83, 1.28; $P=0.78$) for an increment of 100 $\mu\text{g/day}$ of dietary folate intake
 195 (approximately 1 SD). When folate intake was categorised into quartiles, higher dietary folate
 196 intake showed a borderline statistically significant association with lower risk of pancreatic
 197 cancer (Model 1, **Table 2**). The trend became attenuated and did not reach statistical
 198 significance in Model 2 after multivariable adjustment (**Table 2**): the multivariable HR of
 199 pancreatic cancer for those in the highest category of folate intake ($\geq 353 \mu\text{g/day}$) compared
 200 with the lowest category of intake ($< 241 \mu\text{g/day}$) was 0.81 (95% CI: 0.51, 1.31; $P_{\text{trend}} = 0.38$).
 201 Among the variables included in the Model 2, smoking and dietary fibre intake changed the
 202 unadjusted risk estimate most. Our non-linear multivariable modelling of the association
 203 using cubic spline confirmed no significant trend (**Figure 1**). Further analysis using
 204 continuous folate intake with a quadratic term provided no evidence of a non-linear
 205 association between folate intake and pancreatic cancer risk ($P_{\text{quadratic term}}=0.56$).

206 When we investigated the association according to alcohol consumption, there was no
 207 evidence of a differential relationship according to levels of alcohol intake ($P_{\text{interaction}}= 0.82$,
 208 **Table 2**). In a subgroup analysis by smoking status we observed an increased risk of
 209 pancreatic cancer with increasing folate intake in current smokers while no significant
 210 associations were observed in never and former smokers: the multivariable HR of pancreatic
 211 cancer among current smokers and those who had folate intake between 292 and 352 $\mu\text{g/day}$
 212 and those who consumed more than 353 $\mu\text{g/day}$ compared with the lowest category were 4.52
 213 (95% CI: 1.59, 12.88) and 4.42 (95% CI: 1.05, 18.62), respectively ($P_{\text{trend}} = 0.01$, **Table 2**).
 214 The results did not differ when alcohol was additionally adjusted for. Nonetheless, an

interaction test with smoking and folate intake for the risk of pancreatic cancer was not statistically significant ($P_{\text{interaction}} = 0.99$).

The characteristics of the participants in this study varied according to their smoking status. Current smokers were younger, more likely to be men, to have a lower educational level, and less likely to be diabetic at baseline, tended to have lower folate, fibre, fruit and vegetable intakes and consumed more alcohol compared to never smokers (data not shown). Further alcohol adjustment in the main models did not change the risk estimates stratified by smoking status. When we considered smoking intensity in current smokers by adjusting for number of cigarettes smoked per day, the results showed a greater than five-fold increased risk in those who had a folate intake of more than 292 $\mu\text{g}/\text{d}$ (data not shown).

The observed increased risk of pancreatic cancer with higher folate intake in current smokers was further explored by choosing one single reference category (≥ 330 $\mu\text{g}/\text{day}$ of dietary folate intake and never smoker) and combined effects were determined for tertiles of folate intakes in combination with categories of smoking status in relation to pancreatic cancer risk (**Figure 2**). A more than 50% increase in pancreatic cancer risk was observed in current smokers regardless of the levels of folate intake, although a significantly higher risk was observed among those who consumed more than 258 $\mu\text{g}/\text{day}$ of folate.

We also explored the effect of very high (≥ 500 $\mu\text{g}/\text{d}$) or very low folate intake (< 150 $\mu\text{g}/\text{d}$) in relation to pancreatic cancer risk, and we did not observe any significant associations in the multivariable adjusted model, possibly due to limited statistical power (**Table 3**).

When we conducted a sensitivity analysis among those who reported not to take any dietary supplements ($n=237,113$), the results did not change substantially (HR of 0.90, 95% CI: 0.60, 1.35; $P=0.61$ for an increment of 100 $\mu\text{g}/\text{day}$ folate, HR for the highest quartile vs. lowest: 0.61, 95% CI: 0.26, 1.44; $P_{\text{trend}} = 0.28$). Similarly, the results hardly changed when

we excluded the pancreatic cancer cases incident within the first 2 years of follow-up (n=90 cases) and repeated the analyses (HR of 1.03, 95% CI: 0.82, 1.29; $P=0.79$ for an increment of 100 µg/day, HR for the highest quartile vs. lowest: 0.79, 95% CI: 0.48, 1.30; $P_{\text{trend}} = 0.32$). A sensitivity analysis restricting the analyses to the microscopically confirmed cases did not alter the results.

Discussion

To our knowledge, this study that analysed 865 incident pancreatic cancer cases, is the largest single study so far that investigated the association between dietary folate intake and pancreatic cancer risk. Within a unique international setting of European populations with diverse dietary habits and lifestyle characteristics, we found no overall association between dietary folate intake and pancreatic cancer risk.

There have been relatively few published single prospective studies that examined the association between dietary folate intake and pancreatic cancer risk (summarised in **Table 4**). Previous studies showed inconsistent results: they were heterogeneous by sex, ranges of dietary folate intake, supplement use, and confounding factors that were adjusted for in the analyses (**Table 4**). Including studies conducted in the US, where dietary supplement use is widespread, there is little evidence that folic acid intake from supplements was associated with pancreatic cancer risk, while dietary folate intake was shown to be possibly related with lower risks in some, but not in all studies (**Table 4**). Only one study from the US was able to distinguish the difference in dietary folate intake from natural sources and from folic acid fortification¹². In this study, no association was found in men, while women in the highest quartile of food folate intake showed a significant 53% reduction in pancreatic cancer risk

compared with those in the lowest quartile. No significant association was found between supplemental folic acid use and pancreatic cancer risk ¹².

Three meta-analyses^{15, 35, 36} have reported a generally decreased risk of pancreatic cancer with increasing dietary folate intake based on the above mentioned cohort studies together with case-control studies, with significant heterogeneity reported in the two^{15, 36}. The 2012 Continuous Update Project (CUP) Report of the WCRF/AICR Expert Report weakened the conclusions from the 2007 Expert Report, after reviewing evidence from five prospective cohort studies, concluding the evidence is too inconsistent to allow a firm conclusion to be drawn⁴.

A large pooled analysis of 14 prospective cohort studies showed that dietary folate intake was not associated with overall risk of pancreatic cancer ¹⁶. The summary relative risk for the highest vs. the lowest quintile of folate intake was 1.06 (95% CI: 0.90-1.25, $P_{\text{heterogeneity}}=0.15$)¹⁶. In the pooled analysis, folate data may be heterogeneous across studies and countries as studies rely on each country's own food-composition data which tend to vary in terms of availability and quality of folate values ^{37, 38}. This may have an influence in a potential relationship. It has been pointed out that there is a lack of clarity and consistency in the terminology and definitions used for folate information in the food composition tables available in Europe due to the specific complexity of folate ³⁹. A recent critical evaluation of folate data in 18 European and international databases concluded that a lack of comparability still exists between countries ⁴⁰. To overcome this, our study results came from the standardised food and nutrient data linked to the ENDB with recently updated folate information.

Despite the recent increasing use of dietary supplements in many European countries, the use of folic acid supplement was not a common practice when our baseline data were collected ^{10, 41}. Indeed, folic acid-containing supplements were not among the most frequently

consumed types of supplements in the EPIC study according to the 24 hour-recall data that were collected in a sub group of participants with more detailed information on supplement use at the baseline ⁴¹. In addition, folic acid fortification was not widespread at the time of the baseline information collection in Europe²¹. Thus, we had the unique opportunity to assess the association between baseline dietary folate intake and pancreatic cancer risk, with minimum influence of folic acid fortification or supplementation in the EPIC study. This was confirmed when we conducted a sensitivity analysis excluding ever users of vitamin and mineral supplements and the results did not materially change.

In our study, we observed a greater than four-fold elevated risk in current smokers with higher dietary folate intake while in never or former smokers the risks were lower and non-significant. Current smokers have a higher chance to harbour precursor lesions in the pancreas, and increased availability of folate may promote proliferation of already existing neoplastic cells ^{42, 43}. We investigated this further by excluding cases diagnosed within the first two or three years of follow-up which did not alter the results. Previous studies have shown inconsistent results with regards to smoking status. While a previous EPIC nested case-control study that investigated plasma folate levels in relation to pancreatic cancer risk did not show any heterogeneity across smoking status¹⁸, an inverse association with pancreatic cancer risk was reported with both dietary folate intake¹⁴ and serum folate levels¹⁹ in a cohort of Finnish male smokers. In the large pooled analysis of cohort studies, there was no effect modification by smoking status with dietary folate intake¹⁶. Although increased pancreatic cancer risk observed in current smokers in our study, especially in participants with dietary folate intake higher than 292 µg/day may be worth exploring further in future studies, few cases included, no statistically significant interaction found, potential role of residual confounding or chance require cautious interpretation of the results.

The current study has limitations. We did not have information on occurrence of pancreatitis in the study population which might have affected pancreatic cancer risk. Neither did we have repeated information during the follow-up period to assess any potential changes in dietary intakes over time. In addition, we relied on self-reported dietary folate intake. However, the overall results did not substantially differ from the previous EPIC study that investigated plasma folate level in association with pancreatic cancer risk¹⁸. In our study, the range of dietary folate intake was quite narrow, as shown previously in the results using the 24-hour dietary recall methods³⁴, with too few participants with either very low or very high intakes. It was therefore not possible to explore the effect of extreme folate intake on pancreatic cancer risk with sufficient statistical power. We used self-reported smoking status which might be inaccurate. However, a recent EPIC study of plasma cotinine level and pancreatic cancer risk compared the cotinine level against self-reported smoking status and concluded that self-reported smoking status was sufficient to establish a causal relationship and did not underestimate its relationship with pancreatic cancer risk⁴⁴.

In conclusion, using standardised data from this large, multi-centre prospective study, we found no association between dietary folate intake and pancreatic cancer risk.

Data sharing statement: For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at <http://epic.iarc.fr/access/index.php>.

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