

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

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Running head: Dietary folate intake and pancreatic cancer risk

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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.

1 Abstract

2 Pancreatic cancer (PC) has an exceptionally low survival rate and primary prevention
3 strategies are limited. Folate plays an important role in one-carbon metabolism and has been
4 associated with the risk of several cancers, but not consistently with PC risk. We aimed to
5 investigate the association between dietary folate intake and PC risk, using the standardised
6 folate database across 10 European countries. A total of 477,206 participants were followed
7 up for 11 years, during which 865 incident primary PC cases were recorded. Folate intake
8 was energy-adjusted using the residual method. Hazard ratios (HRs) and 95% confidence
9 intervals (CIs) were estimated using Cox proportional hazards models. In multivariable
10 analyses stratified by age, sex, study centre and adjusted for energy intake, smoking status,
11 BMI, educational level, diabetes status, supplement use and dietary fibre intake, we found no
12 significant association between folate intake and PC risk: the HR of PC risk for those in the
13 highest quartile of folate intake (≥ 353 $\mu\text{g}/\text{d}$) compared with the lowest (< 241 $\mu\text{g}/\text{d}$) was 0.81
14 (95% CI: 0.51, 1.31; $P_{\text{trend}} = 0.38$). In current smokers, a positive trend was observed in PC
15 risk across folate quartiles (HR=4.42 (95% CI: 1.05, 18.62) for ≥ 353 $\mu\text{g}/\text{d}$ vs. < 241 $\mu\text{g}/\text{d}$,
16 $P_{\text{trend}} = 0.01$). Nonetheless, there was no significant interaction between smoking and dietary
17 folate intake ($P_{\text{interaction}} = 0.99$). We found no association between dietary folate intake and PC
18 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 instability⁷⁻⁹.

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 supplements¹⁰⁻¹⁴. A recent meta-
39 analysis supported this observation¹⁵ whereas a large pooled analysis of 14 prospective cohort
40 studies found no association¹⁶. 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

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

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

58 **Subjects and Methods**

59 *Study population*

60 The EPIC study is a multicentre prospective cohort study designed to investigate the
61 associations between diet and various lifestyle, environmental risk factors and the incidence
62 of different cancers and other chronic diseases. The full rationale and methods of the study
63 were reported elsewhere^{22,23}. Briefly, the EPIC cohort consists of 23 study centres in 10
64 European countries (Denmark, France, Greece, Germany, Italy, Netherlands, Norway, Spain,
65 Sweden, and the UK), with over 521,330 participants. EPIC participants were mostly
66 recruited from the general population residing within defined geographical areas between
67

68 1992 and 2000, with some exceptions: women members of a health insurance for school
69 employees (France); women attending breast cancer screening (Utrecht, the Netherlands,
70 Florence, and Italy); blood donors (centres in Italy and Spain) and a cohort with a large
71 proportion (approximately 50%) of vegetarians ('health conscious' cohort in Oxford, UK).
72 The participants completed dietary and lifestyle questionnaires and had their anthropometric
73 measurements recorded by trained health professionals (self-reported in France, Norway and
74 Oxford). All participants gave their written informed consent, and the study was approved by
75 the local ethics committee in the participating countries and the Ethics Committee of the
76 Internal Agency for Research on Cancer, Lyon, France.

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

83 *Diet and lifestyle data*

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

90 Dietary folate intake was estimated using the updated EPIC Nutrient DataBase (ENDB)²¹.
91 The ENDB project was initiated and nutrient databases were harmonized using common
92

93 procedures and guidelines, with support from the local national compilers in 10 countries in
94 EPIC²⁵. The ENDB was first completed for 26 priority components to provide a standardised
95 reference instrument for calibrating the EPIC dietary measurements at the nutrient level²⁶.

96 This work has been extended to cover other nutrients including folate and other B vitamins²¹

97 Although the ENDB values were obtained from country-specific food composition tables,
98 they were standardized as much as possible across the EPIC countries by matching of EPIC
99 foods to the national databases according to the recommendation given in a recent review²¹.

100 In particular, a microbiological assay was chosen as the reference analytical method for folate
101 values in the ENDB. Folate values of unavailable foods were derived by recipe calculation or
102 borrowed from similar foods²¹. During the ENDB compilation for folate, to address the issue
103 of voluntary fortification of breakfast cereals particularly in the UK and France where cereal
104 consumption was substantially higher, aggregation was re-done taking into account the brand
105 names and folic acid fortification levels of cereals²¹.

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

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

117

118 *Endpoints*

119 Incident pancreatic cancer cases were identified through population cancer registries
120 (Denmark, Italy, Netherlands, Norway, Spain, Sweden, and the UK) or by active follow-up
121 (France, Germany, Naples, and Greece). The active follow-up procedure used a combination
122 of different strategies, including health insurance records, cancer and pathology registries,
123 and contacts with participants and their next of kin²³. Participants were followed up from
124 study entry until cancer diagnosis (except non-melanoma skin cancer), death, emigration or
125 until the end of the follow-up period, whichever occurred first. Forty-five cases were
126 censored because the tumours were neuroendocrine (n = 42), benign (n = 1), carcinoma in
127 situ (n = 1), or with uncertain primary origin (n = 1). After a mean follow-up of
128 approximately 11 years, 865 first incident pancreatic cancers were available for analysis and
129 were classified corresponding to the International Classification of Diseases 10th revision as
130 C25 (C25.0–C25.3 and C25.7–C25.9).

131 *Statistical analysis*

132 Multivariable Cox proportional hazard models were fitted to estimate the hazard ratios (HRs)
133 and 95% confidence intervals (CIs). Disease models were fitted with intake of folate and
134 other B vitamins as continuous variables and categorisation of the variables in quartiles based
135 on the distribution of the whole study population. Dietary folate intake and other nutrients
136 were energy adjusted using the residual method³¹. To preserve the geographical specificity in
137 the dietary assessment in EPIC, centre-specific residuals for total dietary folate were
138 computed. Centre-specific mean values were then added to residuals to recuperate the
139 original scale and ease interpretability.
140

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

143 active), smoking status (never/former/current), education (<secondary school/ ≥secondary
144 school), ever use of vitamin or mineral supplements (no/yes), history of diabetes (no/yes) and
145 intake of other dietary factors (g/day) including dietary fibre, carbohydrate, and alcohol. In
146 the multivariable models, the variables that changed the unadjusted risk estimate by at least
147 ~10% were considered as confounders and adjusted for. These include smoking status, BMI,
148 educational level, history of diabetes, supplement use and dietary fibre intake. Energy intake
149 was further included in the model for complete energy adjustment³¹ even though it did not
150 alter the unadjusted risk substantially.

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

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

167 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
168 pancreatic cancer risk was additionally explored, with the reference category set at 200-300
169 $\mu\text{g}/\text{d}$, which was observed to be the average intake range in a previous EPIC study comparing
170 standardised dietary folate intake across ten participating countries³⁴.

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

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

180 All statistical tests were two-sided and analyses were performed using STATA (version
181 13, Stata Corporation, College Station, Texas).

182

183 **Results**

184 A total of 477,206 participants without any history of cancer and with complete dietary folate
185 information were included in the analysis among which 397 men and 468 women developed
186 a first primary pancreatic cancer during an average of 11 years of follow-up. **Table 1** shows
187 baseline characteristics of the participants according to quartiles of energy-adjusted dietary
188 folate intake. Participants in the highest category of folate intake tended to be more educated,
189 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 $\mu\text{g}/\text{day}$ 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 ($\geq 353 \mu\text{g}/\text{day}$) compared
200 with the lowest category of intake ($< 241 \mu\text{g}/\text{day}$) 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 $\mu\text{g}/\text{day}$
212 and those who consumed more than 353 $\mu\text{g}/\text{day}$ 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

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

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

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

232 We also explored the effect of very high (≥ 500 $\mu\text{g}/\text{d}$) or very low folate intake (<150
233 $\mu\text{g}/\text{d}$) in relation to pancreatic cancer risk, and we did not observe any significant associations
234 in the multivariable adjusted model, possibly due to limited statistical power (**Table 3**).

235 When we conducted a sensitivity analysis among those who reported not to take any
236 dietary supplements ($n=237,113$), the results did not change substantially (HR of 0.90, 95%
237 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.
238 lowest: 0.61, 95% CI: 0.26, 1.44; $P_{\text{trend}}=0.28$). Similarly, the results hardly changed when

239 we excluded the pancreatic cancer cases incident within the first 2 years of follow-up (n=90
240 cases) and repeated the analyses (HR of 1.03, 95% CI: 0.82, 1.29; $P=0.79$ for an increment of
241 100 $\mu\text{g}/\text{day}$, HR for the highest quartile vs. lowest: 0.79, 95% CI: 0.48, 1.30; $P_{\text{trend}} = 0.32$). A
242 sensitivity analysis restricting the analyses to the microscopically confirmed cases did not
243 alter the results.

245 Discussion

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

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

262 compared with those in the lowest quartile. No significant association was found between
263 supplemental folic acid use and pancreatic cancer risk¹².

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

271 A large pooled analysis of 14 prospective cohort studies showed that dietary folate intake
272 was not associated with overall risk of pancreatic cancer¹⁶. The summary relative risk for the
273 highest vs. the lowest quintile of folate intake was 1.06 (95% CI: 0.90-1.25, $P_{\text{heterogeneity}}=0.15$)
274¹⁶. In the pooled analysis, folate data may be heterogeneous across studies and countries as
275 studies rely on each country's own food-composition data which tend to vary in terms of
276 availability and quality of folate values^{37, 38}. This may have an influence in a potential
277 relationship. It has been pointed out that there is a lack of clarity and consistency in the
278 terminology and definitions used for folate information in the food composition tables
279 available in Europe due to the specific complexity of folate³⁹. A recent critical evaluation of
280 folate data in 18 European and international databases concluded that a lack of comparability
281 still exists between countries⁴⁰. To overcome this, our study results came from the
282 standardised food and nutrient data linked to the ENDB with recently updated folate
283 information.

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

287 consumed types of supplements in the EPIC study according to the 24 hour-recall data that
288 were collected in a sub group of participants with more detailed information on supplement
289 use at the baseline⁴¹. In addition, folic acid fortification was not widespread at the time of the
290 baseline information collection in Europe²¹. Thus, we had the unique opportunity to assess
291 the association between baseline dietary folate intake and pancreatic cancer risk, with
292 minimum influence of folic acid fortification or supplementation in the EPIC study. This was
293 confirmed when we conducted a sensitivity analysis excluding ever users of vitamin and
294 mineral supplements and the results did not materially change.

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

311 The current study has limitations. We did not have information on occurrence of
312 pancreatitis in the study population which might have affected pancreatic cancer risk. Neither
313 did we have repeated information during the follow-up period to assess any potential changes
314 in dietary intakes over time. In addition, we relied on self-reported dietary folate intake.
315 However, the overall results did not substantially differ from the previous EPIC study that
316 investigated plasma folate level in association with pancreatic cancer risk¹⁸. In our study, the
317 range of dietary folate intake was quite narrow, as shown previously in the results using the
318 24-hour dietary recall methods³⁴, with too few participants with either very low or very high
319 intakes. It was therefore not possible to explore the effect of extreme folate intake on
320 pancreatic cancer risk with sufficient statistical power. We used self-reported smoking status
321 which might be inaccurate. However, a recent EPIC study of plasma cotinine level and
322 pancreatic cancer risk compared the cotinine level against self-reported smoking status and
323 concluded that self-reported smoking status was sufficient to establish a causal relationship
324 and did not underestimate its relationship with pancreatic cancer risk⁴⁴.

325 In conclusion, using standardised data from this large, multi-centre prospective study, we
326 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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Reference List

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin D, Forman D, Bray F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: <http://globocan.iarc.fr>, accessed on 6/February/2018., 2013.
2. Maisonneuve P, Lowenfels AB. Risk factors for pancreatic cancer: a summary review of meta-analytical studies. *Int J Epidemiol* 2015;44: 186-98.
3. Raimondi S, Maisonneuve P, Lowenfels AB. Epidemiology of pancreatic cancer: an overview. *Nat Rev Gastroenterol Hepatol* 2009;6: 699-708.
4. World Cancer Research Fund / American Institute for Cancer Research. Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Pancreatic Cancer. Available at <http://www.dietandcancerreport.org>, 2012.
5. Hart AR, Kennedy H, Harvey I. Pancreatic cancer: a review of the evidence on causation. *Clin Gastroenterol Hepatol* 2008;6: 275-82.
6. Stolzenberg-Solomon RZ, Graubard BI, Chari S, Limburg P, Taylor PR, Virtamo J, Albanes D. Insulin, glucose, insulin resistance, and pancreatic cancer in male smokers. *JAMA* 2005;294: 2872-8.
7. Kim YI. Folate and DNA Methylation: A Mechanistic Link between Folate Deficiency and Colorectal Cancer? *Cancer Epidemiol Biomarker Prev* 2004;13: 511-9.
8. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: Implications for cancer and neuronal damage. *Proc Natl Acad Sci U S A* 1997;94: 3290-5.
9. Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA Methylation: A Review of Molecular Mechanisms and the Evidence for Folate's Role. *Adv Nutr* 2012;3: 21-38.

10. Keszei AP, Verhage BA, Heinen MM, Goldbohm RA, van den Brandt PA. Dietary folate and folate vitamers and the risk of pancreatic cancer in the Netherlands cohort study. *Cancer Epidemiol Biomarkers Prev* 2009;18: 1785-91.
11. Larsson SC, Hakansson N, Giovannucci E, Wolk A. Folate intake and pancreatic cancer incidence: a prospective study of Swedish women and men. *J Natl Cancer Inst* 2006;98: 407-13.
12. Oaks BM, Dodd KW, Meinhold CL, Jiao L, Church TR, Stolzenberg-Solomon RZ. Folate intake, post-folic acid grain fortification, and pancreatic cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am J Clin Nutr* 2010;91: 449-55.
13. Skinner HG, Michaud DS, Giovannucci EL, Rimm EB, Stampfer MJ, Willett WC, Colditz GA, Fuchs CS. A prospective study of folate intake and the risk of pancreatic cancer in men and women. *Am J Epidemiol* 2004;160: 248-58.
14. Stolzenberg-Solomon RZ, Pietinen P, Barrett MJ, Taylor PR, Virtamo J, Albanes D. Dietary and other methyl-group availability factors and pancreatic cancer risk in a cohort of male smokers. *Am J Epidemiol* 2001;153: 680-7.
15. Lin HL, An QZ, Wang QZ, Liu CX. Folate intake and pancreatic cancer risk: an overall and dose-response meta-analysis. *Public Health* 2013;127: 607-13.
16. Bao Y, Michaud DS, Spiegelman D, Albanes D, Anderson KE, Bernstein L, van den Brandt PA, English DR, Freudenheim JL, Fuchs CS, Giles GG, Giovannucci E, Goldbohm RA, Hakansson N, Horn-Ross PL, Jacobs EJ, Kitahara CM, Marshall JR, Miller AB, Robien K, Rohan TE, Schatzkin A, Stevens VL, Stolzenberg-Solomon RZ, Virtamo J, Wolk A, Ziegler RG, Smith-Warner SA. Folate Intake and Risk of Pancreatic Cancer: Pooled Analysis of Prospective Cohort Studies. *Journal of the National Cancer Institute* 2011;103: 1840-50.
17. Vollset SE, Clarke R, Lewington S, Ebbing M, Halsey J, Lonn E, Armitage J, Manson JE, Hankey GJ, Spence JD, Galan P, Bonna KH, Jamison R, Gaziano JM, Guarino P, Baron JA, Logan RF, Giovannucci EL, den Heijer M, Ueland PM, Bennett D, Collins R, Peto R. Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: meta-analyses of data on 50000 individuals. *The Lancet* 2013;381: 1029-36.
18. Chuang SC, Stolzenberg-Solomon R, Ueland PM, Vollset SE, Midttun O, Olsen A, Tjonneland A, Overvad K, Boutron-Ruault MC, Morois S, Clavel-Chapelon F, Teucher B, Kaaks R, Weikert C, Boeing H, Trichopoulou A, Benetou V, Naska A, Jenab M, Slimani N, Romieu I, Michaud DS, Palli D, Sieri S, Panico S, Sacerdote C, Tumino R, Skeie G, Duell EJ, Rodriguez L, Molina-Montes E, Huerta JM, Larranaga N, Gurrea AB, Johansen D, Manjer J, Ye W, Sund M, Peeters PH, Jeurink S, Wareham N, Khaw KT, Crowe F, Riboli E, Bueno-de-Mesquita B, Vineis P. A U-shaped relationship between plasma folate and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition. *Eur J Cancer* 2011;47: 1808-16.

19. Stolzenberg-Solomon RZ, Albanes D, Nieto FJ, Hartman TJ, Tangrea JA, Rautalahti M, Sehlub J, Virtamo J, Taylor PR. Pancreatic cancer risk and nutrition-related methyl-group availability indicators in male smokers. *J Natl Cancer Inst* 1999;91: 535-41.
20. Schernhammer E, Wolpin B, Rifai N, Cochrane B, Manson JA, Ma J, Giovannucci E, Thomson C, Stampfer MJ, Fuchs C. Plasma folate, vitamin B6, vitamin B12, and homocysteine and pancreatic cancer risk in four large cohorts. *Cancer Res* 2007;67: 5553-60.
21. Nicolas G, Witthöft CM, Vignat J, Knaze V, Huybrechts I, Roe M, Finglas P, Slimani N. Compilation of a standardised international folate database for EPIC. *Food Chem* 2016;193: 134-40.
22. Riboli E, Kaaks R. The EPIC Project: rationale and study design. *European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol* 1997;26: S6.
23. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondiere UR, Hemon B, Casagrande C, Vignat J, Overvad K, Tjonneland A, Clavel-Chapelon F, Thiebaut A, Wahrendorf J, Boeing H, Trichopoulos D, Trichopoulou A, Vineis P, Palli D, Bueno-de-Mesquita HB, Peeters PHM, Lund E, Engeset D, Gonzalez CA, Barricarte A, Berglund G, Hallmans G, Day NE, Key TJ, Kaaks R, Saracci R. European prospective investigation into cancer and nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002;5: 1113-24.
24. Kaaks R, Slimani N, Riboli E. Pilot phase studies on the accuracy of dietary intake measurements in the EPIC project: overall evaluation of results. *European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol* 1997;26: S26.
25. Slimani N, Deharveng G, Unwin I, Southgate DA, Vignat J, Skeie G, Salvini S, Parpinel M, Moller A, Ireland J, Becker W, Farran A, Westenbrink S, Vasilopoulou E, Unwin J, Borgejordet A, Rohrmann S, Church S, Gnagnarella P, Casagrande C, van Bakel M, Niravong M, Boutron-Ruault MC, Stripp C, Tjonneland A, Trichopoulou A, Georga K, Nilsson S, Mattisson I, Ray J, Boeing H, Ocke M, Peeters PH, Jakszyn P, Amiano P, Engeset D, Lund E, de Magistris MS, Sacerdote C, Welch A, Bingham S, Subar AF, Riboli E. The EPIC nutrient database project (ENDB): a first attempt to standardize nutrient databases across the 10 European countries participating in the EPIC study. *Eur J Clin Nutr* 2007;61: 1037-56.
26. Slimani N, Margetts Be. Nutrient intake and patterns in the EPIC cohorts from 10 European countries. *Eur J Clin Nutr* 2009;63.
27. Slimani N, Kaaks R, Ferrari P, Casagrande C, Clavel-Chapelon F, Lotze G, Kroke A, Trichopoulos D, Trichopoulou A, Lauria C, Bellegotti M, Ocke MC, Peeters PHM, Engeset D, Lund E, Agudo A, Larranaga N, Mattisson I, Andren C, Johansson I, Davey G, Welch AA, Overvad K, Tjonneland A, van Staveren WA, Saracci R, Riboli E. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. *Public Health Nutr* 2002;5: 1125-45.
28. Friedenreich C, Cust A, Lahmann PH, Steindorf K, Boutron-Ruault MC, Clavel-Chapelon F, Mesrine S, Linseisen J, Rohrmann S, Boeing H, Pischon T, Tjonneland A, Halkjaer J, Overvad K, Mendez M, Redondo ML, Garcia CM, Larranaga N, Tormo MJ,

Gurrea AB, Bingham S, Khaw KT, Allen N, Key T, Trichopoulou A, Vasilopoulou E, Trichopoulos D, Pala V, Palli D, Tumino R, Mattiello A, Vineis P, Bueno-de-Mesquita HB, Peeters PHM, Berglund G, Manjer J, Lundin E, Lukanova A, Slimani N, Jenab M, Kaaks R, Riboli E. Anthropometric factors and risk of endometrial cancer: the European prospective investigation into cancer and nutrition. *Cancer Causes Control* 2007;18: 399-413.

29. Haftenberger M, Lahmann PH, Panico S, Gonzalez CA, Seidell JC, Boeing H, Giurdanella MC, Krogh V, Bueno-de-Mesquita H, Peeters PHM, Skeie G, Hjartaker A, Rodriguez M, Quiros JR, Berglund G, Janlert U, Khaw KT, Spencer EA, Overvad K, Tjønneland A, Clavel-Chapelon F, Tehard B, Miller AB, Klipstein-Grobusch K, Benetou V, Kiriaki G, Riboli E, Slimani N. Overweight, obesity and fat distribution in 50-to 64-year-old participants in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutr* 2002;5: 1147-62.

30. Haftenberger M, Schuit AJ, Tormo N, Boeing H, Wareham N, Bueno-de-Mesquita HB, Kumle M, Hjartaker A, Chirlaque MD, Ardanaz E, Andren C, Lindahl B, Peeters PHM, Allen NE, Overvad K, Tjønneland A, Clavel-Chapelon F, Linseisen J, Bergmann MM, Trichopoulou A, Lagiou P, Salvini S, Panico S, Riboli E, Ferrari P, Slimani N. Physical activity of subjects aged 50-64 years involved in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutr* 2002;5: 1163-77.

31. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997;65: 1220S-8S; discussion 9S-31S.

32. Heinzl H, Kaider A. Gaining more flexibility in Cox proportional hazards regression models with cubic spline functions. *Comput Methods Programs Biomed* 1997;54: 201-8.

33. Halsted CH, Villanueva JA, Devlin AM, Chandler CJ. Metabolic interactions of alcohol and folate. *J Nutr* 2002;132: 2367S-72S.

34. Park JY, Nicolas G, Freisling H, Biessy C, Scalbert A, Romieu I, Chajes V, Chuang SC, Ericson U, Wallstrom P, Ros MM, Peeters PHM, Mattiello A, Palli D, Huerta JM, Amiano P, Halkjaer J, Dahm CC, Trichopoulou A, Orfanos P, Teucher B, Feller S, Skeie G, Engeset D, Boutron-Ruault MC, Clavel-Chapelon F, Crowe F, Khaw KT, Vineis P, Slimani N. Comparison of standardised dietary folate intake across ten countries participating in the European Prospective Investigation into Cancer and Nutrition. *Br J Nutr* 2012;108: 552-69.

35. Larsson SC, Giovannucci E, Wolk A. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. *Gastroenterology* 2006;131: 1271-83.

36. Tio M, Andrici J, Cox MR, Eslick GD. Folate intake and the risk of upper gastrointestinal cancers: a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2014;29: 250-8.

37. deBree A, vanDusseldorp M, Brouwer IA, vanhetHof KH, SteegersTheunissen RPM. Folate intake in Europe: recommended, actual and desired intake. *Eur J Clin Nutr* 1997;51: 643-60.

38. Elmadfa I, Meyer A, Nowak V, Hasenegger V, Putz P, Verstraeten R, Remaut-DeWinter AM, Kolsteren P, Dostalova J, Dlouhy P, Trolle E, Fagt S, Biloft-Jensen A, Mathiessen J,

Velsing Groth M, Kambek L, Gluskova N, Voutilainen N, Erkkila A, Vernay M, Krems C, Strassburg A, Vasquez-Caicedo AL, Urban C, Naska A, Efstathopoulou E, Oikonomou E, Tsiotas K, Bountziouka V, Benetou V, Trichopoulou A, Zajkas G, Kovacs V, Martos E, Heavey P, Kelleher C, Kennedy J, Turrini A, Selga G, Sauka M, Petkeviciene J, Klumbiene J, Holm Totland T, Andersen LF, Halicka E, Rejman K, Kowrygo B, Rodrigues S, Pinhao S, Ferreira LS, Lopes C, Ramos E, Vaz Almeida MD, Vlad M, Simcic M, Podgrajsek K, Serra Majem L, Roman Vinas B, Ngo J, Ribas Barba L, Becker V, Fransen H, Van Rossum C, Ocke M, Margetts B. European Nutrition and Health Report 2009. *Forum Nutr* 2009;62: 1-405.

39. Deharveng G, Charrondiere UR, Slimani N, Southgate DA, Riboli E. Comparison of nutrients in the food composition tables available in the nine European countries participating in EPIC. *European Prospective Investigation into Cancer and Nutrition. Eur J Clin Nutr* 1999;53: 60-79.

40. Bouckaert KP, Slimani N, Nicolas G, Vignat J, Wright AJ, Roe M, Witthoft CM, Finglas PM. Critical evaluation of folate data in European and international databases: recommendations for standardization in international nutritional studies. *Mol Nutr Food Res* 2011;55: 166-80.

41. Skeie G, Braaten T, Hjartaker A, Lentjes M, Amiano P, Jakszyn P, Pala V, Palanca A, Niekerk EM, Verhagen H, Avloniti K, Psaltopoulou T, Niravong M, Touvier M, Nimptsch K, Haubrock J, Walker L, Spencer EA, Roswall N, Olsen A, Wallstrom P, Nilsson S, Casagrande C, Deharveng G, Hellstrom V, Boutron-Ruault MC, Tjonneland A, Joensen AM, Clavel-Chapelon F, Trichopoulou A, Martinez C, Rodriguez L, Frasca G, Sacerdote C, Peeters PHM, Linseisen J, Schienkiewitz A, Welch AA, Manjer J, Ferrari P, Riboli E, Bingham S, Engeset D, Lund E, Slimani N. Use of dietary supplements in the European Prospective Investigation into Cancer and Nutrition calibration study. *Eur J Clin Nutr* 2009;63: S226-S38.

42. Smith AD, Kim YI, Refsum H. Is folic acid good for everyone? *The American Journal of Clinical Nutrition* 2008;87: 517-33.

43. Ulrich CM, Potter JD. Folate and cancer - Timing is everything. *Jama-Journal of the American Medical Association* 2007;297: 2408-9.

44. Leenders M, Chuang SC, Dahm CC, Overvad K, Ueland PM, Middtun O, Vollset SE, Tjonneland A, Halkjaer J, Jenab M, Clavel-Chapelon F, Boutron-Ruault MC, Kaaks R, Canzian F, Boeing H, Weikert C, Trichopoulou A, Bamia C, Naska A, Palli D, Pala V, Mattiello A, Tumino R, Sacerdote C, van Duijnhoven FJ, Peeters PH, van Gils CH, Lund E, Rodriguez L, Duell EJ, Perez MJ, Molina-Montes E, Castano JM, Barricarte A, Larranaga N, Johansen D, Lindkvist B, Sund M, Ye W, Khaw KT, Wareham NJ, Michaud DS, Riboli E, Xun WW, Allen NE, Crowe FL, Bueno-de-Mesquita HB, Vineis P. Plasma cotinine levels and pancreatic cancer in the EPIC cohort study. *Int J Cancer* 2012;131: 997-1002.

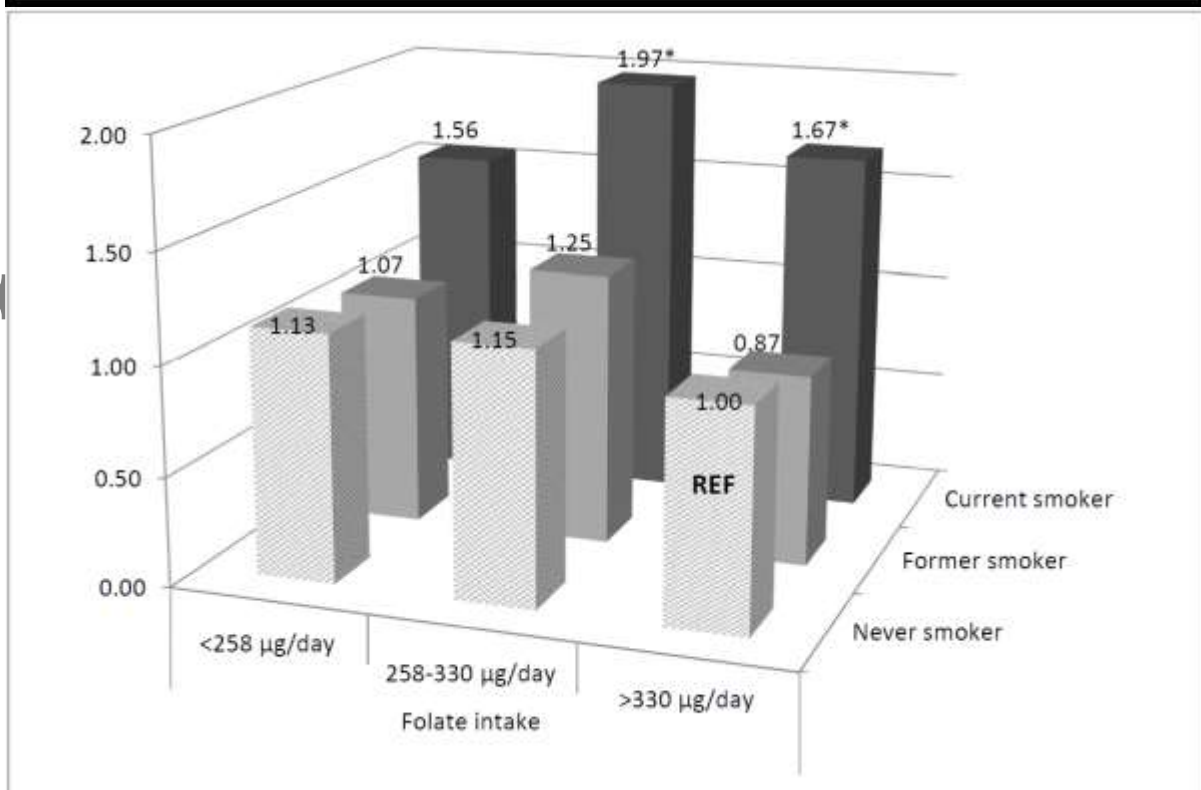
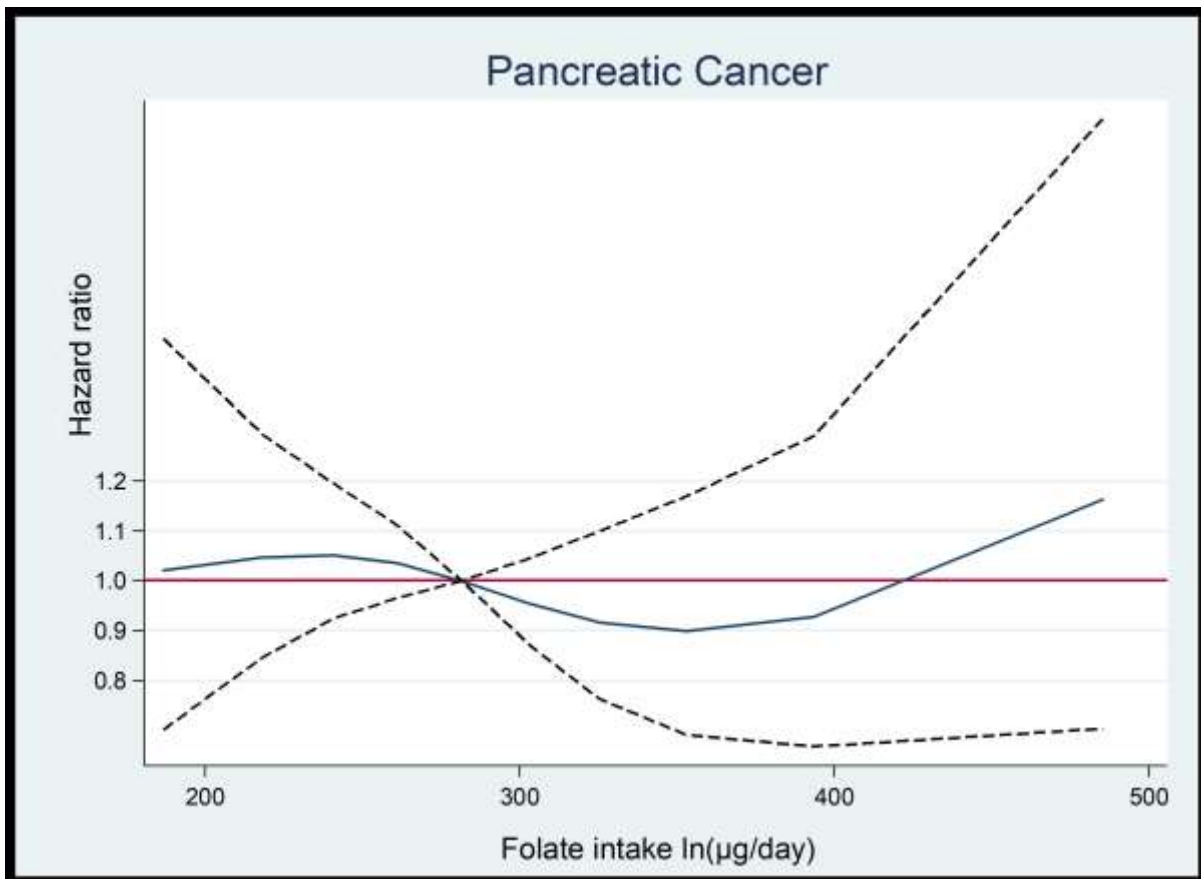


Table 1 Baseline characteristics of participants by energy-adjusted dietary folate intake, the EPIC study

	Energy-adjusted folate intake ($\mu\text{g}/\text{d}$)			
	Q1	Q2	Q3	Q4
Range	0-241	241-292	292-353	≥ 353
Cases/all participants	231/119,302	234/119,301	228/119,302	172/119,301
Men	133/44,758	106/38,789	95/33,284	63/25,397
Women	98/74,544	128/80,512	133/86,018	109/93,904
Person-years	1,320,250	1,321,789	1,309,460	1,313,115
Sex				
Men	37.5	32.5	27.9	21.3
Women	62.5	67.5	72.1	78.7
Age (years)	50.2 (9.0)	51.5 (9.3)	52.3 (9.9)	50.9 (11.3)
BMI (kg/m^2)	25.4 (4.1)	25.6 (4.3)	25.6 (4.4)	25.0 (4.4)
Smoking status				
Never (%)	42.4	46.6	53.1	58.2
Former (%)	27.4	27.8	26.0	27.4
Current (%)	30.2	25.6	20.9	14.4
Educational level				
<Secondary school (%)	62.1	57.5	53.7	42.7
\geq Secondary school (%)	37.9	42.5	46.3	57.3
Physical activity				
< moderately inactive (%)	58.1	56.4	56.9	55.6
> moderately active (%)	41.9	43.6	43.1	44.4
Dietary supplement user				
No (%)	67.4	63.0	57.5	52.9
Yes (%)	32.6	37.0	42.5	47.1
Self-reported diabetes				
No (%)	98.1	97.4	96.6	96.4
Yes (%)	1.9	2.6	3.4	3.7
Energy intake (Kcal)	2,062 (678)	2,064 (614)	2,067 (602)	2,092 (611)
Alcohol intake (units/week)^{1,2}	6.20 (15.27)	8.36 (16.15)	8.13 (13.89)	6.23 (11.17)
Fibre intake (g/day)²	18.39 (4.16)	21.61 (4.45)	23.59 (4.93)	27.54 (6.67)
Folate intake ($\mu\text{g}/\text{d}$)^{1,2}	211.82 (35.04)	266.36 (25.44)	319.59 (29.62)	408.31 (89.83)
Vitamin B-12 intake (μg)^{1,2}	5.51 (2.68)	5.96 (3.19)	6.09 (3.74)	6.02 (4.55)
Vitamin B-6 intake (mg)²	1.53 (0.32)	1.76 (0.31)	1.95 (0.33)	2.25 (0.44)
Riboflavin intake (mg)²	1.47 (0.36)	1.70 (0.42)	1.93 (0.50)	2.34 (0.72)
Meat and meat products intake (g/day)¹	93.5 (68.3)	97.7 (69.2)	94.4 (71.6)	78.7 (91.9)
Vegetable intake (g/day)¹	97.9 (69.6)	145.6 (94.8)	210.4 (134.1)	321.3 (192.7)
Fruits, nuts and seeds intake (g/day)¹	123.5 (137.7)	178.9 (176.1)	232.9 (204.1)	292.5 (246.9)

¹Intakes are all dietary and shown as median values with interquartile ranges, otherwise values are mean (SD) for continuous or percentages for categorical variables. ²Nutrients were energy adjusted.

Table 1 Hazard ratios of pancreatic cancer, with 95% confidence intervals (CIs), according to quartiles of energy-adjusted dietary folate intake

	Quartile of energy-adjusted dietary folate intake ¹				P for trend	P for interaction
	>0 to <241 µg/d	241 to <292 µg/d	292 to <353 µg/d	≥353 µg/d		
No. of all participants	119,302	119,301	119,302	119,301		
Pancreatic cancer cases	231	234	228	172		
Person-years	1,320,250	1,321,789	1,309,460	1,313,115		
Model1 ²	1.00	0.94 (0.70 - 1.25)	0.86 (0.62 - 1.18)	0.70 (0.48 - 1.03)	0.06	
Model2 ³	1.00	1.01 (0.74 - 1.37)	0.97 (0.67 - 1.41)	0.81 (0.51 - 1.31)	0.38	
Sex						
Men	1.00	0.90 (0.57 - 1.42)	0.97 (0.54 - 1.75)	0.76 (0.35 - 1.65)	0.57	0.49
Women	1.00	1.13 (0.73 - 1.74)	0.98 (0.60 - 1.62)	0.80 (0.43 - 1.50)	0.38	
Tertiles of alcohol intake						
T1 (no. cases), Median 0.8 g/day P10-P90 (0-3.0)	77	69	57	63		
	1.00	1.24 (0.62 - 2.48)	1.50 (0.60 - 3.78)	1.37 (0.33 - 5.75)	0.52	0.82
T2 (no. cases), Median 7.2 g/day(4.3-11.1)	54	70	69	61		
	1.00	0.80 (0.33 - 1.94)	0.74 (0.25 - 2.20)	0.51 (0.11 - 2.43)	0.41	
T3 (no. cases), Median 22.5 g/day (13.6-50.9)	100	95	102	48		
	1.00	0.97 (0.48 - 1.97)	1.89 (0.83 - 4.33)	1.33 (0.45 - 3.94)	0.28	
Smoking status						
Never smokers (no. cases)	71	79	100	86		
	1.00	0.79 (0.41 - 1.51)	1.03 (0.48 - 2.18)	0.55 (0.21 - 1.41)	0.24	0.99
Former smokers (no. cases)	67	65	55	52		
	1.00	0.79 (0.31 - 2.02)	0.71 (0.23 - 2.12)	0.55 (0.13 - 2.27)	0.41	
Current smokers (no. cases)	90	89	68	28		
	1.00	1.85 (0.82 - 4.17)	4.52 (1.59 - 12.88)	4.42 (1.05 - 18.62)	0.01	

¹Folate intake was adjusted for energy using the residual method. ²Model1 was with stratification of sex, age and centre. ³Model2 was adjusted for smoking status, energy intake and BMI, education, diabetes status (self-reported), supplement use and fibre intake, and age, sex and centre being included as stratification variables.

Table 3 Hazard ratios of pancreatic cancer, with 95% confidence intervals (CIs), according to different categories of energy-adjusted dietary folate intake, with 200-300 µg/d of folate intake being a reference

	Energy-adjusted folate intake ¹					<i>P</i> for trend
	<150 µg/d	150 to <200 µg/d	200 to <300 µg/d	300 to <500 µg/d	≥500 µg/d	
No. of all participants	4,013	36,330	216,143	200,438	20,282	
Pancreatic cancer cases	17	58	420	344	26	
Person-years	45,102	401,302	2,392,501	2,200,878	224,725	
Model1 ²	2.72 (1.16 - 6.39)	0.81 (0.53 - 1.24)	1.00	0.82 (0.64 - 1.05)	1.17 (0.55 - 2.52)	0.34
Model2 ³	1.82 (0.70 - 4.73)	0.73 (0.47 - 1.14)	1.00	0.89 (0.67 - 1.18)	1.61 (0.67 - 3.86)	0.77

¹Folate intake was adjusted for energy using the residual method. ²Model1 was with stratification of sex, age and centre. ³Model2 was adjusted for smoking status, energy intake and BMI, education, diabetes status (self-reported), supplement use and fibre intake, and age, sex and centre being included as stratification variables.

Table 4 Previous prospective studies that investigated the associations between dietary folate intake and pancreatic cancer risk

Study	Study data	Study participants		Exposure of interest	Average follow-up time (years)	Study outcome	Main statistical methods	Confounding factors adjusted	Main results	Conclusions	Comments on folic acid supplements
		Number (as in the analysis)	Age at baseline (years)								
Oaks et al, 2011 (US)	The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial cohort	51,988 men and 57,187 women	55-74	Dietary food folate (natural + fortified) intake (124-item FFQ data)	6.5	266 incident pancreatic cancer cases	Cox proportional hazard models	Age, total energy intake, smoking, self-reported diabetes, BMI, and saturated fat intake	0.47 (95% CI, 0.23-0.94; $P_{\text{trend}}=0.09$) for Q4 (>253.3 µg/d) vs. Q1 (<179.1 µg/d) in women and 1.20 (0.70-2.04; $P_{\text{trend}}=0.67$) for Q4 (>229.6 µg/d) vs. Q1 (<158.0 µg/d) in men	The results support an association between higher food (natural+fortified) and total folate intakes and decreased risk of pancreatic cancer in women , but not in men	The findings show no association between folic acid added to foods and pancreatic cancer risk (and also do not support the hypothesis that folic acid fortification increases the risk of pancreatic cancer)
Keszei et al, 2009 (The Netherlands)	The Netherlands Cohort Study	5,000 men and women (case-cohort design)	55-69	Dietary folate intake (150-item FFQ data)	13.3	363 incident pancreatic cancer cases	Cox proportional hazard models	Age, sex, smoking status, number of years smoked, number of cigarettes smoked, intake of vegetables and added sugar	1.37 (95% CI, 0.97-1.94; $P_{\text{trend}}=0.07$) for Q5 (>259.1 µg/d in men, >233.1 µg/d in women) vs. Q1 (<176.3 µg/d in men, <154.1 µg/d in women)	The results do not support a protective association of total dietary folate intake on the risk of pancreatic cancer	Folic acid in vitamin supplements was not allowed until the mid nineties, the effect of folic acid supplementation is therefore negligible
Larsson et al, 2006 (Sweden)	The Swedish Mammography Cohort+the Cohort of Swedish Men	81,922 men and women	45-83	Dietary folate intake (96-item FFQ data)	6.8	135 incident pancreatic cancer cases	Cox proportional hazard models	Age, sex, smoking status, pack-years of smoking, education, BMI, exercise, history of diabetes, intakes of total energy, alcohol, and carbohydrate and fruit and vegetable consumption	0.25 (95% CI, 0.11-0.59; $P_{\text{trend}}=0.002$) for Q5 (>350 µg/d) vs. Q1 (<200 µg/d)	The results support an association between increased intake of food folate and a reduced risk of pancreatic cancer	An inverse association was observed between intake of folate from foods (combining dietary and supplemental sources), but not from supplements, and the risk of pancreatic cancer
Skinner et al, 2000 (US)	The Nurses' Health Study + the Health Professionals Follow-up Study	77,640 women and 47,840 men	30-75	Dietary folate intake (131-item FFQ data)	14	326 incident pancreatic cancer cases	Cox proportional hazard models	Age, energy intake, cigarette smoking, BMI, diabetes and height	0.66 (95% CI, 0.37-1.18; $P_{\text{trend}}=0.17$) for Q5 (>500 µg/d) vs. Q1 (<300 µg/d)	The results from two large cohorts do not support a strong association between energy-adjusted folate intake and the risk of pancreatic cancer	No influence of supplemental folic acid, a nonsignificant inverse trend for folate from food sources
Stolzenberg-Solomon et al, 2001 (Finland)	The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study	27,101 male smokers	50-69	Dietary folate intake (276-item FFQ data)	13	157 incident pancreatic cancer cases	Cox proportional hazard models	Age and intervention adjusted	0.52 (95% CI, 0.31-0.87; $P_{\text{trend}}=0.05$) for Q5 (>373 µg/d) vs. Q1 (<280 µg/d)	The results support an inverse association between energy-adjusted dietary folate intake and the risk of pancreatic cancer in male smokers	No significant association found between folic acid supplement consumption and pancreatic cancer risk

Inadequate folate intake is suspected of playing a role in the development of anomalies in DNA methylation, thereby contributing to carcinogenesis. In the case of pancreatic cancer, however, associations with folate status are unclear. The present investigation examined incident pancreatic cancer and dietary folate intake among subjects enrolled in the EPIC study, a multicentre prospective cohort study in Europe. Overall, no significant association was identified between dietary folate and pancreatic cancer risk. While a positive trend in risk association was detected among current smokers with high dietary folate, interactions between smoking and folate intake were not statistically significant.

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