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Plasma carotenoids, vitamin C, retinol and tocopherols levels and pancreatic cancer risk within the European Prospective Investigation into Cancer and Nutrition: A nested case–control study

Plasma micronutrients and pancreatic cancer risk

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Evidence of a protective effect of several antioxidants and other nutrients on pancreatic cancer risk is inconsistent. The aim of this study was to investigate the association for prediagnostic plasma levels of carotenoids, vitamin C, retinol and tocopherols with risk of pancreatic cancer in a case–control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC). 446 incident exocrine pancreatic cancer cases were matched to 446 controls by age at blood collection, study center, sex, date and time of blood collection, fasting status and hormone use. Plasma carotenoids (α- and β-carotene, lycopene, β-cryptoxanthin, canthaxanthin, zeaxanthin and lutein), α- and γ-tocopherol and retinol were measured by reverse phase high-performance liquid chromatography and plasma vitamin C by a colorimetric assay. Incidence rate ratios (IRRs) with 95% confidence intervals (95%CIs) for pancreatic cancer risk were estimated using a conditional logistic regression analysis, adjusted for smoking status, smoking duration and intensity, waist circumference, cotinine levels and diabetes status. Inverse associations with pancreatic cancer risk were found for plasma β-carotene (IRR highest vs. lowest quartile 0.52, 95%CI 0.31–0.88, p for trend = 0.02), zeaxanthin (IRR highest vs. lowest quartile 0.53, 95%CI 0.30–0.94, p for trend = 0.06) and α-tocopherol (IRR highest vs. lowest quartile 0.62, 95%CI 0.39–0.99, p for trend = 0.08. For α- and β-carotene, lutein, sum of carotenoids and γ-tocopherol, heterogeneity between geographical regions was observed. In conclusion, our results show that higher plasma concentrations of β-carotene, zeaxanthin and α-tocopherol may be inversely associated with risk of pancreatic cancer, but further studies are warranted.

What's new?

Fruits and vegetables may play a role in the prevention of pancreatic cancer, but associations between the antioxidants those foods contain and disease risk remain unclear. In this study, pancreatic cancer risk was inversely associated with increased prediagnostic plasma concentrations of the antioxidants β-carotene, zeaxanthin, and α-tocopherol. Geographic variations were also detected. In Northern European countries, inverse associations with risk were found for blood levels of several carotenoids, whereas the association was strongest for γ-tocopherol in Southern European countries. The role of carotenoids and vitamins should be considered in subsequent investigations of the etiology of pancreatic cancer.

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Pancreatic cancer is the eighth most common cause of cancer death in Europe[1] and the fourth in the United States.[2] Because pancreatic cancer is most often diagnosed at a late stage, prognosis is poor with 1-year survival rates of 20% and 5-year survival rates of only 4–5%.[2,3]

Several risk factors have been consistently associated with the risk of developing pancreatic cancer, including family history of pancreatic cancer,[4] chronic pancreatitis,[5] cigarette smoking,[6] diabetes mellitus[7] and obesity.[8] According to the 2012 WCRF/AIRC expert report, evidence for the relation

between vegetable and fruit consumption and pancreatic cancer risk is limited and inconsistent,[9] with inverse associations found in case–control studies and no associations found in cohort studies. Vegetables and fruits may play a role in the prevention of pancreatic cancer because they contain potentially protective substances, such as carotenoids, vitamin C and tocopherols, folate and other phytochemicals.[10] Potential mechanisms of such bioactive compounds include protection against free radical damage to DNA, enhancing immune function and inhibiting insulin-like growth factor (IGF) by binding to IGF-receptors.[11] In addition, some carotenoids, such as α-carotene, β-carotene and β-cryptoxanthin, are metabolized to retinol, which is involved in cell differentiation. Furthermore, both retinol and tocopherols, especially found in animal products like liver, eggs, cheese and milk, prevent lipid peroxidation and protect against cell damage caused by oxidative stress.[12] Nevertheless, levels of these biomarkers have also been suggested to be correlated to gender, age and smoking status, with lower levels of vitamin C and carotenoids in smokers and higher levels in women, especially younger women.[13–15]

In the studies included in the WCRF report, dietary intake was assessed by dietary questionnaires and concentrations of vitamins in foods were calculated using food composition tables. These methods are, however, prone to measurement error and may not fully take into account the bioavailability of dietary nutrients. Blood levels may be a better way to measure the association with pancreatic cancer risk for micronutrients because they better represent their actual bioavailability. However, studies on prediagnostic blood levels of antioxidants and other nutrients and subsequent development of pancreatic cancer are limited. To date, only two prospective studies[16,17] have investigated the association between antioxidant blood levels and pancreatic cancer risk and showed that higher blood concentrations of α-tocopherol[17] and lycopene[16] were inversely associated with pancreatic cancer risk. However, these studies investigated only a few micronutrients within a limited cohort of male smokers[17] or included a small patient population.[16]

Within the EPIC cohort, the association between blood levels of selected micronutrients and cancer has been investigated for bladder cancer, colorectal cancer and breast cancer. It was found that higher plasma levels of carotenoids and lutein may reduce the risk for bladder cancer.[18] For colorectal cancer inverse associations were found for plasma levels of retinol and dietary intake of b-carotene, vitamin C and vitamin E.[19] Preliminary results for breast cancer showed inverse associations between plasma concentrations of several carotenoids and vitamin C and breast cancer risk.[20]

The aim of this study was to examine the association for prediagnostic plasma levels of carotenoids, vitamin C, retinol and tocopherols with the risk of pancreatic cancer in a case–control study nested within the European into Cancer and Nutrition (EPIC).

Materials and Methods

Study population and data collection

EPIC is an ongoing multicenter cohort study, designed to investigate the relations between diet, lifestyle, metabolic and environmental factors and the incidence of chronic diseases. The total cohort consists of 521,468 participants, with a mean age of 51.2 (±9.9 SD) years at baseline, recruited in 23 centres in 10 European countries. In each of the participating centres, blood was drawn from all participants. The populations and methods have been described in full elsewhere.[21–24] The last follow-up update was performed in June 2010.

The study was approved by the local ethics committee in the participating countries and the internal review board of the International Agency for Research on Cancer (IARC, Lyon).

Nested case–control study design and selection of participants

Cases included first primary pancreatic adenocarcinoma (ICD-Oncology third edition codes C25.0-C25.3 and C25.7-C25.9). The case definition did not include endocrine pancreatic tumors (ICD-O-3 C25.4, histology type and morphology codes 8150, 8151, 8153, 8155, 8240 and 8246), because the etiology of these cancers may be different. We also excluded participants with missing blood samples. All identified cases were matched to one control by center (to account for center-specific differences such as questionnaire design and blood collection procedures), sex, age at blood collection (±3 years), date of blood donation (±3 months), time of blood donation (±2 hr), fasting status (<3, 3–6 and >6 hr after last meal) and among women use of hormones (oral contraceptive pills, hormone replacement therapy or estrogen replacement therapy). Matched control participants were alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the index case (incidence density matching). For ten cases and nine controls not enough plasma was available to perform accurate laboratory measurements (including all four participants from Norway), resulting in a loss of 11 matched case–control sets. In total, 446 case–control risk sets were included in the analyses. Of the 446 pancreatic cancer cases, 333 (75%) were microscopically confirmed, based on histology of the primary tumor ($n = 163$), histology of the metastasis ($n = 38$), cytology ($n = 98$) or autopsy ($n = 34$). Diagnosis for the remaining 113 cases (25%) was self-reported ($n = 1$) or based on clinical symptoms, physical examination and imaging results ($n = 112$).

Laboratory assay

Analysis was performed at the National Institute for Public Health and the Environment (Bilthoven, the Netherlands). High-performance liquid chromatography (HPLC) using an HPLC column (250 × 4.6 mm, ChromSpher 5 Mm C18, Varian Assoc., Middelburg, the Netherlands) was used to

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Table 1. Baseline characteristics by case–control status within the nested case–control study within EPIC[1,2]

	Cases ($N = 446$)	Matched controls ($N = 446$)
Participating countries (*n*)[3]		
France	9	9
Italy	42	42
Spain	39	39
United Kingdom	43	43
The Netherlands	36	36
Greece	23	23
Germany	53	53
Sweden	117	117
Denmark	84	84
Cases only		
Age at diagnosis	63.1 ± 8.0	
Length of follow-up (years)	5.25 ± 2.98	
Characteristics		
Men, *n* (%)	219 (49)	219 (49)
BMI (kg/m^2)	26.8 ± 4.3	26.0 ± 4.1
Waist circumference (cm)[4]	90.4 ± 12.9	88.8 ± 13.1
Physical active (yes, %)[5]	169 (38)	157 (35)
Age at recruitment (y)	57.9 ± 7.8	57.9 ± 7.8
Age at blood collection (y)	58.0 ± 7.8	58.0 ± 7.8
Fasting status at blood collection, *n* (%)		
<3 hr after last meal	173 (48)	177 (49)
3–6 hr after last meal	73 (20)	73 (20)
>6 hr after last meal	114 (32)	111 (31)
Use of hormones[6] at blood collection (yes, %)	30 (15)	33 (16)
Season of blood collection, *n* (%)		
Spring	131 (29)	136 (30)
Summer	88 (20)	88 (20)
Autumn	132 (30)	129 (29)
Winter	95 (21)	93 (21)
Self-reported diabetes (yes), *n* (%)	31 (7)	18 (4)
Smoking status		
Never smokers (%)	158 (35)	194 (44)
Former smokers (%)	141 (32)	146 (33)
Lifetime number of cigarettes (cig/day)	9.5 ± 9.3	8.8 ± 10.0
Smoking duration (y)	22.7 ± 12.2	21.3 ± 12.5
Age at start smoking (y)	19.9 ± 6.5	19.2 ± 6.2
Time since quitting smoking (y)	15.6 ± 11.7	16.7 ± 10.4
Current smokers (%)	142 (32)	100 (22)
Lifetime number of cigarettes (cig/day)	13.4 ± 7.6	12.7 ± 8.8
Smoking duration (y)	35.3 ± 9.3	34.8 ± 11.9
Age at start smoking (y)	19.9 ± 6.8	19.8 ± 8.2
Cotinine blood levels, nmol/L	428.0 ± 664.6	274.5 ± 560.0
Educational level, *n* (%)		
None and primary school	191 (43)	181 (41)

Table 1. Baseline characteristics by case–control status within the nested case–control study within EPIC (Continued)

	Cases ($N = 446$)	Matched controls ($N = 446$)
Technical/professional school	113 (25)	115 (26)
Secondary school	43 (10)	54 (12)
University degree	87 (20)	82 (18)
Not specified	12 (2)	14 (2)
Diet		
Total energy (kcal/d)	2147.5 ± 687.5	2148.4 ± 671.6
Energy from fat (kcal/d)	762.3 ± 296.7	759.0 ± 292.1
Energy from nonfat (kcal/d)	1385.1 ± 451.0	1389.3 ± 436.9
Alcohol consumption (g/day)	14.0 ± 19.0	14.4 ± 22.2
Red and processed meat (g/day)	86.8 ± 55.4	84.1 ± 53.2
Total fruits (g/day)	213.2 ± 163.1	223.5 ± 178.9
Total vegetables (g/day)	181.9 ± 121.9	179.5 ± 129.0
Vitamin supplement use (yes, %)[7]	161 (41)	165 (42)
Plasma antioxidants (median, 5th–95th percentile)		
α-Carotene (nmol/L)	81.7 (32.0–241.9)	95.9 (32.0–287.7)
β-Carotene (nmol/L)[8]	352.8 (135.9–1071.8)	386.6 (128.8–1353.8)
Lycopene (nmol/L)	261.0 (67.2–714.2)	259.0 (71.9–667.6)
β-cryptoxanthin (nmol/L)	257.2 (57.1–979.4)	295.3 (70.0–1022.9)
Zeaxthantin (nmol/L)	63.7 (28.0–170.0)	64.0 (31.9–167.8)
Lutein (nmol/L)	259.0 (74.4–758.7)	267.9 (87.4–684.6)
Sum of carotenoids (nmol/L)[8]	1381.9 (654.1–3238.2)	1496.1 (682.6–3527.8)
Vitamin C (μmol/L)	36.4 (5.2–64.1)	38.2 (7.2–73.5)
Retinol (μmol/L)	2.0 (1.2–3.2)	2.0 (1.3–3.2)
α-Tocopherol (μmol/L)	27.3 (15.6–46.6)	27.9 (17.6–45.1)
γ-Tocopherol (μmol/L	3.0 (1.0–9.2)	3.1 (1.0–8.6)
Serum one-carbon metabolites		
Methionine (μmol/L	25.9 ± 6.9	25.9 ± 6.6
Folate (nmol/L)	16.1 ± 13.0	15.6 ± 10.2
Pyridoxal phosphate (nmol/L)[9]	42.5 ± 38.9	47.4 ± 55.7

[1]Mean ± SD, unless otherwise stated.
[2]Cases and controls are matched for age at blood collection, study center, sex, date of blood collection, time of blood collection, fasting status and hormone use.
[3]Participants of Norway were excluded as not enough plasma was available for analyses.
[4]Data on waist was missing for 35 cases and 36 controls.
[5]Active and moderately active according to the Cambridge Physical Activity Index incorporates occupational and nonoccupational physical activity.
[6]Pill, hormone replacement therapy and estrogen replacement therapy.
[7]Missing data of 60 cases and 61 controls.
[8]Significant difference between cases and controls.
[9]Metabolite for vitamin B6.

determine plasma concentrations of carotenoids (at 450 nm), retinol (at 325 nm) and tocopherols (with fluorescence) as previously described.[25]

Cantaxanthin concentrations were close or below the limit of detection for most samples and were therefore not used in the present study. About 1% of the concentrations of the other carotenoids were found under the limit of detection (20 nmol/L) and all were set at the detection limit. For quality check, one control sample, with concentrations similar to that of the controls, was added to each series of analysis for the assessment of the between batch reproducibility. Coefficients of variation were 14.5% for α-carotene, 8.9% for β-carotene, 6.3% for β-cryptoxanthin, 11.8% for lycopene, 8.9% for lutein, 8.4% for zeaxanthin. Plasma vitamin C concentration was determined on an LX20-Pro autoanalyzer (Beckman-Coulter, Woerden, the Netherlands) using a

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Table 2. Plasma levels of carotenoids and the risk of pancreatic cancer in the nested case–control study within EPIC[1]

	Ca/co[2]	Unadjusted IRR	Adjusted IRR[3]
α-Carotene (nmol/L)			
<55.67	115/112	1.00	1.00
56.19–95.34	152/109	1.32 (0.91–1.91)	1.61 (1.06–2.44)
95.86–144.55	80/108	0.68 (0.45–1.04)	0.76 (0.47–1.24)
>147.16	93/111	0.79 (0.53–1.19)	1.14 (0.71–1.85)
p for trend		0.03	0.64
Log_2 transformed		0.84 (0.72–0.98)	0.93 (0.78–1.12)
β-Carotene (nmol/L)			
<252.35	140/113	1.00	1.00
252.56–386.62	113/111	0.76 (0.52–1.12)	0.83 (0.55–1.27)
386.64–672.50	131/111	0.86 (0.59–1.24)	1.05 (0.69–1.61)
>678.86	62/111	0.39 (0.25–0.60)	0.52 (0.31–0.88)
p for trend		<0.001	0.02
Log_2 transformed		0.76 (0.65–0.89)	0.85 (0.71–1.02)
Lycopene (nmol/L)			
<163.14	115/112	1.00	1.00
163.51–258.59	108/111	0.94 (0.65–1.37)	0.96 (0.62–1.47)
258.99–394.82	116/110	1.02 (0.70–1.48)	1.08 (0.71–1.66)
>396.50	105/111	0.90 (0.59–1.37)	1.00 (0.62–1.62)
p for trend		0.71	0.84
Log_2 transformed		1.00 (0.86–1.17)	1.04 (0.87–1.23)
B-cryptoxanthin (nmol/L)			
<150.17	122/112	1.00	1.00
150.81–295.16	131/110	1.06 (0.72–1.55)	1.09 (0.70–1.70)
295.28–492.27	102/111	0.78 (0.52–1.18)	0.84 (0.52–1.37)
>495.52	89/111	0.63 (0.40–0.99)	0.66 (0.39–1.13)
p for trend		0.01	0.06
Log_2 transformed		0.87 (0.76–0.99)	0.89 (0.76–1.05)
Zeaxthantin (nmol/L)			
<46.24	134/113	1.00	1.00
46.52–63.64	88/110	0.65 (0.43–0.97)	0.62 (0.39–0.98)
63.99–96.91	133/109	0.94 (0.64–1.39)	0.83 (0.53–1.28)
>97.50	87/110	0.54 (0.33–0.88)	0.53 (0.30–0.94)
p for trend		0.04	0.06
Log_2 transformed		0.80 (0.63–1.00)	0.81 (0.61–1.06)
Lutein (nmol/L)			
<183.06	135/111	1.00	1.00
184.40–266.23	100/111	0.74 (0.52–1.08)	0.96 (0.64–1.45)
267.94–388.88	109/108	0.81 (0.55–1.18)	1.01 (0.65–1.58)
>390.84	96/110	0.66 (0.43–1.02)	0.90 (0.54–1.50)
p for trend		0.07	0.70
Log_2 transformed		0.87 (0.74–1.03)	0.97 (0.81–1.17)
Sum of carotenoids (nmol/L)			
<1089.69	135/112	1.00	1.00

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Table 2. Plasma levels of carotenoids and the risk of pancreatic cancer in the nested case–control study within EPIC (Continued)

	Ca/co[2]	Unadjusted IRR	Adjusted IRR[3]
1092.62–1495.26	115/110	0.82 (0.56–1.21)	0.76 (0.49–1.18)
1496.09–2168.68	110/109	0.77 (0.53–1.12)	0.78 (0.50–1.20)
>2174.47	81/110	0.51 (0.33–0.80)	0.61 (0.36–1.04)
p for trend		<0.01	0.08
Log_2 transformed		0.69 (0.55–0.87)	0.78 (0.59–1.03)

[1]Analyses are conditioned for the matching factors age at blood collection, study center, sex, date of blood collection, time of blood collection, fasting status and hormone use.
[2]Numbers of cases and controls correspond to the adjusted IRRs.
[3]The analyses were further adjusted for smoking status, duration and intensity of smoking, cotinine levels, waist circumference and diabetes status.

colorimetric assay.[26] The limit of sensitivity of the method is 2.5 μmol/L and about 2.5% of the vitamin C concentrations were under the limit of detection and were all set at the detection limit. The coefficient of variation (between-batch) for vitamin C was 4.1% at a vitamin C concentration of 45.8 μmol/L. Laboratory technicians were blinded to the case–control status of the samples.

Statistical methods

Results were expressed as mean ± SD and median with range (5–95%), as appropriate. Odds ratios (ORs) and 95% confidence intervals (95%CI) for pancreatic cancer risk in relation to plasma concentrations and dietary intake were calculated by conditional logistic regression, stratified by the case–control set. With an incidence density sampling design, the OR is an unbiased approximation of the incidence rate ratio (IRR).[27] IRRs were calculated for quartile categories with cut-off points based on the distribution of each specific antioxidant in the controls. For all models, linear trend tests were determined using a variable with values equal to the median of the quartile grouping. Data were also analyzed continuously by a *log*-transformation (log_2) to normalize skewed data. The IRR for a log_2-transformed IRR variable corresponds to the change in pancreatic cancer risk by doubling the plasma concentration. The sum of carotenoids was created as the sum of plasma α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin.

The effect of potential confounders, other than matching criteria, which are controlled for by design, were examined by inclusion into the logistic regression models. Although cotinine did not significantly change the results, we included cotinine as confounder as it may present smoking status and to prevent residual confounding by passive smoking as good as possible.[6,28] Based on previous studies, we selected waist circumference as confounding factor instead of BMI for anthropometry.[8,29] Other potential confounders [waist hip ratio, physical activity, energy intake (total, fat and nonfat), red and processed meat intake, alcohol intake, educational level, vitamin supplement use[30] and 1-carbon metabolites[31]] each on its own did not substantially alter the risk estimates (more than 10%) and these variables were therefore not included in the final models. Correlations between micronutrients and risk factors (age, smoking status, gender and region) and between micronutrients were analyzed using (partial) Pearson correlation en Spearman correlation tests, respectively.

Sensitivity analyses were performed for microscopically confirmed cases and their matched controls. In addition, all analyses were performed excluding the first 2 years of follow-up, to diminish the influence of possible changes in food patterns or metabolic changes influencing the level of antioxidants due to preclinical disease.

To account for regional differences in food patterns, analyses were stratified by European region. Possible heterogeneity of effects between regions was tested using the heterogeneity statistics derived from the inverse variance method.

Interactions between the investigated nutrients and waist circumference (<83, 83–94 and >94 cm), smoking status (current, former and never) and cotinine levels (<55, 55–1,004 and >1,004 nmol/L) were examined in a joint effects model. Statistical interaction on a multiplicative scale was tested by introducing a product term between plasma nutrient levels (continuous) and smoking status, waist circumference or cotinine levels, respectively.

Finally, the association for dietary intake of β-carotene, retinol, vitamin C and vitamin E with pancreatic cancer risk was analyzed using conditional logistic regression, stratified by the case–control risk set.

All statistical analyses were conducted using SAS version 9.2 (SAS Institute, Cary, North Carolina) and two-sided *p* values <0.05 were considered statistically significant.

Results

The mean age at recruitment of cases and controls was 57.9 years and 49% were male. Compared to controls, cases were more often current smokers, had higher cotinine levels, reported more often being diabetic and had lower average blood concentrations of β-carotene and the sum of carotenoids (Table 1). Correlation analyses showed that most micronutrients were inversely correlated with smoking status (correlation coefficients varying between −0.11 and −0.16) with higher levels in never smokers compared to former and current smokers. Zeaxanthin, lutein, α-tocopherol and γ-tocopherol were positively correlated with age (correlation coefficients between 0.07 and 0.10). Gender was positively

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Table 3. Plasma levels of vitamin C, retinol and tocopherols and the risk of pancreatic cancer in the nested case–control study within EPIC[1]

	Ca/co[2]	Unadjusted IRR	Adjusted IRR[3]
Vitamin C (μmol/L)			
<22.55	107/113	1.00	1.00
23.21–38.06	126/107	1.22 (0.84–1.78)	1.22 (0.79–1.89)
38.17–50.49	123/111	1.16 (0.77–1.74)	1.35 (0.83–2.19)
>50.71	86/111	0.78 (0.50–1.21)	0.91 (0.55–1.51)
p for trend		0.29	0.77
Log_2 transformed		0.92 (0.79–1.07)	0.96 (0.80–1.16)
Retinol (μmol/L)			
<1.64	109/113	1.00	1.00
1.64–2.03	120/111	1.12 (0.76–1.65)	1.04 (0.68–1.59)
2.03–2.49	108/110	1.01 (0.67–1.53)	0.91 (0.58–1.44)
>2.49	107/110	1.00 (0.63–1.58)	0.84 (0.50–1.40)
p for trend		0.87	0.43
Log_2 transformed		0.90 (0.62–1.32)	0.71 (0.46–1.11)
α-tocopherol (μmol/L)			
<23.32	144/112	1.00	1.00
23.34–27.84	89/111	0.59 (0.40–0.88)	0.59 (0.38–0.94)
27.89–32.83	91/111	0.60 (0.40–0.90)	0.57 (0.36–0.90)
>32.83	121/111	0.79 (0.53–1.20)	0.62 (0.39–0.99)
p for trend		0.47	0.08
Log_2 transformed		0.72 (0.51–1.01)	0.60 (0.40–0.88)
γ-Tocopherol (μmol/L)			
<1.86	112/113	1.00	1.00
1.86–3.07	122/110	1.12 (0.77–1.64)	1.07 (0.69–1.65)
3.07–4.62	81/111	0.74 (0.48–1.13)	0.53 (0.32–0.87)
>4.63	130/111	1.22 (0.78–1.91)	0.92 (0.55–1.54)
p for trend		0.36	0.76
Log_2 transformed		1.04 (0.88–1.24)	0.88 (0.72–1.08)

[1]Analyses are conditioned for the matching factors age at blood collection, study center, sex, date of blood collection, time of blood collection, fasting status and hormone use.
[2]Numbers of cases and controls correspond to the adjusted IRRs.
[3]The analyses were further adjusted for smoking status, duration and intensity of smoking, cotinine levels, waist circumference and diabetes status.

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correlated with α-carotene, β-carotene, cryptoxanthin, α-tocopherol and vitamin C (correlation coefficient between 0.11 and 0.19) with higher levels in women and inversely correlated with retinol (correlation coefficient −0.14) with lower levels in men. Strong correlations were found between micro-nutrients and region (correlation coefficients varying between 0.29 and −0.47) with higher levels of lutein, cryptoxanthin and a-tocopherol in southern European countries and higher levels of retinol and y-tocopherol in Northern European countries. Correlations between micronutrients were found between almost all micronutrients (see Supporting Information 3).

A significant inverse association with pancreatic cancer risk was found for plasma β-carotene (adjusted IRR highest *vs.* lowest quartile 0.52, 95%CI 0.31–0.88, *p* for trend=0.02) and zeaxanthin (adjusted IRR highest *vs.* lowest quartile 0.53; 95%CI 0.30–0.94, *p* for trend = 0.06; Table 2). Log_2-transformation showed a statistically borderline significant reduction in risk for each doubling of β-carotene (adjusted IRR 0.85, 95%CI 0.71–1.02), of the sum of carotenoid levels (adjusted IRR 0.78, 95%CI 0.59–1.03) and of zeaxanthin (adjusted IRR 0.81, 95%CI 0.61–1.06). Blood levels of α-carotene, β-cryptoxanthin, lycopene and lutein were not associated with pancreatic cancer risk. Elimination of the first 2 years of follow-up did not change the results for overall and individual carotenoids.

We observed an inverse association for plasma α-tocopherol (adjusted IRR highest *vs.* lowest quartile 0.62, 95%CI 0.39–0.99, *p* for trend = 0.08; Table 3). Log_2-transformation showed a 40% reduction in risk with each

Table 4. Adjusted incidence rate ratios (and 95%CIs) for pancreatic cancer for plasma carotenoids, vitamin C, retinol and tocopherols (log2 transformed), stratified by European region within EPIC

	European region			
	North[1] (201/201)	Middle[2] (141/141)	South[3] (104/104)	p for heterogeneity
α-Carotene				
Median (5th–95th percentile; nmol/L)	112.69 (32.0–337.0)	100.84 (32.7–264.3)	70.72 (29.8–164.9)	
Adjusted IRR[4]	0.72 (0.54–0.96)	1.08 (0.77–1.52)	1.54 (0.98–2.42)	0.01
β-Carotene				
Median (5th–95th percentile; nmol/L)	457.1 (136.5–1353.8)	390.34 (144.8–1254.4)	316.00 (115.3–1429.6)	
Adjusted IRR[4]	0.70 (0.52–0.94)	0.78 (0.55–1.12)	1.37 (0.90–2.07)	0.03
Lycopene				
Median (5th–95th percentile; nmol/L)	283.76 (105.0–615.5)	198.84 (55.5–657.2)	266.16 (76.8–710.9)	
Adjusted IRR[4]	0.86 (0.63–1.17)	1.19 (0.88–1.60)	1.21 (0.86–1.71)	0.24
β-Cryptoxanthin				
Median ($5^{th}-95^{th}$ percentile), nmol/L	256.30 (54.0–827.1)	282.19 (87.2–920.2)	421.21 (76.4–1629.4)	
Adjusted IRR[4]	0.70 (0.53–0.94)	1.06 (0.798–1.43)	1.04 (0.78–1.37)	0.09
Zeaxanthin				
Median (5th–95th percentile; nmol/L)	63.63 (35.3–128.9)	63.00 (29.6–153.6)	66.82 (31.4–196.5)	
Adjusted IRR[4]	0.32 (0.17–0.59)	1.15 (0.71–1.86)	1.39 (0.81–2.40)	<0.01
Lutein				
Median (5th–95th percentile; nmol/L)	268.86 (130.3–561.3)	241.44 (45.1–636.2)	292.45 (75.5–1320.4)	
Adjusted IRR[4]	0.64 (0.40–1.01)	0.86 (0.64–1.17)	1.51 (1.05–2.17)	0.01
Sum of carotenoids				
Median (5th–95th percentile; nmol/L)	1584.43 (794.2–3325.4)	1364.34 (630.6–3432.8)	1508.93 (674.2–4699.5)	
Adjusted IRR[4]	0.43 (0.26–0.73)	0.88 (0.52–1.48)	1.53 (0.88–2.68)	<0.01
Vitamin C				
Median (5th–95th percentile; μmol/L)	36.85 (6.4–73.7)	39.66 (11.9–78.2)	34.87 (3.5–54.3)	
Adjusted IRR[4]	0.93 (0.71–1.24)	0.82 (0.57–1.18)	1.31 (0.84–2.04)	0.27
Retinol				
Median (5th–95th percentile; μmol/L)	2.40 (1.5–3.5)	1.77 (1.3–2.7)	1.70 (1.1–2.5)	
Adjusted IRR[4]	0.66 (0.32–1.36)	0.89 (0.42–1.93)	0.59 (0.24–1.46)	0.76
α-Tocopherol				
Median (5th–95th percentile; μmol/L)	25.78 (17.6–40.9)	29.27 (16.8–45.7)	32.11 (21.0–47.5)	
Adjusted IRR[4]	0.61 (0.29–1.28)	0.64 (0.35–1.18)	0.44 (0.19–1.07)	0.78
γ-Tocopherol				
Median (5th–95th percentile; μmol/L)	4.02 (1.3–9.1)	2.91 (0.8–8.5)	1.87 (1.0–5.8)	
Adjusted IRR[4]	1.23 (0.84–1.81)	0.96 (0.69–1.33)	0.45 (0.28–0.75)	0.01

[1]The category "North" includes Norway, Sweden and Denmark.
[2]The category "Middle" includes United Kingdom, The Netherlands, Germany and France.
[3]The category "South" includes Italy, Spain and Greece.
[4]Analyses are conditioned for the matching factors age at blood collection, study center, sex, date of blood collection, time of blood collection, fasting status and hormone use and were further adjusted for smoking status, duration and intensity of smoking, cotinine levels, waist circumference and diabetes status.

doubling of α-tocopherol levels (IRR 0.60, 95%CI 0.40–0.88). Blood concentrations of γ-tocopherol, vitamin C and retinol were not associated with pancreatic cancer risk. Exclusion of the first 2 years of follow-up did not change the results.

The magnitude of the associations for carotenoids, vitamin C, retinol and tocopherols with pancreatic cancer did not change when analyses were performed in microscopically confirmed cases only, however, they were no longer significant. No heterogeneity by gender was found. In addition,

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when models for β-carotene, zeaxanthin and α-tocopherol were mutual adjusted for β-carotene, zeaxanthin and α-tocopherol, it did not change our results (data not shown).

The test for interaction with region was statistically significant for several nutrients of interest, *i.e.*, for α-carotene (p for interaction 0.01), β-carotene (p 0.03), zeaxanthin ($p < 0.01$), lutein (p 0.01), sum of carotenoids ($p < 0.01$) and γ-tocopherol (p 0.01; Table 4). For plasma carotenoids a gradient from North to South Europe was observed with strongest, often statistically significant inverse associations in the North, some moderately reduced risks—albeit statistically nonsignificant—in the Central European region, and essentially no association at all in the South. In addition, plasma lutein showed a significant positive association with pancreatic cancer risk in Southern European countries and a nonsignificant inverse association in the North. Blood concentrations of γ-tocopherol, however, were statistically significantly inversely associated with risk in Southern European countries only.

For all tested nutrients no clear heterogeneity by waist circumference, smoking status or cotinine levels was found and none of the tests for multiplicative interaction was statistically significant (p for interaction >0.05; Supporting Information 1).

Analyses for dietary intake of antioxidants showed a borderline significant inverse association between of β-carotene and pancreatic cancer risk ($\log_2$ transformation adjusted IRR 0.89 95%CI 0.75–1.05). No associations with risk of pancreatic cancer were seen for dietary intake of vitamin C, retinol and vitamin E.

Discussion

This nested case–control study showed inverse associations for prediagnostic plasma levels of β-carotene, zeaxanthin and α-tocopherol with risk of developing pancreatic cancer. Blood levels of most carotenoids were inversely associated with risk of pancreatic cancer in Northern European countries, whereas an inverse association for γ-tocopherol was seen in Southern European countries.

A case–control study reported an inverse association for dietary β-carotene with risk in nonsmokers.[32] In our study, dietary β-carotene intake was borderline significantly inversely associated with pancreatic cancer risk. However, the correlation coefficient between plasma levels and dietary intake was only 0.13 for β-carotene. This weak correlation may be explained by errors in the food composition table, biases in the assessment of dietary intake, the metabolism of carotenoids that can affect their blood concentrations and/or the fact that only a single plasma measurement was available. Yet, the pancreatic cancer risk estimates for quartiles of plasma levels and dietary intake of β-carotene are quite similar despite low correlations.

Part of the β-carotene effect may be ascribed to its provitamin A activity, as vitamin A has been shown to modify metabolism of carcinogens, and stimulate cell differentiation.[33] However, we did not find an inverse relationship for plasma retinol with pancreatic cancer risk. Moreover, adjustment for retinol in the β-carotene model did not change our results. It is therefore unlikely that the inverse association for β-carotene is explained by its provitamin A activity.

Zeaxanthin, which does not possess provitamin A activity, may act as an antioxidant and is also related to many eye diseases. Although no previous studies have been performed investigating the association between pancreatic cancer and plasma levels of zeaxanthin, other studies showed that zeaxanthin may be inversely related to risk of breast cancer.[34]

An inverse association for plasma α-tocopherol levels with pancreatic cancer risk was observed. This association was also found in a recently published cohort study investigating serum α-tocopherol levels in male smokers and pancreatic cancer risk.[17] That study included participants from Finland. Therefore, the inverse association between pancreatic cancer and α-tocopherol observed in our study may possibly be restricted to northern European countries. Nevertheless, analyses by European region did not confirm this. It is suggested that α-tocopherol inhibits pancreatic carcinogenesis by preventing cellular damage of polyunsaturated fatty acids caused by free radicals.[35] Although the Finnish study also found a stronger inverse association in participants with high dietary intake of polyunsaturated fat, our results did not change with additional adjustment for dietary PUFA's (data not shown).

The analyses by European region suggested stronger inverse associations for α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin and the sum of carotenoids in Northern European countries and conversely a stronger inverse association for γ-tocopherol in Southern countries. In addition, our analyses showed a positive association between lutein and pancreatic cancer in southern European countries. A possible explanation may be the difference in food patterns between Northern and Southern countries. Root vegetables (*e.g.*, carrots) and cabbages (*e.g.*, kale, broccoli and Brussels sprout), are major sources of carotenoids, whereas γ-tocopherols are more common in vegetable oils, tomato sauce and nuts and seeds. Within EPIC the intake of root vegetables and cabbages was higher in Northern countries,[36] while the consumption of vegetable oils, nuts and seeds and tomato sauce was higher in southern countries.[37] This was confirmed in our nested case–control population (Supporting Information 2). This may lead to more variation in exposure and therefore a higher chance of finding a significant difference. The difference may also be related to greater use of vitamin supplement use in Northern countries.[30] Additional adjustment for vitamin supplement use did however not noticeably change our results. In addition, separate analyses of non-supplement users did not change the magnitude of the inverse associations. However, it was unknown what type and dose of supplement was used by the participants. Lastly, the difference may have been caused by a difference in power between Northern and Southern Europe.

According to clinical expertise sensitivity and specificity of case diagnosis is generally considered as moderate. Although the magnitude of the inverse associations for β-carotene, zeaxanthin and α-tocopherol did not change when only microscopically confirmed pancreatic cancer cases and their matched controls were included, they were no longer significant. This may be due to the loss of power, as 25% of the cases were based on clinical or physical examination or imaging only. Therefore, we believe that the results for our total study population are accurate.

The key advantage of this study, aside from detailed lifestyle and dietary questionnaire data, and large sample size, is that nutrients were measured in blood prior to diagnosis of disease and analyses were performed at the same laboratory. This study also has a number of limitations. First, it is possible that potential risk factors may have changed during follow-up, which could lead to residual confounding. We could, however, not take into account changes in dietary and lifestyle factors as replicate measurements of these factors are not available in our study. Although analyses were corrected for several lifestyle factors, residual confounding cannot be excluded. In addition, the inverse associations we found between plasma levels and pancreatic cancer risk may be the result of multiple testing.

Second, plasma levels of antioxidants are sensitive to degradation over time. It has previously been demonstrated, however, that long-term stability of vitamin C even without a stabilizing agent like meta-phosphoric acid, remains reasonably reliable after 7–11 years of storage at −196°C. In addition, short term losses of blood antioxidant levels during initial collection and handling of blood samples, have been shown to be minimal.[38] Excluding participants from Oxford (UK), because blood samples were transported by post without being cooled, did however not change our results.

Third, analyses were based on single measurements of plasma nutrient levels and may not sufficiently reflect the usual blood concentrations during the long-term preclinical stage. An examination of the repeatability of serum carotenoids, retinol and α-tocopherol over 15 years showed that repeatability was similar, with Spearman's correlation coefficients of 0.3–0.5, which suggested that these measures should be reliable enough to detect moderate to strong associations, should they exist.[39] A recent study in 30 participants of the Dutch subcohort of EPIC reported intraclass correlation coefficients for carotenoids, vitamin C, retinol and tocopherols over a 2- to 5-year period that ranged from 0.37 to 0.55 (data not shown) which suggests moderate repeatability of measurements of plasma levels.[40] We examined the extent to which our risk estimates might be attenuated by using the intraclass correlation coefficients of above study as attenuation factors. This resulted in a strengthening of the original risk estimates, except for α-carotene (*e.g.*, β-carotene IRR highest quintile *vs*. the lowest 0.19; results not shown). These findings indicate that the true inverse association between all antioxidants and pancreatic cancer risk may be stronger. It is however important to recognize that the reliability of repeated measures was only examined in Dutch EPIC participants and that the attenuation factor may be different in the different EPIC study populations.

Finally, carotenoids and tocopherols are transported in blood by lipoproteins. Therefore, plasma levels of antioxidants may depend on the type and amount of fat consumed.[41] Although data on blood lipids were not available in this study, further adjustment for dietary fat intake did not change our results.

In conclusion, our results show that higher plasma concentrations of β-carotene, zeaxanthin and α-tocopherol may be inversely associated with pancreatic cancer. Further research using larger sample sizes is warranted.

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