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A U-shaped relationship between plasma folate and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition

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ABSTRACT

Folate intake has shown an inverse association with pancreatic cancer; nevertheless, results from plasma measurements were inconsistent. The aim of this study is to examine the association between plasma total homocysteine, methionine, folate, cobalamin, pyridoxal 5′-phosphate, riboflavin, flavin mononucleotide and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). We conducted a nested case–control study in the EPIC cohort, which has an average of 9.6 years of follow-up (1992–2006), using 463 incident pancreatic cancer cases. Controls were matched to each case by center, sex, age (±1 year), date (±1 year) and time (±3 h) at blood collection and fasting status. Conditional logistic regression was used to calculate the odds ratios (OR) and 95% confidence intervals (CI), adjusting for education, smoking status, plasma cotinine concentration, alcohol drinking, body mass index and diabetes status. We observed a U-shaped association between plasma folate and pancreatic cancer risk. The ORs for plasma folate ⩽5, 5–10, 10–15 (reference), 15–20, and >20 nmol/L were 1.58 (95% CI = 0.72–3.46), 1.39 (0.93–2.08), 1.0 (reference), 0.79 (0.52–1.21), and 1.34 (0.89–2.02), respectively. Methionine was associated with an increased risk in men (per quintile increment: OR = 1.17, 95% CI = 1.00–1.38) but not in women (OR = 0.91, 95% CI = 0.78–1.07; p for heterogeneity <0.01). Our results suggest a U-shaped association between plasma folate and pancreatic cancer risk in both men and women. The positive association that we observed between methionine and pancreatic cancer may be sex dependent and may differ by time of follow-up. However, the mechanisms behind the observed associations warrant further investigation.

1. Introduction

Pancreatic cancer is the 5th most common cause of death from cancer in Europe in 2008 (95,200 deaths, representing 5.5% total cancer deaths).[1] There is no effective screening test for the malignancy; it is often diagnosed at an advanced stage, which leads to a 5-year survival rate as low as 6% in Europe.[2] Few risk factors for pancreatic cancer have been consistently identified. Cigarette smoking,[3] obesity,[4] diabetes mellitus[5] and chronic pancreatitis[6] increase the risk of pancreatic cancer.

Evidence has been mounting for folate being a potentially important micronutrient in the prevention of cancer. Prospective studies in Finland[7] and Sweden[8] showed an inverse

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association between folate intake and pancreatic cancer, while the US NHS and the HPFS studies[9] showed that the inverse association was from foods and not from supplements. The PLCO study[10] further suggested that the inverse association was only observed in women but not in men. The World Cancer Research Fund (WCRF) systematically reviewed the literature and concluded that the folate intake probably protects against pancreatic cancer (2007).[11]

To date, there have been two studies investigating the association between blood biomarkers of one-carbon metabolism and pancreatic cancer. In a cohort of Finnish male smokers (ATBC cohort, 126 cases), a significant twofold reduction in pancreatic cancer risk was observed in participants with higher serum folate (>4.45 ng/mL) and pyridoxal 5′-phosphate (PLP, the coenzyme form of vitamin B6, >39.46 nmol/L), compared to those with lower concentrations (⩽3.33 ng/mL for folate and ⩽26.34 nmol/L for PLP).[12] However, the results from a pooled analysis based on four US cohorts (208 cases) showed no association[13]; this lack of association between folate concentrations and risk of pancreatic cancer may be because very few participants had less than adequate serum folate concentrations due to the folic acid grain fortification of wheat flour that has been mandatory in the US since 1998.

The aim of this study was to assess the association between nutrition related one-carbon metabolites, namely plasma total homocysteine (tHcy), methionine, folate, cobalamin, PLP, riboflavin, flavin mononucleotide (FMN) and pancreatic cancer in a case–control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC).

2. Methods

2.1. The EPIC cohort

EPIC is a multicenter prospective cohort study, which recruited 520,000 healthy volunteers from 23 centers in 10 countries (Sweden, Denmark, Norway, the Netherlands, United Kingdom, France, Germany, Spain, Italy and Greece) between 1992 and 2000. The cohort was described in detail previously.[14] In brief, the study population included volunteers aged 25–70 years at the time of recruitment. Informed consent forms were filled at each local center and the study was approved by the Institutional Review Board at the International Agency for Research on Cancer (IARC) and the local ethical committees. Lifestyle questionnaires included questions on dietary items, education, occupation, previous illness, alcohol and tobacco consumption and physical activity.

2.2. Ascertainment of cases and control selection

The follow-up was based on population cancer registries in Denmark, Italy, the Netherlands, Norway, Spain, Sweden and the United Kingdom. In France, Germany and Greece, a combination of health insurance records, cancer and pathology registries and active follow-up through participants and their next-of-kin were used. Mortality data were also obtained at regional or national levels. All participants were followed from recruitment (1992–2000) until cancer development, death, emigration or the end of the follow-up period (1992–2006).

Over 15 years of follow-up, 638 incident pancreatic cancer (ICD-0-2:C25) cases were identified. One hundred and seventy cases were excluded due to lack of blood samples ($N = 122$), benign tumor ($N = 1$), carcinoma in situ ($N = 1$), uncertain whether it is a primary or metastatic tumor ($N = 1$), neuroendocrine tumors ($N = 15$) and secondary tumors ($N = 30$). For each incident case, one control was selected by incidence density sampling and matched by center, gender, age at blood collection (±1 year), date (±1 year) and time (±3 h) at blood collection, and fasting status. Five cases and four controls were further excluded because the blood samples were not sufficient for the assay. Thus, the current analysis included 463 incident pancreatic cancer cases and 464 controls.

2.2.1. Biological samples and laboratory analyses

The blood samples were collected according to a standardised protocol.[14] Measurements of 25 markers, directly or indirectly involved in one-carbon metabolism,were made at Bevital A/S (http://www.bevital.no), Bergen, Norway. Plasma concentrations tHcy, methionine, PLP, riboflavin, FMN and cotinine (a nicotine metabolite and established marker for tobacco smoke exposure) were determined by mass spectrometry based methods,[15,16] while microbiological methods were used to determine concentrations of folate[17] and cobalamin.[18]

Samples were analysed in batches of 86 and quality control included six calibration samples, two control samples and one blank sample in each batch. Samples from case and control participants were kept at −80 °C and analysed in random order. The within and between day coefficients of variance (CV) were about 3–9% for folate, vitamin B12, PLP, riboflavin, FMN and about 0.9% for within-day CV and 2% for between-day CV for tHcy and methionine.[19] All staff at the Bevital laboratory were blinded to the case–control status of the blood samples.

2.3. Statistical analysis

Quintiles of each one-carbon nutrient biomarker were categorised based on distributions of the controls. Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for pancreatic cancer, with the lowest quintile serving as the reference category. ORs were also calculated by increasing quintiles as ordinal variables. All models included the following variables: education (no degree/primary school, technical or professional school, secondary school, university degree, not specified/missing), smoking status (never, former, current and unknown), cotinine plasma concentration, baseline alcohol drinking (never/former, current less than 6, 6–18 and greater than 18 g/d), body mass index (BMI, kg/m^2), and self-reported diabetes status at the baseline (Yes versus No). We examined effect modification by sex, region (northern-, central-, southern Europe), smoking status, cotinine plasma concentration (<5 indicating non-smoking, 5–85 indicating passive smoking and ⩾85 nmol/L indicating current smoking), lifetime alcohol drinking, BMI and physical activity (low/medium and high/very high) in stratified analyses.

We also evaluated the potential effect of subclinical disease on the association between the biomarkers and pancreatic cancer with time stratified analysis (⩽4 versus

>4 years[20,21] since blood was drawn). When necessary, we broke the matching sets and used unconditional logistic regression models, adjusted for the matching variables as well as the potential confounding variables described above. P-values for heterogeneity across sex and follow-up years were assessed by χ^2 statistics.

All analyses were performed using SAS 9.1. All tests were two sided and statistical significance was assessed at the level of 0.05.

3. Results

Table 1 shows the characteristics of the cases and controls. The study population includes about the same number of men and women. Almost half of the cases and controls were from Northern European countries (44%). The average follow-up was 9.6 years. Men tended to be smokers, to drink more and to be more often overweight than women.

The current analyses focused on the associations for the key one-carbon metabolites, tHcy, methionine, folate, PLP, cobalamin, riboflavin, FMN and risk of pancreatic cancer, overall and by sex (Table 2). Methionine was positively associated with pancreatic cancer risk in men (for each quintile increment: OR = 1.17, 95% CI = 1.00–1.38) but not in women (OR = 0.91, 95% CI = 0.78–1.07); heterogeneity between sex was detected ($p < 0.01$). Plasma folate tended to have a U-shaped dose–response relationship with pancreatic cancer risk: comparing the 4th to the 1st quintile, an inverse association was observed (OR = 0.46, 95% CI = 0.28–0.77), while no clear association was observed for the 5th versus 1st quintile (OR = 0.81, 95% CI = 0.49–1.35). The effect size was about the same for both men and women (p for heterogeneity = 1.00). Folate deficiency or high folate (defined as >20 nmol/L) were associated with higher risk of pancreatic cancer, but this was not statistically significant. As plasma concentrations of methionine result from the combination of dietary intake and the metabolism of folate intake, we calculated the folate/methionine ratio and examined its association with pancreatic cancer. Similar to the plasma folate, a U-shaped dose–response relationship was observed with the lowest OR in the 4th quintile (OR = 0.49, 95% CI = 0.30–0.81 and the 5th quintile OR = 0.68, 95% CI = 0.41–1.13, data not shown). Plasma PLP was inversely associated with pancreatic cancer in women (for each quintile increment, OR = 0.84, 95% CI = 0.72–0.97) but not in men (OR = 1.05, 95% CI = 0.87–1.24); however, the test for heterogeneity between men and women was not statistically significant (p for heterogeneity = 0.62).

No heterogeneity in the association between one-carbon metabolism biomarkers and pancreatic cancer risk was evident according to follow-up time except for methionine (Table 3). Using sex-specific quintiles, methionine was positively associated with risk of pancreatic cancer in men who had more than 4 years of follow-up between blood sample collection and diagnosis of pancreatic cancer (each quintile increment, OR=1.35, 95% CI = 1.06–1.71) but not among men with a shorter period of follow-up (⩽4 years: OR = 0.99, 95% CI = 0.76–1.29). On the other hand, methionine was inversely associated with pancreatic cancer risk in women, especially among women with shorter follow-up times (OR = 0.66, 95% CI = 0.46–0.94).

No heterogeneity across smoking status, cotinine concentration, alcohol drinking, BMI, or physical activity was detected (data not shown). Blood samples from Oxford and Norway cohorts were exposed at ambient temperatures for up to 48 h, and metabolites might be partly degraded due to such handling.[22] Excluding the two centers did not change the results materially.

4. Discussion

Overall, no clear pattern of association was observed for one-carbon metabolites and pancreatic cancer. Our results suggest a weak inverse, U-shaped association between pancreatic cancer risk and folate and an inverse relation with PLP in women. Methionine was inversely associated with the risk of pancreatic cancer in women who had a shorter period of follow-up but positively associated in men, especially for those with longer follow-up times.

The previous studies[12,13] clarified that the decreasing trend of serum folate and PLP may be only among non-multivitamin supplement users. The information for multivitamin use is not available in the EPIC study; however, in a subset of the EPIC cohort (~36,000 subjects), information on supplement use was collected.[23] Results from the sub-cohort indicated that participants from more northern countries (Denmark, Norway, Sweden and UK) reported higher supplement use than more southern countries (Greece, Italy, Spain) and women reported a higher use than men.[24] Results from the present study showed no evidence of regional (data not shown) or gender differences in the folate- and PLP-pancreatic cancer associations although the PLP effects appeared to be stronger in women.

Inadequate folate intake has been associated with several cancer risks but results have been inconsistent. The US folic acid fortification program has shown great success on increasing the plasma folate status in the general population.[25] The plasma folate is correlated better with folic acid supplements than with natural folate (bioavailability).[26] A tolerable upper intake of 1000 μg/d of folate has been set in the US and Europe because high folate levels could mask megaloblastic anaemia associated with vitamin B12 deficiency.[27] Moreover, there is rising concern for a dual function of folate, i.e. while folate deficiency could increase the risk of neoplastic transformation, folate supplementation could promote the progression of existing cancerous or pre-cancerous lesions.[20,28] Our observation suggested a U-shaped dose–response relationship. Although the effect was not significantly different from the null, those with high plasma folate concentrations (>20 nmol/L) had a similar risk as those with moderate deficiency (5–10 nmol/L) comparing to those with adequate level (10–15 nmol/L).

This is the first report on plasma methionine and pancreatic cancer risk. Serum methionine was previously reported to be inversely associated with lung cancer; in that study, the effects were consistent across sex and years of follow-up.[29] Dietary methionine intake based on food questionnaires was inversely associated with pancreatic cancer risk in a Swedish cohort study.[30] The ATBC[7] and the US Health Profes-

Table 1 – Characteristics of cases and controls.

	Men				Women				P[a]
	Controls		Cases		Controls		Cases		
	N	%	N	%	N	%	N	%	
Country									
France		0		0	15	6	15	6	
Italy	18	8	18	8	24	10	24	10	
Spain	17	8	17	8	22	9	22	9	
UK	20	9	20	9	24	10	24	10	
Netherlands	10	4	10	5	29	12	29	12	
Greece	14	6	14	6	9	4	9	4	
Germany	34	15	34	15	21	9	21	9	
Sweden	55	25	54	24	62	26	63	26	
Denmark	55	25	55	25	33	14	32	13	
Norway		0		0	2	1	2	1	
Education									
None	8	4	4	2	18	7	14	6	<0.01
Primary school completed	74	33	84	38	83	34	95	39	
Technical/professional school	52	23	50	23	67	28	66	27	
Secondary school	29	13	29	13	29	12	17	7	
University	55	25	50	23	35	15	42	17	
Not specified/missing	5	2	5	2	9	4	7	3	
Smoking status									
Never	59	26	54	24	141	59	111	46	<0.01
Former	98	44	91	41	59	24	55	23	
Quit over 10 years	71	32	52	23	41	17	32	13	
Quit within 10 years	27	12	39	18	18	7	23	10	
Current	62	28	74	33	40	17	73	30	
<15 cigarettes/d	35	16	37	17	17	7	38	16	
15–25 Cigarettes/d	20	9	24	11	17	7	27	11	
>25 Cigarettes/d	7	3	13	6	6	2	8	3	
Unknown	4	2	3	1	1	0	2	1	
Drinking status									
None drinker	6	3	2	1	14	6	17	7	<0.01
Former drinker	3	1	12	5	16	7	11	5	
Current drinker	207	93	198	89	199	83	193	80	
<6 g/d	61	27	53	24	106	44	92	38	
6–18 g/d	55	25	58	26	63	26	64	27	
18–30 g/d	41	18	37	17	16	7	19	8	
30–60 g/d	31	14	36	16	12	5	15	6	
>60 g/d	19	9	14	6	2	1	3	1	
Unknown	7	3	10	5	12	5	20	8	
BMI									
<18.5	1	0	0	0	9	4	5	2	<0.01
18.5–25	73	33	77	35	126	52	105	44	
25–30	109	49	106	48	71	29	90	37	
⩾30	40	18	39	18	35	15	41	17	
Diabetes at baseline									
No	206	92	194	87	218	98	217	97	0.57
Yes	11	5	20	9	9	4	13	6	
Missing	6	3	8	4	14	6	11	5	
Median (5th–95th percentile)									
Age at blood drawing	58 (43–70)		59 (43–70)		59 (44–70)		59 (44–70)		0.38
Age at diagnosis/end of follow-up	67 (53–79)		63 (48–75)		68 (54–79)		63 (50–76)		0.14
Metabolites in plasma									
Total homocysteine (μmol/L)	10.11 (6.73–16.44)		9.91 (6.64–17.47)		8.79 (5.51–16.12)		9.15 (5.34–15.43)		<0.01
Methionine (μmol/L)	26.26 (17.93–41.75)		26.73 (17.92–40.37)		23.54 (16.46–35.64)		23.72 (15.74–34.25)		<0.01
Folate (nmol/L)	12.47 (5.62–35.29)		11.98 (4.60–31.95)		13.45 (4.20–40.58)		13.10 (3.87–51.08)		0.09
Cobalamin (pmol/L)	342.60 (182.40–633.90)		344.75 (181.60–631.90)		376.90 (192.70–691.60)		379.30 (195.50–711.60)		0.05
Pyridoxal phosphate (nmol/L)	35.30 (16.36–98.59)		34.56 (17.35–102.20)		35.89 (15.59–111.41)		29.54 (12.72–104.22)		0.57
Riboflavin (nmol/L)	15.15 (5.74–71.21)		15.27 (6.19–56.26)		17.37 (7.45–62.32)		16.46 (6.49–59.31)		<0.01
Flavin mononucleotide (nmol/L)	6.71 (3.16–18.29)		6.52 (1.94–15.15)		7.09 (3.46–21.09)		6.63 (3.45–17.79)		0.51

[a] The *p* compares men and women among controls; χ^2 tests for categorical data and Wilcoxon Rank Sum tests for continuous data.

sional and Nurses' Health cohort[9] reported a null association between methionine intake and pancreatic cancer risk. Concerning other cancer sites, the effect of methionine intake has been inconsistent.

In our study, plasma methionine was positively associated with pancreatic cancer in men, especially for those with longer follow-up times but no such association was observed among women. We are uncertain what might explain this dif-

Table 2 – Odds ratios and 95% confidence intervals for biomarker levels (quintiles) and risk of pancreatic cancer by sex.

	All				Men					Women			
	Controls	Cases	OR	95% CI	Controls	Cases	OR	95% CI		Controls	Cases	OR	95% CI
Total homocysteine (μmol/L)													
⩽7.6	92	99	1.00		29	31	1.00			63	68	1.00	
7.6–8.8	92	77	0.72	(0.46–1.13)	35	37	1.01	(0.49–2.07)		57	40	0.48	(0.25–0.90)
8.8–10.2	92	95	1.28	(0.78–2.10)	50	49	1.19	(0.55–2.58)		42	46	1.33	(0.65–2.70)
10.2–12.3	93	105	0.93	(0.58–1.50)	59	56	0.88	(0.43–1.80)		34	49	1.04	(0.51–2.10)
>12.3	92	85	0.73	(0.67–1.46)	48	48	0.96	(0.44–2.11)		44	37	0.44	(0.19–1.01)
Every quintile			0.96	(0.85–1.09)			0.97	(0.81–1.15)				0.94	(0.79–1.13)
P for heterogeneity									0.75				
Methionine (μmol/L)													
⩽20.7	92	109	1.00		38	36	1.00			54	73	1.00	
20.7–23.0	92	62	0.56	(0.34–0.91)	34	25	1.01	(0.45–2.26)		58	37	0.35	(0.18–0.69)
23.0–26.3	92	92	0.87	(0.54–1.39)	39	40	1.68	(0.78–3.62)		53	52	0.49	(0.25–0.95)
26.3–0.2	93	98	0.93	(0.59–1.53)	54	53	1.55	(0.77–3.12)		39	45	0.63	(0.32–1.25)
>30.2	92	100	0.96	(0.66–1.42)	56	67	1.86	(0.90–3.87)		36	33	0.52	(0.26–1.06)
Every quintile			1.03	(0.93–1.15)			1.17	(1.00–1.38)				0.91	(0.78–1.07)
P for heterogeneity									<0.01				
Folate (nmol/L)													
⩽9.0	92	123	1.00		48	63	1.00			44	60	1.00	
9.0–11.6	92	79	0.56	(0.34–0.92)	46	41	0.55	(0.27–1.10)		46	38	0.61	(0.28–1.30)
11.6–14.8	93	88	0.62	(0.38–1.04)	46	43	0.57	(0.28–1.16)		47	45	0.62	(0.23–1.36)
14.8–20.2	92	75	0.46	(0.28–0.77)	46	36	0.46	(0.23–0.92)		46	39	0.50	(0.23–1.10)
>20.2	92	97	0.81	(0.49–1.35)	35	39	0.88	(0.40–1.90)		57	58	0.84	(0.40–1.78)
Every quintile			0.95	(0.85–1.06)			0.93	(0.79–1.10)				0.99	(0.84–1.16)
P for heterogeneity									1.00				
Folate (nmol/L), clinically relevant cut-points													
⩽5	25	30	1.58	(0.72–3.46)	9	12	1.55	(0.46–5.23)		16	18	1.47	(0.49–4.43)
5–10	106	125	1.39	(0.93–2.08)	61	70	1.50	(0.85–2.66)		45	55	1.32	(0.71–2.44)
10–15	150	139	1.00		71	66	1.00			79	73	1.00	
15–20	87	71	0.79	(0.52–1.21)	44	35	0.83	(0.45–1.51)		43	36	0.79	(0.41–1.51)
>20	93	97	1.34	(0.89–2.02)	36	39	1.49	(0.76–2.94)		57	58	1.35	(0.76–2.38)
P for heterogeneity									1.00				
Cobalamin (pmol/L)													
⩽267.3	92	102	1.00		46	57	1.00			46	45	1.00	
267.3–330.0	92	86	0.87	(0.55–1.36)	51	44	0.62	(0.33–1.17)		41	42	1.38	(0.69–2.76)
330.0–391.7	93	90	0.85	(0.54–1.32)	46	43	0.61	(0.32–1.15)		47	47	1.03	(0.53–2.01)
391.7–493.6	92	98	1.04	(0.65–1.66)	41	41	0.66	(0.32–1.35)		51	57	1.49	(0.75–2.95)
>493.6	92	86	0.94	(0.57–1.54)	37	37	0.63	(0.30–1.33)		55	49	1.35	(0.67–2.71)
Every quintile			1.01	(0.90–1.13)			0.92	(0.78–1.08)				1.08	(0.92–1.27)
P for heterogeneity									0.27				
Pyridoxal phosphate (nmol/L)													
⩽23.8	91	131	1.00		36	47	1.00			55	84	1.00	
23.8–31.6	92	89	0.65	(0.41–1.01)	48	46	0.68	(0.35–1.30)		44	43	0.59	(0.31–1.12)
31.6–39.3	92	67	0.53	(0.34–0.84)	46	36	0.65	(0.33–1.27)		46	31	0.44	(0.23–0.85)
39.3–54.8	92	94	0.72	(0.45–1.15)	49	49	0.94	(0.46–1.95)		43	45	0.61	(0.32–1.18)
>54.8	92	77	0.68	(0.43–1.10)	40	43	1.13	(0.55–2.32)		52	34	0.42	(0.21–0.83)
Every quintile			0.93	(0.83–1.03)			1.05	(0.87–1.24)				0.84	(0.72–0.97)
P for heterogeneity									0.62				
Riboflavin (nmol/L)													
⩽10.2	91	106	1.00		52	58	1.00			39	48	1.00	
10.2–14.0	92	88	0.90	(0.58–1.40)	47	42	0.83	(0.45–1.53)		45	46	0.95	(0.49–1.85)
14.0–18.3	92	77	0.79	(0.49–1.26)	45	35	0.73	(0.36–1.49)		47	42	0.94	(0.48–1.86)
18.3–29.2	92	105	1.04	(0.65–1.65)	40	44	1.11	(0.57–2.17)		52	61	1.01	(0.51–2.00)
>29.2	92	82	0.88	(0.54–1.44)	35	42	1.28	(0.61–2.70)		57	40	0.72	(0.36–1.45)
Every quintile			0.99	(0.89–1.10)			1.08	(0.91–1.27)				0.94	(0.80–1.10)
P for heterogeneity									0.98				
Flavin mononucleotide (nmol/L)													
⩽4.7	88	94	1.00		48	56	1.00			40	38	1.00	
4.7–6.1	89	92	0.90	(0.56–1.43)	36	38	0.80	(0.40–1.59)		53	54	0.98	(0.50–1.95)
6.1–8.1	88	99	1.05	(0.64–1.71)	42	53	1.12	(0.55–2.31)		46	46	1.05	(0.51–2.14)
8.1–11.6	89	81	0.76	(0.45–1.30)	52	35	0.46	(0.21–1.03)		37	46	1.31	(0.60–2.85)
>11.6	88	75	0.73	(0.43–1.25)	36	34	0.73	(0.32–1.64)		52	41	0.82	(0.38–1.76)
Every quintile			0.93	(0.82–1.05)			0.89	(0.74–1.07)				0.98	(0.83–1.17)
P for heterogeneity									0.83				

The OR were estimated from conditional logistic regression based on matching factors and further adjustments for education, smoking status, cotinine concentration in plasma, baseline alcohol drinking, BMI and self-reported diabetes status at baseline.

Table 3 – Odds ratios and 95% confidence intervals for methionine levels (sex-specific quintiles) and risk of pancreatic cancer by years of follow-up before cancer diagnosis.

Methionine (μmol/L)	All				⩽4 years				>4 years			
	Controls	Cases	OR	95% CI	Controls	Cases	OR	95% CI	Controls	Cases	OR	95% CI
q1	92	106	1.00		31	49	1.00		61	57	1.00	
q2	92	75	0.82	(0.52–1.32)	31	36	0.59	(0.25–1.40)	61	39	0.95	(0.52–1.75)
q3	93	84	0.80	(0.50–1.27)	36	20	0.29	(0.11–0.74)	57	64	1.26	(0.70–2.25)
q4	92	93	1.01	(0.62–1.66)	33	29	0.45	(0.17–1.14)	59	64	1.42	(0.77–2.64)
q5	92	103	1.00	(0.64–1.59)	40	39	0.44	(0.19–1.02)	52	64	1.53	(0.85–2.75)
Every quintile			1.02	(0.92–1.14)			0.84	(0.70–1.02)			1.13	(0.99–1.30)
P for heterogeneity								0.02				
Men												
⩽21.0	44	36	1.00		14	18	1.00		30	18	1.00	
21.0–24.4	44	46	2.33	(1.11–4.89)	16	20	1.37	(0.43–4.35)	28	26	4.06	(1.37–12.0)
24.4–27.9	45	44	1.84	(0.89–3.80)	17	12	0.66	(0.18–2.45)	28	32	3.86	(1.37–10.8)
27.9–31.5	44	38	2.15	(0.96–4.83)	18	11	0.62	(0.15–2.53)	26	27	5.09	(1.61–16.1)
>31.5	44	57	2.54	(1.20–5.40)	21	27	1.18	(0.36–3.92)	23	30	4.93	(1.64–14.8)
Every quintile			1.17	(0.99–1.38)			0.99	(0.76–1.29)			1.35	(1.06–1.71)
P for heterogeneity								0.05				
Women												
⩽20.4	48	70	1.00		17	31	1.00		31	39	1.00	
20.4–22.4	48	29	0.32	(0.15–0.66)	15	16	0.20	(0.03–1.19)	33	13	0.32	(0.12–0.78)
22.4–25.2	48	40	0.40	(0.20–0.80)	19	8	0.11	(0.02–0.69)	29	32	0.59	(0.24–1.44)
25.2–28.6	48	55	0.56	(0.27–1.14)	15	18	0.31	(0.06–1.52)	33	37	0.72	(0.30–1.73)
>28.6	48	46	0.49	(0.26–0.96)	19	12	0.08	(0.01–0.51)	29	34	0.80	(0.36–1.78)
Every quintile			0.90	(0.77–1.04)			0.66	(0.46–0.94)			1.01	(0.84–1.21)
P for heterogeneity								0.28				
P for heterogeneity by sex				<0.01			0.11				<0.01	

The OR were estimated from conditional logistic regression based on matching factors and further adjustments for education, smoking status, cotinine concentration in plasma, baseline alcohol drinking, BMI and self-reported diabetes status at baseline.

ference by gender. It is possible that the association could be explained by residual confounding from other risk factors, such as smoking and drinking, because the distribution of risk factors was different in men and women (Table 1). However, no heterogeneity across smoking ($p = 0.62$) or drinking status ($p = 0.20$) was detected. Methionine status could also be a marker of certain dietary patterns, behaviors, or disease status that could explain our association.

We could not exclude the possibility that the observed associations were due to residual confounding from other dietary components or dietary patterns or risk behaviors. Cigarette smoking is by far the most consistent risk factor for pancreatic cancer. Furthermore, alcohol intake is known to interfere with folate metabolism and could be a possible risk factor for pancreatic cancer. Nevertheless, the observed associations did not differ across smoking status, the average lifetime alcohol consumption, BMI and physical activity. Adjusting and not adjusting for diabetes and restricting to non-diabetic participants did not change the results materially, which might imply that diabetes status is not an important confounder or effect modifier of the associations we have observed. The observed sex-dependent methionine-pancreatic cancer associations could be explained by different residual confounding between sexes. Whether there is gender-specific susceptibility needs further investigation.

Our results have to be interpreted with caution due to several limitations. First, we cannot rule out the possibility of chance findings because multiple associations were tested. In addition, the methionine-pancreatic cancer association patterns changed after 4 years of follow-up for both men and women; this could suggest reverse causality, i.e. participants changed their behaviors because of pre-diagnostic cancer-related symptoms. We do not have pancreatitis information in our dataset, and this might be an intermediate between alcohol and pancreatic cancer. Changes in lifestyle and risk factors for pancreatic cancer over time could alter the associations between metabolite concentrations and pancreatic cancer risk. Neither did we have information on one-carbon nutrient metabolites at different points in time during the follow-up period, which limits our ability to address the potential changes in dietary intakes and of levels of metabolites over time within individuals. On the other hand, the larger sample size compared to previous studies, and the diversity of the EPIC population gave us greater power and the opportunity to test for potential heterogeneity across different strata. The prospective nature of dietary assessment and blood collection and the long follow-up of the current study enabled us to test whether the observed lower levels of B-vitamins are a result of cancer development. In addition, folic acid fortification is infrequent in Europe (except in the UK where it is voluntary).

In conclusion, our results suggest a U-shaped association between folate and pancreatic cancer risk in both men and women, and an inverse association between PLP and pancreatic cancer risk in women but not in men. The positive association that we observed between methionine and pancreatic cancer may be sex dependent and may differ by time period. However, these effects warrant further investigation.

Conflict of interest statement

None declared.

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