Circulating Concentrations of Folate and Vitamin B_{12} in Relation to Prostate Cancer Risk: Results from the European Prospective Investigation into Cancer and Nutrition Study

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Abstract

Background: **Determinants of one-carbon metabolism, such as folate and vitamin B_{12}, have been implicated in cancer development. Previous studies have not provided conclusive evidence for the importance of circulating concentrations of folate and vitamin B_{12} in prostate cancer etiology. The aim of the present study was to investigate the relationship between prostate cancer risk and circulating concentrations of folate and vitamin B_{12} in a large prospective cohort.**

Methods: **We analyzed circulating concentrations of folate and vitamin B_{12} in 869 cases and 1,174 controls, individually matched on center, age, and date of recruitment, nested within the European Prospective Investigation into Cancer and Nutrition cohort. Relative risks (RR) for prostate cancer were estimated using conditional logistic regression models.**

Results: **Overall, no significant associations were observed for circulating concentrations of folate (P_{trend} = 0.62) or vitamin B_{12} (P_{trend} = 0.21) with prostate cancer risk. RRs for a doubling in folate and vitamin B_{12} concentrations were 1.03 [95% confidence interval (95% CI), 0.92-1.16] and 1.12 (95% CI, 0.94-1.35), respectively. In the subgroup of cases diagnosed with advanced stage prostate cancer, elevated concentrations of vitamin B_{12} were associated with increased risk (RR for a doubling in concentration, 1.69; 95% CI, 1.05-2.72, P_{trend} = 0.03). No other subgroup analyses resulted in a statistically significant association.**

Conclusion: **This study does not provide strong support for an association between prostate cancer risk and circulating concentrations of folate or vitamin B_{12}. Elevated concentrations of vitamin B_{12} may be associated with an increased risk for advanced stage prostate cancer, but this association requires examination in other large prospective studies.** (Cancer Epidemiol Biomarkers Prev 2008;17(2):279–85)

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Introduction

Folate, primarily supplied by cereals and vegetables, has recently attracted increasing attention as a potential cancer-protective agent (1). Folate is an essential factor in the one-carbon metabolism pathway which is involved in DNA methylation, synthesis, and repair (2).

In colorectal cancer—the most frequently studied cancer site in relation to one-carbon metabolism—a relatively high folate status has generally been associated with a modestly reduced risk for cancer development in prospective studies (3). However, the first randomized controlled trial investigating the potential antineoplastic effect of supplementary folic acid in subjects with colorectal adenomas, reported—at the second follow-up—a 67% increased risk of advanced lesions in the supplementation arm compared with the placebo arm (4). This unexpected result requires confirmation but could support the hypothesis that folic acid possesses a dual modulatory effect on neoplasia depending on the timing of exposure (5).

In the one-carbon metabolism pathway, methyl groups are donated by 5-methylenetetrahydrofolate, the predominant form of folate in the circulation, when homocysteine is converted to methionine. This reaction is catalyzed by vitamin B_{12}. The methionine derivative S-adenosylmethionine is the universal methyl donor for DNA methylation reactions. Patterns of hypomethylation and gene-specific hypermethylation are often observed in tumor tissue (6). In prostate cancer, hypermethylation of the CpG island sequences of the glutathione S-transferase π gene (*GSTP1*) have been observed in >90% of tumor tissues. This is the most frequently reported epigenetic change in prostate cancer, suggesting that hypermethylation may be particularly important in prostate cancer development (7). High concentrations of folate or vitamin B_{12} may therefore increase prostate cancer risk by inducing hypermethylation, even though evidence for this is sparse to date (1).

In addition to its central role in methylation, folate in the form of 5,10-methyltetrahydrofolate may have a protective role in cancer development by promoting the synthesis of thymidylate from uracil, minimizing the misincorporation of uracil into DNA. Excessive uracil could lead to double-strand breaks and possibly to cancer development (8).

In studies of prostate cancer, a previous prospective study reported an increased risk associated with elevated circulating concentrations of folate and vitamin B_{12} (9). This association was particularly strong for vitamin B_{12} with subjects in the highest quartile showing an almost 3-fold increase in risk compared with the bottom quartile. The study also reported a risk increase for elevated concentrations of folate, but this association disappeared after adjusting for vitamin B_{12} concentrations. In addition, a statistically significant increase in prostate cancer risk was observed in the folate supplementation arm of the randomized controlled folate trial, although the numbers were small (4). Only one additional prospective study has been conducted on prostate cancer in relation to plasma folate and vitamin B_{12}, and this study reported null results for both circulating folate and vitamin B_{12} (10). Interestingly, an additional analysis of dietary intake of folate and vitamin B_{12} from the same Finnish cohort reported an increased prostate cancer risk for dietary intake of vitamin B_{12} (11).

The aim of the present study was to investigate variations in circulating concentrations of folate and vitamin B_{12} in relation to prostate cancer risk. The study was conducted within the European Prospective Investigation into Cancer and Nutrition study, using a nested case-control design including 869 prostate cancer cases and 1,174 individually matched controls.

Materials and Methods

Study Cohort. European Prospective Investigation into Cancer and Nutrition study recruitment procedures and collection of questionnaire data, anthropometric measurements, and blood samples have been described in detail elsewhere (12). In brief, standardized questionnaire data on dietary and nondietary variables were collected between 1992 and 2006 from 519,978 individuals across Europe, including 153,457 men of whom 137,001 provided a blood sample. The present study included prostate cancer cases diagnosed after blood collection and matched control subjects from 7 of the 10 participating countries: Germany, Greece, Italy, the Netherlands, Spain, Sweden, and the United Kingdom. France and Norway were not included in the present study because these cohorts only included women, and Denmark was not included because serum was not available in the Danish cohorts.

Blood samples were collected according to a standardized protocol. Filled syringes were kept at 5°C to 10°C, protected from light, and transferred to a local laboratory for further processing and aliquoting, with the exception of subjects recruited through the Oxford center. Here, blood samples were collected throughout the United Kingdom and transported to a laboratory in Norfolk by mail at ambient temperature. Blood fractions (serum, plasma, red cells, and buffy coat) were aliquoted into 0.5 mL straws, which were then heat-sealed and stored in liquid nitrogen tanks at −196°C, except in Umeå Sweden, where samples were stored in 1.8 mL plastic tubes in −80°C freezers.

Follow-up for Cancer Incidence and Vital Status. In Italy, the Netherlands, Spain, Sweden, and the United Kingdom, incident cancer cases were identified through record linkage with regional or national cancer registries. In Germany and Greece, follow-up was based on a combination of methods, including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin. Data on vital status in most European Prospective Investigation into Cancer and Nutrition study centers were collected from mortality registries at the regional or national level, in combination with data collected by active follow-up (Greece). For each European Prospective Investigation into Cancer and Nutrition study center, closure dates of the study period were defined as the latest dates of complete follow-up for both cancer incidence and vital status (dates varied between centers, from June 1999 to January 2006).

Selection of Case and Control Subjects. In total, the seven subcohorts contributing to the present study

Table 1. Baseline characteristics by case-control status

Characteristic	Cases (n = 869)	Controls (n = 1,174)
Anthropometric measurements, mean (SD)		
Age at blood collection (y)	59.1 (6.7)	58.3 (6.8)
Weight (kg)	79.5 (11.3)	80.4 (11.6)
Height (cm)	173.3 (6.7)	174.2 (7.0)
Body mass index (kg/m^2)	26.5 (3.4)	26.5 (3.5)
Smoking, n (%)		
Never	311 (36.7%)	470 (41.3%)
Former	334 (39.4%)	389 (34.2%)
Current	202 (23.8%)	279 (24.5%)
Alcohol consumption, n (%)		
<8 (g/d)	256 (41.4%)	315 (45.2%)
8-15 (g/d)	119 (19.3%)	150 (21.5%)
16-39 (g/d)	148 (23.9%)	140 (20.1%)
≥40 (g/d)	95 (15.4%)	92 (13.2%)
Physical activity, n (%)		
Inactive	100 (18.6%)	78 (14.4%)
Moderately inactive	188 (34.9%)	176 (32.5%)
Active	251 (46.6%)	287 (53%)
Marital status, n (%)		
Married or cohabiting	669 (88.1%)	924 (87.6%)
Not married or cohabiting	90 (11.9%)	131 (12.4%)
Educational attainment, n (%)		
Primary or equivalent	432 (52.0%)	655 (58.4%)
Secondary	227 (27.3%)	272 (24.2%)
Degree	172 (20.7%)	195 (17.4%)
Cases only		
Years from blood collection to diagnosis, median (range)	5 (0-18)	
Stage of disease, n (%)		
Localized	365 (42.0%)	
Advanced	161 (18.5%)	
Unknown	343 (39.5%)	
Grade of disease, n (%)		
Gleason sum <7 or well or moderately differentiated	476 (54.8%)	
Gleason sum ≥7 or poorly differentiated or undifferentiated	250 (28.8%)	
Unknown	143 (16.5%)	

included 1,289 men diagnosed with incident prostate cancer by the end of each center's follow-up period. Cases with no available blood sample and those who had missing information on the date of blood donation or who had a history of another cancer (except non–melanoma skin cancer) at the time of blood donation were excluded. After these exclusions, laboratory measurements for the current analysis were available for 1,107 cases: 61 cases in Italy, 182 in Germany, 9 in Greece, 24 in the Netherlands, 94 in Spain, 560 in Sweden, and 177 in the United Kingdom. For each case, one male control (two in Umeå) was chosen at random from appropriate risk sets consisting of all cohort members alive and free of cancer (except non–melanoma skin cancer) at the time of diagnosis of the index case. Matching criteria were study center, age at enrollment (±6 months), time of day of blood collection (±1 h), and time between blood draw and last consumption of food or drink (<3, 3-6, >6 h). Two hundred and thirty-eight cases from the Swedish cohort had been used in a previous study on the relation between prostate cancer and circulating concentrations of folate and vitamin B_{12} (9).

These subjects and their matched controls were therefore excluded from the main data analysis of the present study. In total, 869 cases and 1,174 controls were included in the present study. All participants gave written informed consent to participate in the study and the research was approved by the local ethics committees in the participating countries and the Internal Review Board of the IARC.

Data on stage and grade of disease were collected from each center where possible. Tumor stage was categorized as localized (tumor-node-metastasis categories T_0 or T_1 or T_2, and N_0 or N_X, and M_0, or stage coded in the recruitment center as localized), advanced (T_3 or T_4 and/or N_{1+} and/or M_1, or stage coded in the recruitment center as metastatic), or unknown. Histologic grade was categorized as Gleason sum <7 or equivalent (tumors coded as well-differentiated or moderately differentiated), Gleason sum ≥7 or equivalent (tumors coded as poorly differentiated or undifferentiated), or unknown.

Biochemical Analyses. Cases were analyzed in the same batch as their matched controls. All assays were done by laboratory personnel who were blinded as to the case-control status of the blood samples. Folate concentrations were determined by a *Lactobacillus casei* microbiological assay (13) and vitamin B_{12} concentrations were determined by a *Lactobacillus leichmannii* microbiological assay (14, 15). Both folate and vitamin B_{12} assays were adapted to a microtiter plate format and carried out by a robotic workstation (Microlab AT plus 2; Hamilton Bonaduz). The within- and between-run variations were 6.0% and 6.3%, respectively, for folate, and 5.4% and 6.7%, respectively, for vitamin B_{12}.

Statistical Analyses. Circulating concentrations of folate and vitamin B_{12} were categorized into quintiles

Table 2. Median circulating concentrations of folate and vitamin B_{12} by country

	Country	Cases/Controls	Cases*	Controls*
Folate (nmol/L)	Germany	180/183	13.7 (8.2-30.4)	15.3 (8.2-28.6)
	Greece	9/9	10.7 (6.0-52.8)	11.4 (7.3-22.4)
	Italy	61/60	10.2 (6.4-19.3)	9.5 (4.8-23.4)
	Netherlands	24/23	11.6 (6.7-21.4)	10.1 (6.9-21.0)
	Spain	93/92	14.8 (8.6-26.3)	13.4 (8.2-25.9)
	Sweden	322/619	5.8 (3.3-11.1)	5.6 (3.4-10.0)
	United Kingdom	175/178	15.2 (8.2-33.0)	15.2 (7.9-38.6)
Vitamin B_{12} (pmol/L)	Germany	176/181	291 (189-400)	290 (190-452)
	Greece	9/9	361 (37-557)	295 (156-525)
	Italy	61/61	318 (204-539)	284 (203-408)
	Netherlands	24/25	356 (223-496)	294 (187-481)
	Spain	93/93	382 (254-579)	368 (208-506)
	Sweden	321/620	344 (241-475)	349 (233-484)
	United Kingdom	176/179	296 (177-487)	279 (175-443)

*Median (10-90th percentile).

with cut-points based on the concentration distribution in the control subjects for the whole cohort. We calculated odds ratios as estimates of relative risks (RR) for prostate cancer in relation to concentrations of folate and vitamin B_{12} using conditional logistic regression models. Overall significance and trends in RRs were calculated by replacing the categorical quintile variables with the base 2 logarithm of the observed concentration in the logistic regression model, thus achieving a trend RR associated with a doubling in concentration. The effects of potential confounders were examined by including dummy variables for additional covariates in the logistic regression models: smoking (never, past, current); alcohol intake (<8, 8-15, 16-39, 40+ g/d), body mass index (kg/m^2; in quartiles), physical activity (index of combined recreational, household, and occupational physical activity: inactive, moderately inactive, and active), marital status (married/cohabiting, not married/cohabiting), and education level (primary school or none, secondary school or equivalent, university degree). For each of these variables, a small proportion of values were unknown, and these were included in the analyses as unknown categories.

χ^2 tests were used to examine heterogeneity between subgroups in the association of prostate cancer risk with concentrations of folate and vitamin B_{12}. All P values presented are two-sided and $P < 0.05$ was considered statistically significant. Statistical analyses were done using Statistical Analysis System software (SAS Institute).

Results

Baseline Characteristics. Baseline characteristics of cases and controls are shown in Table 1. Overall, we observed no major differences in characteristics between cases and controls. The median age at blood collection was 59.1 years for cases and 58.3 years for controls. Cases were less likely to smoke, less physically active, and had a lower body weight than the controls.

The median time between blood collection and case diagnosis was 5 years. Stage of disease was known for 60.5% of the cases, of which 30.6% were classified as having advanced disease. Grade of disease was known for 83.5% of cases, of which 34.4% were classified as having high-grade disease.

Median concentrations of folate and vitamin B_{12} by country and case-control status are shown in Table 2. Folate concentrations differed substantially between the countries, with Sweden having low concentrations (median, 5.6 nmol/L in controls) and Germany and the

Table 3. Relative risks for prostate cancer in relation to concentrations of folate and vitamin B_{12}

Circulating concentrations*	Cases/controls	Unadjusted		Adjusted †	
		RR (95% CI)	P_{trend} ‡	RR (95% CI)	P_{trend} ‡
Folate (nmol/L)					
<4.82	117/229	1.00 (reference)	0.62	1.00 (reference)	0.46
4.83-6.77	119/232	0.99 (0.73-1.36)		1.02 (0.74-1.39)	
6.78-9.87	162/229	1.15 (0.83-1.61)		1.18 (0.84-1.66)	
9.89-16.52	250/233	1.52 (1.06-2.18)		1.62 (1.12-2.34)	
≥16.55	207/227	1.23 (0.84-1.81)		1.30 (0.88-1.93)	
Vitamin B_{12} (pmol/L)					
<247.4	174/231	1.00 (reference)	0.21	1.00 (reference)	0.16
247.4-299.7	176/230	1.13 (0.84-1.52)		1.15 (0.85-1.55)	
299.9-352.7	169/227	1.14 (0.85-1.53)		1.15 (0.85-1.55)	
353.0-424.8	170/232	1.23 (0.90-1.68)		1.23 (0.90-1.69)	
≥425.0	165/229	1.16 (0.85-1.58)		1.19 (0.87-1.63)	

*Groups divided into quintiles according to concentrations of distribution in the controls.
† Adjusted for body mass index, smoking status, alcohol intake, physical activity, marital status, and education level.
‡ P_{trend} values relate to the test of trend obtained by replacing the categorical variables by the logarithm of the concentrations.

Table 4. Unadjusted relative risks for prostate cancer in relation to concentrations of folate stratified by non-Swedish and Swedish subjects

Folate concentration (nmol/L)	Cases/controls	RR (95% CI)	P_{trend}*
Non-Swedish subjects †			
<8.98	96/104	1.00 (reference)	0.99
8.99-11.91	101/108	1.02 (0.69-1.53)	
11.92-16.21	135/109	1.37 (0.93-2.01)	
16.33-22.70	84/104	0.88 (0.59-1.32)	
≥22.71	118/109	1.20 (0.80-1.80)	
Swedish subjects ‡			
<4.03	61/121	1.00 (reference)	0.41
4.04-5.11	63/124	1.03 (0.66-1.6)	
5.12-6.16	53/124	0.88 (0.56-1.38)	
6.16-8.00	65/123	1.08 (0.7-1.68)	
≥8.03	79/124	1.33 (0.85-2.08)	

*P_{trend} values relate to the test of trend obtained by replacing the categorical variables by the logarithm of folate concentration.
† Groups divided into quintiles according to the concentration distribution in non-Swedish control subjects.
‡ Groups divided into quintiles according to the concentration distribution in Swedish control subjects.

United Kingdom having high concentrations (median, 15.3 and 15.2 nmol/L for controls in Germany and the United Kingdom, respectively). No large differences between countries were seen for concentrations of vitamin B_{12}.

Prostate Cancer Risk and Concentrations of Folate and Vitamin B_{12}. Overall relative risks for prostate cancer in relation to quintiles of circulating concentrations of folate and vitamin B_{12} are shown in Table 3. There were no significant associations with overall risk for circulating concentrations of folate or vitamin B_{12} in unadjusted or adjusted logistic regression, as determined by the trend test, although an increase in risk was observed in the fourth folate quintile compared with the lowest quintile. Because the Swedish subjects displayed very low folate concentrations, we also did a logistic regression with the quintiles for the Swedish and non-Swedish subjects defined separately (see Table 4). In these analyses, no association between concentrations of folate and prostate cancer risk were observed for any quintile or for overall trend.

Relative risks associated with a doubling of folate and vitamin B_{12} concentrations for selected subgroups are shown in Tables 5 and 6. We investigated heterogeneity between subgroups defined by tumor stage (localized or advanced), histologic grade (low or high), lag time between blood collection and diagnosis (<4 or ≥4 years), age at diagnosis (<65 or ≥65 years), and country of recruitment (seven countries). For circulating concentrations of folate, no significant heterogeneity between subgroups was observed for any of these factors. For circulating concentrations of vitamin B_{12}, there was borderline significant heterogeneity in risk between localized and advanced stage cases (P = 0.05). A doubling in vitamin B_{12} concentration was associated with a RR of 1.69 [95% confidence interval (95% CI), 1.05-2.72, P = 0.03] of advanced stage disease, whereas vitamin B_{12} concentrations were not associated with risk for localized stage disease (RR, 0.96; 95% CI, 0.71-1.29, P = 0.77). No other subgroup displayed a significant association between prostate cancer risk and circulating concentrations of folate or vitamin B_{12}.

We also separately analyzed the subjects used in the previous Swedish study that were excluded from the analyses described above (9). These subjects were reanalyzed using the same biochemical assay as all other subjects in the present study. This analysis resulted in a highly significant association for vitamin B_{12} in relation to prostate cancer risk (trend RR, 1.83; 95% CI, 1.29-2.61, P = 0.0008), as reported in the original study.

Discussion

In this study, the largest prospective study investigating circulating concentrations of folate and vitamin B_{12} in relation to prostate cancer risk to date, we observed no

Table 5. Unadjusted relative risks for prostate cancer associated with a doubling of folate concentration by subgroup

Study group	Cases/controls	RR (95% CI)	P_{trend}*	$P_{heterogeneity}$ †
Overall	855/1,150	1.03 (0.92-1.16)	0.62	
Lag time ‡ <4 y	279/300	0.96 (0.80-1.15)	0.67	0.34
Lag time ‡ ≥4 y	576/850	1.08 (0.93-1.26)	0.32	
Localized stage cases	357/478	0.96 (0.80-1.15)	0.66	0.88
Advanced stage cases	159/208	1.00 (0.79-1.26)	0.98	
Low grade cases	465/631	1.06 (0.91-1.23)	0.48	0.16
High grade cases	250/359	0.87 (0.70-1.09)	0.23	
Young cases (age <65 at diagnosis)	436/583	1.07 (0.91-1.26)	0.43	0.54
Old cases (age ≥65 at diagnosis)	419/567	0.99 (0.84-1.17)	0.93	
Germany	178/178	0.92 (0.72-1.16)	0.47	0.90
Greece	9/9	0.98 (0.32-3.01)	0.98	
Netherlands	22/22	1.12 (0.45-2.79)	0.81	
Italy	60/60	1.03 (0.71-1.50)	0.88	
Spain	91/91	1.30 (0.81-2.09)	0.28	
Sweden	321/616	1.09 (0.90-1.32)	0.41	
United Kingdom	174/174	1.00 (0.78-1.30)	0.97	

*P_{trend} indicates significance for the logarithm of the concentrations.
† Significance for heterogeneity in trend RRs were assessed by χ^2 statistics.
‡ Years between blood collection and diagnosis.

Table 6. Unadjusted relative risks for prostate cancer associated with a doubling of vitamin B_{12} concentration by subgroup

Study group	Cases/controls	RR (95% CI)	P_{trend}*	$P_{heterogeneity}$†
Overall	854/1,149	1.12 (0.94-1.35)	0.21	
Lag time‡ <4 y	276/297	1.09 (0.80-1.50)	0.57	0.84
Lag time‡ ≥4 y	578/852	1.14 (0.91-1.43)	0.26	
Localized stage cases	361/482	0.96 (0.71-1.29)	0.77	0.05
Advanced stage cases	154/203	1.69 (1.05-2.72)	0.03	
Low grade cases	464/630	1.07 (0.85-1.37)	0.56	0.92
High grade cases	248/358	1.10 (0.76-1.59)	0.61	
Young cases (age <65 at diagnosis)	434/580	1.17 (0.88-1.55)	0.27	0.71
Old cases (age ≥65 at diagnosis)	420/569	1.09 (0.85-1.39)	0.49	
Germany	172/172	0.78 (0.49-1.23)	0.29	0.19
Greece	9/9	1.03 (0.27-3.93)	0.97	
Netherlands	24/24	3.99 (0.84-19.01)	0.08	
Italy	61/61	1.89 (0.94-3.80)	0.07	
Spain	92/92	1.49 (0.86-2.59)	0.16	
Sweden	320/615	1.00 (0.73-1.37)	1.00	
United Kingdom	176/176	1.15 (0.80-1.65)	0.45	

*P_{trend} indicates significance for the logarithm of the concentrations.
† Significance for heterogeneity in trend RRs were assessed by χ^2 statistics.
‡ Years between blood collection and diagnosis.

significant associations with overall prostate cancer risk for concentrations of folate or vitamin B_{12}. However, in subgroup analyses, borderline significant heterogeneity was observed by stage of disease for vitamin B_{12}, with high concentrations of circulating vitamin B_{12} showing a significant association with increased risk of advanced stage prostate cancer, whereas no association was observed among the localized stage cases.

To date, most studies on the relationship between factors of one-carbon metabolism and human cancer have been conducted on colorectal cancer, and prospective studies have generally supported the hypothesis of a protective effect of elevated concentrations of folate in the circulation (3). Recently, however, this association has been questioned in the light of a randomized controlled trial investigating the potential antineoplastic effect of supplementary folate in subjects with colorectal adenomas (4). At the second follow-up of this trial, Cole et al. reported a 67% increased risk of advanced lesions in the supplementation arm compared with the placebo arm (4). This surprising result, together with animal studies showing the antineoplastic effects of folate on normal epithelial tissue and—in contrast—a promoting effect on established neoplasms, has led to the hypothesis of a dual effect of folate on cancer development. The effect of elevated concentrations of folate would depend on the timing of exposure, i.e., if the exposure is exerted before or after neoplastic transformations (16).

To our knowledge, only two previous studies have been conducted on circulating concentrations of folate and vitamin B_{12} in relation to prostate cancer risk. In 2003, Weinstein et al. reported no association with prostate cancer risk for circulating concentrations of folate and vitamin B_{12} in 224 cases and 454 controls nested within the Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention study (10). However, it should be noted that subjects in this study were all heavy smokers, and therefore, the cohort may not be representative of the overall population. In the present study, we did not have enough power to assess smoking as a potential effect modifier. In 2005, Hultdin et al. reported a significant increase in prostate cancer risk for elevated concentrations of vitamin B_{12}, and a positive association of folate with risk, although the latter association disappeared after adjusting for vitamin B_{12} concentration (9). We reanalyzed 94% of the cases included in that study using the same assay as in the present study, and the association between vitamin B_{12} and risk among these subjects remained highly significant. These subjects were excluded from the main analysis of the present study because we anticipated that they would influence the overall result. In the present study, no significant associations were observed for concentrations of folate or vitamin B_{12} with prostate cancer risk overall, however, we observed a significant increase in risk for subjects in the fourth folate quintile. Because the Swedish samples displayed very low concentrations of folate compared with the other countries, we did analyses stratified by Swedish and non-Swedish subjects and, in these analyses, no association was observed overall or for any individual quintile. The only statistically significant trend association with prostate cancer risk in the present study was observed in the group of men diagnosed with advanced stage disease. In this subgroup, a doubling in vitamin B_{12} concentration was associated with a 69% increase in prostate cancer risk. We could not compare this association with the previous studies because neither of them reported results by stage of disease (9, 10). The reason for the association between advanced stage prostate cancer and vitamin B_{12} is unknown; speculatively, it may be related to advanced cases being phenotypically less similar to controls compared with the group of localized cases that might be diluted with clinically less relevant cases. Alternatively, the association might be due to chance. In subgroup analyses by participating countries, the Swedish subjects displayed no association between vitamin B_{12} and prostate cancer risk. This contrasts with the strong association between elevated vitamin B_{12} concentrations and increased prostate cancer risk noted in the previous study from the same subcohort of subjects not included in this study (9). The reason for this apparent change in the association in Sweden is not known, but it could be due to differences in the characteristics of the cases, or that the

association noted in the previous study was due to chance.

One particular feature of the present study was the large differences in folate concentrations observed across the participating countries. The participants in Germany and United Kingdom displayed almost three times the median folate concentrations of Sweden. The reasons for these large differences are not known, but are presumably related to differences between study centers in the dietary intake of folate and in sample handling. Folate, in contrast to vitamin B_{12}, is degraded during storage, and time between blood draw and freezing of the samples varied between the centers (17). In addition, in Västerbotten County, where the Swedish samples were collected, low intake of vegetables has previously been reported which may partly explain the low folate concentrations observed among the Swedish participants (18). It should also be noted that the Swedish samples were plasma and that samples from all the other countries were serum, but this difference between the cohorts would not be expected to result in such large differences in folate concentrations. Overall, the study design of controls individually matched to cases by center, age, and date of blood draw effectively controls for any systematic differences between countries when estimating the overall trends.

Given that prostate cancer is a slowly developing disease with neoplastic transformation occurring many years before diagnosis (7), the direction of the relative risks of folate estimated in prospective studies might depend on time between blood draw and diagnosis (lag time). Even though no heterogeneity in relative risks was observed for folate or for vitamin B_{12} when stratifying on lag-time in the present study, few cases had a lag time of >10 years. It might be useful to investigate prospectively collected prostate cancer samples with very long lag-time in order to assess the potentially complex nature of one-carbon metabolism and prostate cancer (19). Because a single plasma or serum sample only provides an estimate of folate/vitamin B_{12} exposure over the previous days, it would also be useful to study samples from each subject obtained at several time points in order to estimate long-term exposure.

In conclusion, this study does not provide support for the hypothesis that circulating concentrations of folate or vitamin B_{12} are related to prostate cancer risk. Further prospective studies are needed to investigate the possible association between high concentrations of vitamin B_{12} and increased risk of advanced stage prostate cancer.

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