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Serum folate and vitamin B12 concentrations in relation to prostate cancer risk—a Norwegian population-based nested case–control study of 3000 cases and 3000 controls within the JANUS cohort

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Background Although individual studies have been inconsistent, meta-analyses of epidemiological data suggest that high folate and vitamin B12 levels may be associated with increased prostate cancer risk.

Methods Within JANUS, a prospective cohort in Norway ($n = 317\,000$) with baseline serum samples, we conducted a nested case–control study among 3000 prostate cancer cases and 3000 controls, matched on age and time at serum sampling, and county of residence. Using conditional logistic regression, odds ratios (OR) and 95% confidence intervals (CI) for prostate cancer risk were estimated according to quintiles of serum folate, vitamin B12, methylmalonic acid (MMA), total homocysteine (tHcy) and methionine, and according to *MTHFR 677C→T* genotypes. To correct for degradation during sample storage, folate concentration was measured as *p*-aminobenzoylglutamate (pABG) equivalents following oxidation and acid hydrolysis.

Results We observed a weak positive association between folate concentration and prostate cancer risk [OR highest vs lowest quintile = 1.15 (0.97–1.37), *P*-trend = 0.04], which was more pronounced among individuals ⩾50 years at inclusion [OR 1.40 (1.07–1.84), *P*-trend = 0.02]. tHcy showed an inverse trend with risk [OR 0.92 (0.77–1.10), *P*-trend = 0.03]. Vitamin B12, MMA and methionine concentrations were not associated with prostate cancer risk. Compared with the *MTHFR 677CC* genotype, the *CT* and *TT* variants, both of which were related to lower folate concentrations, were associated with reduced prostate cancer risk [OR 0.82 (0.72–0.94) and OR 0.78 (0.64–0.94), respectively].

Conclusion This large-scale population-based study suggests that high serum folate concentration may be associated with modestly increased prostate cancer risk. We did not observe an association between vitamin B12 status and prostate cancer risk.

Keywords Folate, vitamin B12, prostate cancer risk, population-based case–control study

Introduction

Prostate cancer is the second most frequently diagnosed cancer among men globally, with markedly higher incidence rates in developed countries partly as a result of the common practice of prostate-specific antigen (PSA) testing.[1–3] The folate-mediated one-carbon metabolism, which involves B-vitamins as enzymatic co-factors, is characterized by the transfer of methyl groups and has been hypothesized to affect carcinogenesis by inducing epigenomic changes[4] and influencing synthesis of DNA.

The associations of prostate cancer risk with circulating folate and vitamin B12 have been investigated in several large population-based studies.[5–9] Although generally not associated in individual studies, a meta-analysis of prospective cohort studies suggested that relatively high folate concentration is associated with modestly increased prostate cancer risk.[5] In addition, high folate concentrations at prostate cancer diagnosis were associated with increased cancer cell proliferation,[10] or with faster increases of PSA levels during follow-up,[11] suggesting that high folate levels may enhance progression of localized prostate cancer. Furthermore, randomized controlled trials showed increased cancer risk[12,13] including prostate cancer risk[14] among subjects receiving folic acid supplements, although no associations have been observed in two trials.[15,16] Conversely, observational studies suggested that high dietary folate intake is associated with decreased prostate cancer risk.[17–19]

Although one prospective study reported no association,[9] vitamin B12 concentration has been associated with increased prostate cancer risk,[5–7] in agreement with the finding of an inverse association between methylmalonic acid concentration (MMA; an inverse marker for vitamin B12 status) and prostate cancer risk.[8] In addition, dietary vitamin B12 intake has been reported to be associated with increased prostate cancer risk.[20]

In view of potential harmful effects of folate and vitamin B12, and because accumulated evidence on the associations with prostate cancer has been inconsistent, we conducted a large-scale case–control study of 3000 cases and 3000 controls nested within the JANUS serum bank, a Norwegian prospective cohort study of 317 000 individuals with available serum samples. We investigated associations of prostate cancer risk with serum concentrations of folate, vitamin B12, MMA, total homocysteine (tHcy) and methionine, and with *methylenetetrahydrofolate reductase (MTHFR) 677C→T* genotypes. We measured folate concentration as *p*-aminobenzoylglutamate (pABG) equivalents by a newly developed method involving oxidation and mild acid hydrolysis,[21] allowing recovery of most degraded folate in stored serum samples, which has not previously been possible.

Methods

Study population

The JANUS Serum Bank is a population-based bio bank including baseline serum samples of 317 000 individuals who participated in health screening surveys or were Red Cross blood donors in Norway between 1973 and 2004.[22] All demographic data were collected at inclusion in the cohort at the time of blood sampling. Cancer incidence data were obtained from the Cancer Registry of Norway. Approval was given by the Regional Ethical Committee and by the Data Inspectorate who have approved the use of data and serum samples based on a broad consent from each donor.

We conducted a nested case–control study. Incident prostate cancer cases ($n = 3000$) were matched to male controls ($n = 3000$) by age at serum sampling (±6 months), date of serum sampling (±2 months) and county of residence. Additional eligibility criteria for selected controls included being alive at the time of prostate cancer diagnosis of the matched case, resident in Norway and not having a diagnosed cancer other than non-melanoma skin cancer.

Sample handling, biochemical analyses and genotyping

Biochemical and genotyping analyses were conducted at Bevital AS, Bergen, Norway.[23] Serum samples were stored at −25°C. Routines for sample collection and processing have been described previously.[24] Because folate is subject to degradation during sample storage, a novel serum folate assay was used that measures folate and putative degradation products as pABG equivalents following oxidation and mild acid hydrolysis.[21] This method has been evaluated in JANUS serum samples that were stored for up to 29 years, and proved to be superior in accurately measuring folate status compared with methods that measure intact folate species.[25] Moreover, an independent

set of serum samples from healthy Norwegian blood donors suggested that folate in fresh samples measured as pABG equivalents shows a strong linear correlation with folate by LC-MS/MS or the microbiological assay.[26]

Vitamin B12 (cobalamin), MMA and tHcy concentrations were stable,[25] as was the case for total methionine, i.e. the sum of methionine and its degradation product methionine sulfoxide.[27] tHcy and MMA were analysed by methylchloroformate derivatization and gas chromatography–mass spectrometry.[28] Methionine, methionine sulfoxide and creatinine concentrations were analysed by liquid chromatography mass spectrometry.[23] Plasma vitamin B12 was determined by a microbiological assay.[29]

Samples were analysed in batches of 86 and quality control included six calibration samples, two control samples and one blank sample in each batch. Between-day coefficients of variation (CV) were 5% (pABG), 2.1% (tHcy), 2.6% (methionine), 5% (cobalamin), 2.6% (MMA) and 4% (creatinine). Samples from cases and controls were kept at −25°C and analysed in random order. The laboratory staff was blinded to case–control status in both the main analyses as well as in quality control analyses.

MTHFR 677C→T genotypes were determined using matrix-assisted laser-desorption/ionization-time-of-flight mass spectrometry.[30] Owing to insufficient amounts of DNA in some of the samples, genotyping could not be performed for 13% of the individuals. Distribution of *MTHFR 677C→T* genotypes in the study population was not in Hardy–Weinberg equilibrium ($P < 0.001$).

Potential confounders

Cigarette smoking and physical activity have been associated with prostate cancer risk,[31,32] and high body mass index (BMI) with prostate cancer-specific mortality.[33] In addition, smokers may have lower folate and B-vitamin concentrations compared with never smokers.[34] Information on smoking habits (never, former, current), physical activity (sedentary, ⩾4 h/wk moderate, frequent vigorous) and BMI (<25, 25–<30, ⩾30 kg/m^2) was available from health survey data from the Norwegian Institute of Public Health. Data on highest educational level (less than A-levels or high school, equivalent to A-levels or high school, college or university) were obtained from Statistics Norway.

Statistical analyses

Statistical analyses were performed using SAS (version 9.2) and R (version 2.14.1). Potential confounders, *MTHFR 677C→T* genotypes and serum concentrations were compared between cases and controls. Differences of categorical variables were tested by chi-square tests. Because distributions of serum concentrations tended to be right-skewed, median concentrations (5–95th percentiles) were presented.

Conditional logistic regression analyses were conducted to estimate associations between serum concentrations and prostate cancer risk. Exposure variables were categorized into quintiles based on the distribution among controls. Odds ratios (OR) and 95% confidence intervals (CI) were estimated for prostate cancer risk with the lowest quintiles as reference. Tests for linear trend over quintiles were conducted by replacing the ordinal values with the median concentration within each quintile. The association between the *MTHFR 677C→T* polymorphism with prostate cancer risk was estimated taking the *677CC* genotypes as reference.

Crude associations were presented, as well as analyses adjusted for highest education, smoking habits, physical activity and BMI. Because impaired renal function may influence serum concentrations of measured metabolites, and thereby bias the associations with prostate cancer risk, all models included serum creatinine as a marker for renal function.

As has been hypothesized for colorectal cancer,[35] high folate status may protect against formation of neoplasms, but may also enhance growth of existing (undiagnosed) early lesions. For this reason, and because presence of preclinical prostatic lesions theoretically influences exposure status, we stratified the risk analyses by cancer diagnosis occurring early (i.e. in the period below the median of 16 years after inclusion) or later (diagnosis >16 years after inclusion). In addition, to investigate whether the risk may be age-dependent, the analyses were stratified according to age at serum sampling. Analyses were also stratified by *MTHFR 677C→T* genotypes, in which matching variables were used as covariates.

Results

Cases and controls were on average 49.1 years of age at serum sampling, and mean age at prostate cancer diagnosis was 64.8 years (Table 1). Mean sample storage time until biochemical analyses was 24.2 years. Compared with controls, cases more often had higher education and BMI <25, but similar smoking behaviour and physical activity levels. Among cases, we observed slightly higher folate concentrations and lower tHcy concentrations than among controls, whereas serum vitamin B12, MMA, methionine and creatinine were comparable. The *MTHFR 677TT* genotype occurred less frequently among cases than among controls. Carriers of the *677TT* genotype had lower folate and higher tHcy concentrations compared with the *677CC* wild-type (Table 2).

We observed that folate concentration was modestly associated with prostate cancer risk in adjusted analyses (highest vs lowest quintile, OR 1.15, CI 0.97–1.37, $P_{trend} = 0.04$; Table 3). The tHcy concentration showed an inverse trend with prostate cancer risk, although the risk estimates across quintiles did not decrease in a linear fashion. Vitamin B12, MMA and methionine concentrations were not associated with

Table 1 Characteristics of prostate cancer cases and matched controls in the JANUS cohort

Characteristic	Prostate cancer cases	Matched controls	*P*-value[a]
N	3000	3000	
Matching criteria and patient characteristics [mean (SD)/(range)]			
Age at blood sampling (years)	49.1 (8.7)/(25.7–87.8)	49.1 (8.7)/(25.2–88.0)	
Serum sample storage time (years)	24.2 (3.3)/(7.9–29.4)	24.2 (3.3)/(7.9–29.4)	
Age at prostate cancer diagnosis (years)	64.8 (8.0)/(42.7–94.5)		
Time between blood sampling and diagnosis (years)	15.6 (5.1)/(1.1–25.6)		
Potential confounders (*n*, %)			
Highest education			
Less than A-levels or high school	1972 (65.9)	2076 (69.4)	
Equivalent to A-levels or high school	463 (15.5)	457 (15.3)	
College or university	558 (18.6)	459 (15.3)	0.002
No information	7	8	
Smoking status			
Never smoker	803 (27.7)	757 (25.9)	
Former smoker	957 (33.0)	1006 (34.4)	
Current smoker	1142 (39.4)	1159 (39.7)	0.08
No information	98	78	
Physical activity			
Sedentary	479 (16.1)	508 (17.1)	
Moderate physical activity $\geqslant$4 h/wk	1607 (54.0)	1658 (55.6)	
Frequent vigorous physical activity	890 (29.9)	814 (27.3)	0.27
No information	24	20	
Body mass index (BMI, kg/m^2)			
<25.0	1369 (46.0)	1279 (42.9)	
25.0–<30.0	1372 (46.1)	1446 (48.5)	
$\geqslant$30	233 (7.8)	257 (8.6)	0.05
No information	26	18	
Serum concentrations (median, [p5–p95])			
Folate as *p*-aminobenzoylglutamate (nmol/l)[21]	13.98 (8.67–23.60)	13.70 (8.65–23.40)	
Cobalamin (pmol/l)	449.8 (252.2–804.0)	446.5 (252.1–780.3)	
MMA (μmol/l)	0.20 (0.13–0.35)	0.20 (0.13–0.36)	
tHcy (μmol/l)	12.16 (8.59–19.23)	12.41 (8.53–20.12)	
Methionine (μmol/l)	33.05 (20.42–68.40)	32.70 (20.26–70.92)	
Creatinine (μmol/l)	77.70 (61.00–101.00)	77.60 (60.50–101.00)	
MTHFR C677T genotypes (*n*, %)[b]			
CC	1407 (55.8)	1334 (51.2)	
CT	820 (32.5)	929 (35.6)	
TT	295 (11.7)	344 (13.2)	
No data available	478	393	

[a] χ^2 test for difference between cases and controls.
[b] MTHFR, methylene tetrahydrofolate reductase.

prostate cancer risk (Table 3). Crude and adjusted analyses were comparable.

Risk analyses stratified according to time between inclusion and prostate cancer diagnosis did not show substantially different associations (Table 4). Folate concentration was associated with increased prostate cancer risk among individuals $\geqslant$50 years at serum sampling (OR 1.40, CI 1.07–1.84, $P_{trend} = 0.02$),

Table 2 Median serum concentrations among controls across *MTHFR 677C→T* genotypes

Genotype	Folate[a] (nmol/l)	Cobalamin (pmol/l)	MMA (μmol/l)	tHcy (μmol/l)	Methionine (μmol/l)
MTHFR 677C→T genotypes					
CC	14.05	451.6	0.200	12.02	32.73
CT	13.70	446.9	0.196	12.56	33.62
TT	12.65	427.1	0.200	13.86	32.06
P-value[b]	<0.001	0.07	0.43	<0.001	0.17

[a]Folate as *p*-aminobenzoylglutamate.[21]
[b]Differences across genotypes are evaluated by Kruskal–Wallis tests.

whereas no association was observed among those <50 years. The remaining biochemical parameters did not show substantially different risk estimates between these two age groups.

The *CT* and *TT* genotypes of the *MTHFR 677C→T* polymorphism were associated with decreased prostate cancer risk compared with the *677CC* genotype (*CT* genotype, OR 0.82, CI 0.72–0.94; *TT* genotype, OR 0.78, CI 0.64–0.94; Table 3). However, the serum metabolite concentrations were not differentially associated with prostate cancer risk across *MTHFR 677C→T* genotypes (data not shown).

Discussion

Principal findings

In this large-scale Norwegian population-based nested case–control study, high baseline serum folate concentration, as measured by a novel method that corrects for potential degradation during sample storage, was weakly associated with increased prostate cancer risk. tHcy concentration showed an inverse trend with prostate cancer risk. The *T* allele of the *MTHFR 677C→T* polymorphism was associated with reduced risk compared with the common CC genotype. However, neither vitamin B12 nor the marker for vitamin B12 status MMA was associated with prostate cancer risk.

Methodological considerations

This case–control study included 3000 cases and an equal number of controls, which is the largest single case–control study ever conducted on the relation between circulating folate, vitamin B12, related markers of the one-carbon metabolism and prostate cancer risk. This large sample size has minimized the probability of reporting spurious findings and allowed for subgroup analyses with adequate statistical power.

During storage of serum samples before biochemical analyses, biomarkers of the one-carbon metabolism, including folate, are subject to degradation.[27] In this respect, an important strength of the current study is the measurement of total folate as pABG equivalents by a novel method,[21] allowing recovery of most degraded folate in stored samples.[25] Moreover, tHcy, vitamin B12, MMA and total methionine concentrations proved stable across the storage period.[27]

The amount of DNA was generally low in the serum samples, which may have resulted in poor amplification of some PCR products and subsequent misinterpretation of heterozygous genotypes as homozygous genotypes.[36] This may have contributed to the distribution of *MTHFR 677CT* genotypes being not in Hardy–Weinberg equilibrium, and to possible underestimation of the associations with prostate cancer risk. Although pooled analyses did not suggest an association of the *MTHFR 677TT* variant with prostate cancer risk,[37] an inverse association with colorectal carcinoma has been established in both the JANUS cohort[38] and by meta-analysis of published data.[39] In addition, the *677C→T* polymorphism affects the distribution of folate species.[40] In this respect, we observed as expected that carriers of the *T* allele had lower folate and higher tHcy concentrations (Table 2), and were at reduced prostate cancer risk compared with carriers of the wild type CC variant. Moreover, because folate and tHcy are in part complementary factors that in combination are markers of one-carbon balance and folate status, the observed positive association of folate with prostate cancer risk was in agreement with the inverse relation with tHcy concentration. These observations for folate, tHcy and *MTHFR 677C→T* are therefore consistent and indicate integrity of the study data.

A further strength of this study is its nested prospective design, with exposure measurement prior to clinical manifestation of the disease. Although one may argue that the process of carcinogenesis of prostate cancer initiates much earlier, it is unlikely that preclinical disease has had systemic effects on the studied biochemical parameters, and that reverse causation has thus biased the observed associations. Moreover, average time between serum sampling and prostate cancer diagnosis was more than 15 years, and analyses confined to individuals with cancer diagnosis less or more than 16 years after inclusion were comparable.

An important screening tool for early detection of prostate cancer is the PSA test, which became commercially available in the late 1980s, and was followed by increases of prostate cancer incidence.[2,3]

Table 3 Associations of serum concentrations and *MTHFR 677C→T* genotypes with prostate cancer risk

Serum concentration	Quintiles (range)[a] or genotypes	Number of cases/controls	Crude analyses[b]	Adjusted analyses[c]
Folate (nmol/l)[d]	1 (<10.9)	585/577	1.00	1.00
	2 (10.9–<12.8)	566/601	0.93 (0.79–1.10)	0.92 (0.78–1.09)
	3 (12.8–<14.7)	556/596	0.93 (0.79–1.10)	0.92 (0.77–1.09)
	4 (14.7–<17.5)	590/614	0.96 (0.81–1.14)	0.94 (0.80–1.12)
	5 (⩾17.5)	703/612	1.17 (0.99–1.39)	1.15 (0.97–1.37)
	P-trend		0.02	0.04
Cobalamin (pmol/l)	1 (<340)	583/583	1.00	1.00
	2 (340–<412)	557/583	0.96 (0.81–1.14)	0.95 (0.80–1.13)
	3 (412–<483)	574/583	0.98 (0.83–1.15)	0.98 (0.83–1.16)
	4 (483–<581)	581/583	1.00 (0.85–1.19)	0.99 (0.83–1.18)
	5 (⩾581)	627/583	1.10 (0.93–1.30)	1.10 (0.93–1.31)
	P-trend		0.17	0.17
MMA (μmol/l)	1 (<0.16)	507/586	1.00	1.00
	2 (0.16–<0.18)	640/601	1.20 (1.02–1.42)	1.18 (0.99–1.40)
	3 (0.18–<0.21)	609/595	1.17 (0.99–1.38)	1.15 (0.97–1.36)
	4 (0.21–<0.26)	610/564	1.23 (1.04–1.46)	1.22 (1.02–1.45)
	5 (⩾0.26)	611/632	1.11 (0.94–1.32)	1.10 (0.93–1.31)
	P-trend		0.52	0.54
tHcy (μmol/l)	1 (<10.1)	577/594	1.00	1.00
	2 (10.1–<11.7)	688/596	1.21 (1.02–1.42)	1.22 (1.03–1.44)
	3 (11.7–<13.1)	620/597	1.05 (0.89–1.24)	1.06 (0.89–1.26)
	4 (13.1–<15.3)	568/597	0.96 (0.81–1.14)	0.97 (0.82–1.16)
	5 (⩾15.3)	530/598	0.90 (0.76–1.08)	0.92 (0.77–1.10)
	P-trend		0.02	0.03
Methionine (μmol/l)	1 (<25.9)	634/589	1.00	1.00
	2 (25.9–<30.5)	528/591	0.83 (0.70–0.98)	0.84 (0.71–0.99)
	3 (30.5–<35.4)	579/590	0.90 (0.76–1.07)	0.92 (0.78–1.10)
	4 (35.4–<44.1)	624/590	0.97 (0.82–1.16)	0.99 (0.83–1.19)
	5 (⩾44.1)	576/591	0.90 (0.74–1.09)	0.92 (0.76–1.12)
	P-trend		0.80	0.99
MTHFR 677C→T	CC	1407/1334	1.00	1.00
	CT	820/929	0.84 (0.74–0.96)	0.82 (0.72–0.94)
	TT	295/344	0.81 (0.67–0.98)	0.78 (0.64–0.94)
	P-trend		0.004	0.001

[a]Quintiles are based on the distribution of serum concentrations among controls.
[b]Adjusted for serum creatinine concentration.
[c]Adjusted for serum creatinine concentration, education, smoking, physical activity and body mass index.
[d]Folate as *p*-aminobenzoylglutamate.[21]

In our study, the majority of the cases (i.e. >99%) were diagnosed after the introduction of PSA testing, which is likely to have contributed to earlier detection of prostate cancer among cohort members who were screened. In addition, we observed that cases were more often highly educated, possibly because these individuals were more likely to have had a PSA test. However, even though high education was also associated with higher folate status, inclusion of education in the logistic regression models did not materially alter the main exposure estimates.

The JANUS cohort comprises healthy individuals who in part donated consecutive blood samples in addition to the baseline sample. However, for the

Table 4 Associations of serum concentrations with prostate cancer risk, stratified for time between serum sampling and diagnosis, and for age at serum sampling

Serum concentration	Quintiles (range)[a]	Time between serum sampling and diagnosis[b]				Age at serum sampling			
		1.1–16.0 years		16.1–25.8 years		<50 years		⩾50 years	
		ca/co[c]	OR (95% CI)[d]	ca/co[c]	OR (95% CI)[d]	ca/co[c]	OR (95% CI)[d]	ca/co[c]	OR (95% CI)[d]
Folate (nmol/l)[e]	1 (<10.9)	290/280	1.00	295/297	1.00	382/357	1.00	203/220	1.00
	2 (10.9–<12.8)	277/304	0.88 (0.69–1.13)	289/297	0.98 (0.77–1.24)	350/384	0.82 (0.66–1.01)	216/217	1.14 (0.86–1.51)
	3 (12.8–<14.7)	281/294	0.94 (0.74–1.20)	275/302	0.90 (0.71–1.14)	322/341	0.87 (0.70–1.09)	234/255	1.03 (0.79–1.36)
	4 (14.7–<17.5)	298/310	0.94 (0.73–1.20)	292/304	0.95 (0.75–1.21)	359/369	0.88 (0.71–1.10)	231/245	1.06 (0.81–1.40)
	5 (⩾17.5)	361/319	1.14 (0.89–1.45)	342/293	1.17 (0.92–1.50)	377/343	1.03 (0.82–1.28)	326/269	1.40 (1.07–1.84)
	P-trend		0.14		0.14		0.46		0.02
Cobalamin (pmol/l)	1 (<340)	292/283	1.00	291/300	1.00	336/340	1.00	247/243	1.00
	2 (340–<412)	281/303	0.88 (0.69–1.12)	276/280	1.02 (0.80–1.30)	351/355	1.00 (0.80–1.25)	206/228	0.88 (0.67–1.16)
	3 (412–<483)	289/284	0.93 (0.73–1.19)	285/299	0.99 (0.78–1.26)	345/362	0.96 (0.77–1.20)	229/221	0.99 (0.75–1.29)
	4 (483–<581)	289/292	0.92 (0.72–1.18)	292/291	1.00 (0.79–1.28)	356/342	1.07 (0.85–1.34)	225/241	0.88 (0.67–1.15)
	5 (⩾581)	317/307	1.00 (0.78–1.27)	310/276	1.22 (0.96–1.56)	356/348	1.11 (0.89–1.40)	271/235	1.11 (0.85–1.44)
	P-trend		0.77		0.11		0.25		0.35
MMA (μmol/l)	1 (<0.16)	270/307	1.00	237/279	1.00	330/383	1.00	177/203	1.00
	2 (0.16–<0.18)	318/296	1.17 (0.92–1.48)	322/305	1.19 (0.93–1.52)	406/389	1.16 (0.93–1.43)	234/212	1.24 (0.94–1.65)
	3 (0.18–<0.21)	304/314	1.08 (0.85–1.37)	305/281	1.23 (0.96–1.56)	372/367	1.16 (0.94–1.43)	237/228	1.17 (0.88–1.56)
	4 (0.21–<0.26)	292/275	1.22 (0.96–1.56)	318/289	1.23 (0.96–1.58)	359/321	1.27 (1.01–1.59)	251/243	1.15 (0.87–1.51)
	5 (⩾0.26)	310/302	1.17 (0.92–1.49)	301/330	1.04 (0.80–1.34)	309/323	1.13 (0.89–1.42)	302/309	1.08 (0.83–1.42)
	P-trend		0.28		0.81		0.36		0.92
tHcy (μmol/l)	1 (<10.1)	273/311	1.00	304/283	1.00	382/374	1.00	195/220	1.00
	2 (10.1–<11.7)	351/299	1.42 (1.12–1.80)	337/297	1.06 (0.84–1.34)	427/369	1.16 (0.94–1.43)	261/227	1.34 (1.02–1.77)
	3 (11.7–<13.1)	320/282	1.32 (1.03–1.68)	300/315	0.88 (0.69–1.11)	381/362	1.03 (0.83–1.28)	239/235	1.18 (0.89–1.57)
	4 (13.1–<15.3)	289/309	1.06 (0.83–1.36)	279/288	0.89 (0.69–1.14)	308/345	0.84 (0.67–1.05)	260/252	1.20 (0.90–1.79)
	5 (⩾15.3)	262/294	1.03 (0.80–1.33)	268/304	0.82 (0.63–1.06)	281/336	0.85 (0.67–1.08)	249/262	1.07 (0.80–1.42)
	P-trend		0.24		0.05		0.01		0.64
Methionine (μmol/l)	1 (<25.9)	342/305	1.00	292/284	1.00	370/334	1.00	264/255	1.00
	2 (25.9–<30.5)	284/298	0.83 (0.65–1.05)	244/293	0.82 (0.64–1.05)	309/372	0.78 (0.63–0.98)	219/219	0.95 (0.73–1.23)
	3 (30.5–<35.4)	271/298	0.80 (0.63–1.03)	308/292	1.03 (0.80–1.32)	361/352	0.96 (0.77–1.21)	218/238	0.87 (0.66–1.14)
	4 (35.4–<44.1)	295/272	0.94 (0.73–1.21)	329/318	1.04 (0.81–1.34)	386/362	0.99 (0.78–1.25)	238/228	0.98 (0.74–1.29)
	5 (⩾44.1)	281/301	0.80 (0.60–1.06)	295/290	1.03 (0.78–1.35)	328/346	0.88 (0.68–1.15)	248/245	0.97 (0.72–1.31)
	P-trend		0.29		0.40		0.87		0.97

[a]Quintiles are based on the distribution of serum concentrations among controls.
[b]Median time of 16 years used as cut-off value.
[c]Number of cases and controls within categories of exposure.
[d]Adjusted for serum creatinine concentration, education, smoking, physical activity and body mass index.
[e]Folate as *p*-aminobenzoylglutamate.[21]

current study population there are no repeat measurements available. We do appreciate though that there is within-person variability over time. As an indication of the extent to which our OR estimates might have been attenuated by regression dilution, we have data from a parallel project with replicate fasting samples, donated 1–2 years apart, from 40 healthy participants of the Nurses' Health Studies (unpublished data). In that study, folate was measured by a microbiological assay. As folate measured as pABG equivalents, as in Janus, correlates strongly with folate by the microbiological assay,[26] the intraclass correlation coefficient (ICC) for the latter may be relevant. ICCs of 0.61 and 0.71 were observed for folate and tHcy, respectively. After correction, the OR estimates in Janus of the 5th quintiles of folate and tHcy (calculated as described by Clarke *et al.*[41]) were 1.26 for folate and 0.89 for tHcy, suggesting that the true associations were stronger than the ones observed.

Folate and prostate cancer risk

Although folate concentration was modestly associated with increased prostate cancer risk in a meta-analysis of observational studies, results from individual studies included were inconsistent.[5] One reason for the inconsistencies may be variation of folate status between study populations and different concentrations in the reference categories after categorization of exposure. For instance, whereas median folate concentration in JANUS was 13.7 nmol/l among controls, concentration was substantially higher (15.8 nmol/l) in a British cohort[5] and markedly lower in Swedish[6] and Finnish[9] studies (~9 nmol/l), although only the Swedish study provided some evidence for an association with increased prostate cancer risk.[6] Furthermore, folate status was not associated with prostate cancer across European countries with different folate status,[7] suggesting that differences in folate status are not the primary cause of the observed differences in prostate cancer risk between studies. However, an association with prostate cancer risk may be weak, requiring a very large sample size, as in our study, to be demonstrated. Methionine was not associated with prostate cancer risk in our study and in one previous study,[8] suggesting that serum methionine cannot be used as a biomarker of prostate cancer risk.

Randomized controlled trials suggested increased cancer risk[12–14] or no association with cancer risk[15,16] among subjects who received folic acid compared with controls. Meta-analyses on trial data suggested no association,[42] or a positive association with cancer risk.[43] In addition, two recent trials including individuals with a history of cardiovascular events did not suggest that folic acid supplementation was associated with prostate cancer risk.[44,45] However, disadvantages of such experimental settings are the generally limited follow-up period to study cancer incidence, and administration of relatively high doses of folic acid, which is the synthetic form of folate. Observational studies typically have a long follow-up and naturally occurring folates can be investigated. In addition, study populations are generally based on individuals with a history of cancer or cardiovascular disease rather than comprising healthy individuals, as usually included in observational studies. Nevertheless, high folate status was associated with progression of localized prostate cancer in observational studies among prostate cancer patients.[10,11]

Similar to previous observations,[6] we found that the positive association between high folate concentration and prostate cancer risk was more pronounced among the older men (i.e. >50 years) at serum sampling. In this respect, it would have been interesting to investigate whether older individuals were diagnosed with more advanced grade prostate cancer. However, no data were available on Gleason scores, which is a limitation of our study. There was information available on disease staging (categorized as localized tumour, regional spread, or distant metastasis) for 61% of the cases. Although individuals with higher stage cancers were generally older at inclusion among those with available data, folate concentration was not differentially associated with prostate cancer according to different staging (data not shown).

Vitamin B12 status and prostate cancer risk

Unlike previous findings,[5–8,20] vitamin B12 and MMA concentrations were not associated with increased prostate cancer risk in our study. When comparing studies however, it is important to consider age differences across study populations, as vitamin B12 status may be decreased in the elderly and deficiency is typically observed in people >60 years of age.[46] Age of participants and vitamin B12 concentrations were largely similar in the population-based studies conducted, i.e. median age being around 60 years and vitamin B12 concentration approximately 300 pmol/l.[5–7,9] In JANUS, median age at serum sampling was 49 years, and median vitamin B12 concentration was 446 pmol/l among controls. This considerably higher range of vitamin B12 concentrations may explain why we did not observe an association with prostate cancer risk. Even stratification according to age at serum sampling did not reveal associations between vitamin B12 or MMA concentrations with prostate cancer risk.

Conclusions

This large-scale population-based case–control study adds new evidence to the previously suggested positive association between folate status and prostate cancer risk. Vitamin B12 status was not associated with prostate cancer risk in the current study.

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Conflict of interest: None declared.

KEY MESSAGES

- This large-scale Norwegian population-based nested case–control study, in which folate degraded during sample storage was measured as *p*-aminobenzoylglutamate, suggested a weak positive association between serum folate concentration and prostate cancer risk.
- Vitamin B12 status was not associated with prostate cancer risk in the current study.

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