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Plasma folate, vitamin B12, and homocysteine and prostate cancer risk: A prospective study

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The role of folate metabolism in cancer development is a topic of much current interest, with maintenance of adequate folate status tending to show a protective effect. Aberrant methylation, primarily hypermethylation of certain genes including tumor suppressors, has been implicated in prostate cancer development. Folate, vitamin B12 and homocysteine are essential for methyl group metabolism and thus also for DNA methylation. We related plasma levels of these factors to prostate cancer risk in a prospective study of 254 case subjects and 514 matched control subjects. Increasing plasma levels of folate and vitamin B12 were statistically significantly associated with increased prostate cancer risk, with an odds ratio of 1.60 (95% CI = 1.03–2.49; $p_{trend} = 0.02$) for folate and 2.63 (95% CI = 1.61–4.29; $p_{trend} < 0.001$) for vitamin B12 for highest *vs*. lowest quartile. Increasing plasma homocysteine levels were associated with a reduced risk of borderline significance (OR = 0.67; 95% CI = 0.43–1.04; $p_{trend} = 0.08$). After adjustment for the other 2 plasma variables, body mass index and smoking, a statistically significant increased risk remained only for vitamin B12 (OR = 2.96; 95% CI = 1.58–5.55; $p_{trend} = 0.001$). Adjusted OR for folate and homocysteine were 1.30 (95% CI = 0.74–2.24; $p_{trend} = 0.17$) and 0.91 (95% CI = 0.51–1.58; $p_{trend} = 0.60$), respectively. Our results suggest that factors contributing to folate status are not protective against prostate cancer. On the contrary, vitamin B12, associated with an up to 3-fold increase in risk, and possibly also folate, may even stimulate prostate cancer development. These findings are novel and should be explored further in future studies.

Key words: folate; folic acid; vitamin B12; cobalamin; homocysteine; one-carbon metabolism; prostatic neoplasm; prospective

The role of folate metabolism, and specifically DNA methylation, in carcinogenesis has attracted increasing attention, with tumor tissue showing a general pattern of global DNA hypomethylation and gene-specific hypermethylation.[1] In the prostate, *de novo* methylation and consequent silencing of certain genes, including tumor suppressors, appear to be a driving force in cancer development.[2] Methyl group availability is dependent on folate status, with 5-methyltetrahydofolate providing methyl groups for the conversion of homocysteine to methionine, catalyzed by a vitamin B12-containing enzyme. Methionine then becomes S-adenosyl methionine, the universal methyl donor for reactions including DNA methylation.

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 in DNA, and thus also double-strand breaks, and ensuring sufficient mismatch repair.

Maintaining an adequate folate status has generally been shown to be protective against cancer development, with evidence strongest for colorectal cancer.[3] However, despite the potentially important role of folate metabolism, particularly DNA methylation, in prostate tumorigenesis, only one study has addressed the effect of circulating levels of folate, vitamin B12 and homocysteine on prostate cancer development, reporting no association in male smokers.[4]

Two small studies have suggested a possible increase in prostate cancer risk for individuals homozygous for the *methylenetetrahydrofolate reductase* (*MTHFR*) 677 C→T polymorphism, which diverts folate from methylation reactions to nucleotide synthesis, but both were severely underpowered.[5,6] Studies of the same polymorphism in other cancer types have supported varying roles, depending on tumor site.[7–15] The aim of the present study was to relate plasma levels of vitamin B12, folate and homocysteine to prostate cancer risk in a prospective population-based cohort.

Material and methods

Study population and subjects

We conducted a nested case-control study within the Northern Sweden Health and Disease Cohort, which for men includes the Västerbotten Intervention Project (VIP)[16] and the WHO Northern Sweden Monitoring Trends and Determinants of Cardiovascular Disease (MONICA) study.[17] VIP is an ongoing community-based intervention initiated in 1985 with the aim of reducing cardiovascular disease and diabetes through changes in diet and lifestyle. Upon turning 40, 50 and 60 years of age, residents of the county of Västerbotten are called to a health survey, at which time they are also invited to complete an extensive lifestyle questionnaire and to donate blood samples for future research. As part of the MONICA study, 2,000 residents of Västerbotten and Norrbotten, Sweden's 2 northernmost counties, were randomly selected to participate in a similar survey in 1986, 1990, 1994 and 1999. As of July 2001, 37,776 men had been recruited to these 2 subcohorts.

Incident cases of prostate cancer were identified through linkage with the regional cancer registry. Tumor characteristics were obtained from the Primary Prostate Cancer Registry of Northern Sweden at Umeå University Hospital, which has been in operation since 1992. Details were extracted from medical records by a research nurse and verified by the treating physician. No formal screening program has been in effect in the catchment area of the cohort since its inception, and in the present study approximately 12% of cases were asymptomatic and identified through health checkups (Table I). This suggests little exposure to opportunistic prostate-specific antigen screening for early detection of prostate cancer in this region.

For each case subject, 2 control subjects matched for age (± 6 months), recruitment date (± 2 months) and subcohort were randomly selected from eligible subjects alive and free of disease at the time of index case diagnosis. This resulted in 254 case subjects

Abbreviations: MTHFR, methylenetetrahydrofolate reductase; VIP, Västerbotten Intervention Project; MONICA, Monitoring Trends and Determinants of Cardiovascular Disease; PSA, prostate-specific antigen; OR, odds ratio; CI, confidence interval; BMI, body mass index; SD, standard deviation

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TABLE I – CHARACTERISTICS OF PROSTATE CANCER CASE SUBJECTS IN THE NORTHERN SWEDEN HEALTH AND DISEASE COHORT

	Median	25–75th percentile
Age at diagnosis	63.6	60.9–66.4
Follow-up years	4.9	2.9–7.0
S-PSA at diagnosis	11.0	6.8–26.0
Cause of workup[2]	Number case subjects	Percent
Health checkup	31	12.2
Local symptoms	139	54.7
Other causes	32	12.6
Not registered	41	16.1
Missing	11	4.3
Local tumor stage[3]		
Nonpalpable		
T1a, b	9	3.6
T1c	115	45.3
Palpable		
Localized; T2	93	36.6
Nonlocalized; T3, T4	27	10.6
Tx	10	3.9
Lymph node metastasis[4]		
N0, no lymph node metastasis	116	45.7
N1, lymph node metastasis	10	3.9
Nx, no lymph node extirpation	128	50.6
Bone metastasis[5]		
M0, no bone metastasis	187	73.6
M1, bone metastasis	21	8.3
Mx, no bone scan	46	18.2
Tumor differentiation[6]		
Highly differentiated	138	54.3
Intermediately differentiated	80	31.5
Poorly differentiated	28	11.0
Missing	8	3.2

[1]Follow-up time between recruitment at which blood draw was done and prostate cancer diagnosis.– [2]Cause of workup leading to prostate cancer diagnosis, determined from medical charts prior to 2000 and from the Primary Prostate Cancer Registry of Northern Sweden since 2000.–[3]T1a, b tumors detected by histopathologic examination of tissue from transurethral resection; T1c, tumor detected in workup of elevated PSA, not detectable by digital or ultrasound rectal examination; T2, tumor localized and palpable; T3, tumor locally extensive; T4, tumor invading neighboring tissue; Tx, not defined.–[4]Nodal stage at histologic examination of surgical specimen from obturator lymph nodes.–[5]Bone metastasis evaluated by bone scan.–[6]Prior to 2000, pathology reports employed the WHO grading system; since 2000, the Gleason score has been used in virtually all cases. Tumor differentiation was classified as highly differentiated for WHO grade 1 and Gleason score 2–5, intermediate for WHO grade 2 and Gleason score 6–7 and poorly differentiated for WHO grade 3 and Gleason score 8–10.

and 514 control subjects for whom analysis of plasma folate, vitamin B12 and homocysteine was possible. Blood samples, as well as extensive anthropometric and lifestyle information, were obtained before diagnosis of cases (median, 4.9 years; 25–75th percentile, 2.9–7.0). Subjects provided informed consent at the time of recruitment for the use of their blood samples in future research.

Analysis of plasma folate, vitamin B12 and homocysteine

Concentrations of folate and vitamin B12 in heparin plasma stored at −80°C were analyzed by Quantaphase II radioassay (Bio-Rad, Richmond, CA), and total plasma homocysteine was measured by a fluorescence polarization immunoassay on an IMx unit (Abbott Laboratories, Abbott Park, IL). Total coefficients of variation were for folate, 6.7% at 4.5 nM and 6.6% at 17.5 nM; for vitamin B12, 6.7% at 304 pM and 7.4% at 669 pM; and for homocysteine, 1.9% at 12.7 μM and 2.3% at 25.7 μM. Seventy-one percent of samples were collected after a minimum of 8-hr fasting, 26% after 4–8 hr and 3% after less than 4 hr. Plasma levels of folate, vitamin B12 and homocysteine for these fasting durations did not differ significantly (Kruskall-Wallis, $p > 0.55$, 0.79 and 0.28, respectively).

Statistical analysis

To account for skewed distributions of the plasma variables, the Wilcoxon signed-rank test was conducted for all continuous variables, comparing case subjects with the mean value of their 2 matched control subjects. Chi-square analysis was performed on the categorical smoking variable. Associations between variables were assessed using Spearman's rank correlations. Odds ratios (ORs) for disease and 95% confidence intervals (CIs) were calculated by conditional logistic regression for quartiles of folate, vitamin B12 and homocysteine, with quartile cutoff points based on variable distributions of case and control subjects combined. Effect modification was assessed by stratification [employing binary logistic regression for body mass index (BMI) and smoking, which were nonmatching variables] and interaction terms. Tests for trend were also performed; analyte quartiles were assigned their midpoint values and included as continuous variables in the regression analyses. Statistical tests and corresponding *p*-values were 2-sided, and SPSS version 11.5 (SPSS, Chicago, IL) was used for all statistical analyses.

Results

As assessed by the Wilcoxon signed-rank test, plasma levels of folate did not differ significantly between case and control subjects (mean 9.3 ± 5.4 and 8.8 ± 5.3 nM, respectively; $p = 0.48$). Levels were statistically significantly higher for plasma vitamin B12 and lower for plasma homocysteine in case subjects *vs*. control subjects (for vitamin B12, mean 328.9 ± 99.7 *vs*. 299.8 ± 103.5 pM, $p < 0.001$; for homocysteine, mean 12.2 ± 3.1 *vs*. 13.2 ± 7.3 μM, $p = 0.004$; Table II).

TABLE II – BASELINE CHARACTERISTICS OF PROSTATE CANCER CASE AND CONTROL SUBJECTS IN THE NORTHERN SWEDEN HEALTH AND DISEASE COHORT

	Case subjects (n = 254) Mean ± SD	Control subjects (n = 514) Mean ± SD	p (difference)[1]
Age at recruitment, years	58.2 ± 4.9	58.2 ± 3.9	Matched
BMI, kg/m^2	26.1 ± 2.7	26.6 ± 3.5	0.11
P-folate, nM	9.3 ± 5.4	8.8 ± 5.3	0.48
P-B12, pM	328.9 ± 99.7	299.8 ± 103.5	< 0.001
P-homocysteine, μM	12.2 ± 3.1	13.2 ± 7.3	0.004
Smoking frequency[2]	n (%)	n (%)	
Never smoker	115 (48)	230 (50)	
Ex-smoker	79 (33)	132 (29)	
Current smoker	45 (19)	97 (21)	0.47

[1]Wilcoxon signed-rank test for continuous variables; chi-square analysis for the categorical smoking variable.–[2]Smoking data were not available for 15 case and 55 control subjects.

A statistically significant positive correlation was found between folate and vitamin B12 (r = 0.21; $p < 0.001$), whereas inverse associations were observed between folate and homocysteine (r = −0.46; $p < 0.001$) and vitamin B12 and homocysteine (r = −0.23; $p < 0.001$). There was also a weak, albeit statistically significant, positive association between homocysteine and BMI (r = 0.12; $p = 0.001$).

Unadjusted ORs for highest *vs*. lowest quartile of folate and homocysteine were 1.60 (95% CI = 1.03–2.49; $p_{trend} = 0.02$) and 0.67 (95% CI = 0.43–1.04; $p_{trend} = 0.08$), respectively (Table III). Risk of prostate cancer increased for each quartile of vitamin B12 up to an unadjusted OR of 2.63 (95% CI = 1.61–4.29; $p_{trend} < 0.001$) for highest *vs*. lowest quartile (Table III).

Stratification by BMI, smoking, or disease state [advanced disease defined as locally advanced tumor (T3 or T4), lymph node metastasis (N1), metastasis on bone scan (M1), and/or serum prostate-specific antigen (S-PSA) above 50 ng/ml] had no material effect on risk estimates for any of the plasma variables (data not shown), while stratification by baseline age indicated that observed trends were attributable primarily to those aged 59 years or more (Table III). As a result of recruitment to VIP at even decades of age, a large sampling cluster at 59–60 years occurred. Although only 22% of subjects were under the age of 59 at sample collection, using this cutoff rather than the median age of 59.9 years prevented splitting the study group in the middle of the largest cluster.

For folate, increased risk at higher plasma levels was only seen in subjects with follow-up time above the median, 4.9 years, or with S-PSA under 10 ng/ml at diagnosis (Table III). High plasma homocysteine was statistically significantly associated with reduced risk only in case subjects with S-PSA under 10 ng/ml at diagnosis (and their matched control subjects; Table III), while stratification by follow-up time did not affect risk estimates, only reducing their significance. A statistically significant increased risk was observed with increasing plasma vitamin B12 regardless of follow-up time or S-PSA at diagnosis (Table III).

Variables clearly not statistically significant in univariate or multiplicative interaction analysis (p for trend and for individual quartiles > 0.2) were excluded from the multivariate analysis. After adjustment for the other 2 plasma variables, BMI and smoking, the ORs for highest *vs*. lowest quartile of folate and homocysteine were attenuated and not significant: 1.30 (95% CI = 0.74–2.24; $p_{trend} = 0.17$) for folate and 0.91 (95% CI = 0.51–1.58; $p_{trend} = 0.60$) for homocysteine (Fig. 1). In contrast, the corresponding OR for vitamin B12 rose to 2.96 (95% CI = 1.58–5.55; $p_{trend} = 0.001$) in the multivariate model (Fig. 1).

Discussion

In this prospective population-based study, increasing plasma levels of vitamin B12 were statistically significantly associated with an up to 3-fold increase in prostate cancer risk. Although both higher folate and lower homocysteine levels also increased risk in univariate analysis, these effects were attenuated and no longer statistically significant after adjustment for the other plasma variables, BMI and smoking.

The greatest strength of this study lies in its prospective design; blood samples and anthropometric data were collected with follow-up times from recruitment to diagnosis ranging from less than 1 year to 13 years (mean, 4.9 ± 2.8). Due to the slow-growing nature of prostate cancer, a large proportion of our case subjects would almost certainly have had an undiagnosed tumor at the time of sampling. However, this is not likely to have influenced results; 70–80% of vitamin B12 in blood is transported by haptocorrins. Unlike transcobalamin II, the transporter responsible for removing vitamin B12 from blood to cells, haptocorrings have a long half-life and remove vitamin B12 only by receptor-mediated uptake in the liver. Decreased uptake due to a massive tumor burden in the liver at the time of blood sampling is, however, extremely unlikely to have occurred in the present study as most tumors were localized at diagnosis (Table I). Some tumor cells have been reported to upregulate their transcobalamin receptors as a reflection of their increased demand for vitamins to support proliferation,[18] but this might result in reduced plasma levels of vitamin B12, clearly not the case in the present study.

The slow development of prostate cancer also makes it difficult, even in prospective studies, to distinguish between a role of a given variable in tumor initiation and tumor growth. Although our results cannot exclude the possibility of a vitamin B12-rich environment allowing more aggressive tumor progression and leading to earlier diagnosis, increased risk was seen regardless of follow-up time between sampling and diagnosis. Furthermore, the positive association for folate occurred only for subjects with a longer follow-up time or lower S-PSA. Taken together, these observations support an early role in prostate tumorigenesis for factors involved in folate metabolism.

Use of dietary supplements such as multivitamins was not assessed in this study. However, a study by Wahlin *et al*.[19] determining reference values of plasma folate and vitamin B12 in a random sample from the same population as ours (and employing the same laboratory techniques) reported no association between supplement use and either folate or vitamin B12 in plasma. That our findings might reflect confounding of a high status of some other vitamin or mineral due to supplement use is therefore unlikely.

Alcohol consumption, as well as drugs and gastrointestinal disorders interfering with folate or vitamin B12 bioavailability or uptake, such as antacids or certain anticonvulsives, and gastritis or celiac disease, was also not addressed in the present study. However, with the possible exception of calcium in antacids, confounding is unlikely. A high calcium intake has been suggested to increase prostate cancer risk,[20] but given the direction of our observed associations between vitamin B12 and risk, accounting for calcium-containing antacids would not be expected to weaken

TABLE III – ODDS RATIOS FOR PROSTATE CANCER BY QUARTILES OF FOLATE, VITAMIN B12 AND HOMOCYSTEINE IN PLASMA

		Quartiles				
		1 (ref)	2	3	4	p_{trend}[1]
Folate						
Cutoffs, nM		< 5.85	5.85–7.70	7.70–10.30	> 10.30	
Full study group	Case/control	60/130	58/134	58/133	77/111	
	OR (95% CI)	1.00	0.94 (0.60–1.47)	0.95 (0.61–1.48)	1.60 (1.03–2.49)	0.02
Baseline age[2]						
< 59 years	Case/control	12/20	12/22	9/29	14/21	
	OR (95% CI)	1.00	0.7 (0.3–2.1)	0.5 (0.2–1.4)	1.1 (0.4–3.2)	0.81
≥ 59 years	Case/control	48/110	46/112	49/104	63/90	
	OR (95% CI)	1.00	1.0 (0.6–1.6)	1.1 (0.7–1.8)	1.7 (1.1–2.8)	0.013
Follow-up[3]						
< 4.9 years	Case/control	27/54	29/54	29/68	41/68	
	OR (95% CI)	1.00	1.2 (0.6–2.3)	0.9 (0.5–1.7)	1.3 (0.7–2.5)	0.45
≥ 4.9 years	Case/control	33/76	29/80	29/65	36/43	
	OR (95% CI)	1.00	0.8 (0.4–1.4)	1.0 (0.5–1.8)	2.0 (1.1–3.7)	0.012
Serum PSA[4]						
≤ 10 ng/ml	Case/control	23/56	29/67	29/58	37/48	
	OR (95% CI)	1.00	1.1 (0.5–2.2)	1.2 (0.6–2.4)	2.1 (1.1–4.1)	0.018
> 10 ng/ml	Case/control	35/66	28/60	27/69	34/56	
	OR (95% CI)	1.00	0.8 (0.5–1.6)	0.8 (0.4–1.4)	1.2 (0.7–2.2)	0.53
vit. B12						
Cutoffs, pM		< 238	238–302	302–370	> 370	
Full study group	Case/control	43/150	68/129	66/120	76/114	
	OR (95% CI)	1.00	1.88 (1.17–3.01)	2.12 (1.31–3.42)	2.63 (1.61–4.29)	< 0.001
Baseline age[2]						
< 59 years	Case/control	7/20	8/21	15/25	17/27	
	OR (95% CI)	1.00	1.0 (0.3–3.3)	1.5 (0.5–4.0)	1.5 (0.5–4.4)	0.32
≥ 59 years	Case/control	36/130	60/108	51/95	59/87	
	OR (95% CI)	1.00	2.1 (1.3–3.6)	2.3 (1.3–3.9)	2.9 (1.7–5.1)	< 0.001
Follow-up[3]						
< 4.9 years	Case/control	18/67	26/61	38/56	44/64	
	OR (95% CI)	1.00	1.7 (0.8–3.5)	2.7 (1.4–5.4)	3.0 (1.5–6.0)	0.001
≥ 4.9 years	Case/control	25/83	42/68	28/64	32/50	
	OR (95% CI)	1.00	2.0 (1.1–3.8)	1.6 (0.8–3.2)	2.3 (1.2–4.6)	0.037
Serum PSA[4]						
≤ 10 ng/ml	Case/control	16/59	33/60	32/61	37/52	
	OR (95% CI)	1.00	2.1 (1.0–4.2)	2.0 (1.0–4.0)	2.6 (1.3–5.4)	0.015
> 10 ng/ml	Case/control	24/85	33/63	32/50	35/55	
	OR (95% CI)	1.00	1.9 (1.0–3.8)	2.6 (1.3–5.3)	2.8 (1.4–5.9)	0.003
Homocysteine						
Cutoffs, μM		< 10.35	10.35–12.06	12.06–13.85	> 13.85	
Full study group	Case/control	70/122	64/129	68/126	52/137	
	OR (95% CI)	1.00	0.92 (0.60–1.41)	0.97 (0.62–1.51)	0.67 (0.43–1.04)	0.08
Baseline age[2]						
< 59 years	Case/control	15/29	13/21	7/27	12/16	
	OR (95% CI)	1.00	1.4 (0.5–3.6)	0.4 (0.1–1.4)	1.5 (0.6–4.2)	0.61
≥ 59 years	Case/control	55/93	51/108	61/99	40/121	
	OR (95% CI)	1.00	0.8 (0.5–1.4)	1.1 (0.7–1.8)	0.6 (0.4–0.9)	0.033
Follow-up[3]						
< 4.9 years	Case/control	41/72	30/63	31/53	24/60	
	OR (95% CI)	1.00	0.9 (0.5–1.6)	1.0 (0.6–1.9)	0.7 (0.4–1.3)	0.30
≥ 4.9 years	Case/control	29/50	34/66	37/73	28/77	
	OR (95% CI)	1.00	0.9 (0.5–1.7)	0.9 (0.5–1.7)	0.6 (0.3–1.2)	0.14
Serum PSA[4]						
≤ 10 ng/ml	Case/control	36/60	33/53	29/58	20/62	
	OR (95% CI)	1.00	1.1 (0.6–2.0)	0.8 (0.4–1.6)	0.5 (0.3–1.0)	0.034
> 10 ng/ml	Case/control	31/58	28/68	36/63	30/64	
	OR (95% CI)	1.00	0.8 (0.4–1.4)	1.1 (0.6–2.1)	0.9 (0.5–1.7)	0.99

[1]*p* for trend was determined by assigning quartiles their midpoint values and including them as a continuous variable in analysis by conditional logistic regression.–[2]Median age 59.9, but 59 years used as cutoff value to preserve sample clustering at even decades.–[3]Median follow-up time between recruitment and prostate cancer diagnosis.–[4]Patients with S-PSA above 10 ng/ml have a worse prognosis and can thus be regarded as having significant disease.[8]

results. In any case, in Wahlin *et al*.,[19] only 28 of 1,000 subjects were excluded for these reasons, suggesting a minimal impact in the present study.

While our vitamin B12 levels were comparable to those in Wahlin *et al*.,[19] folate levels were substantially lower, possibly due to the nonfasting status of subjects in that study. In comparison with the only other report addressing plasma folate, vitamin B12, homocysteine and prostate cancer risk, we found somewhat lower folate and vitamin B12 levels and, as might be predicted by the vitamin levels, somewhat higher homocysteine levels.[4]

High consumption of meat, the major dietary source of vitamin B12 and a major component of the Western diet, has been linked to increased risk of prostate cancer in some studies.[2,21,22] Plasma levels of vitamin B12 might therefore be a marker of meat intake or some component of meat. In the large Framingham Offspring Study, however, meat intake was not a major contributor to vari-

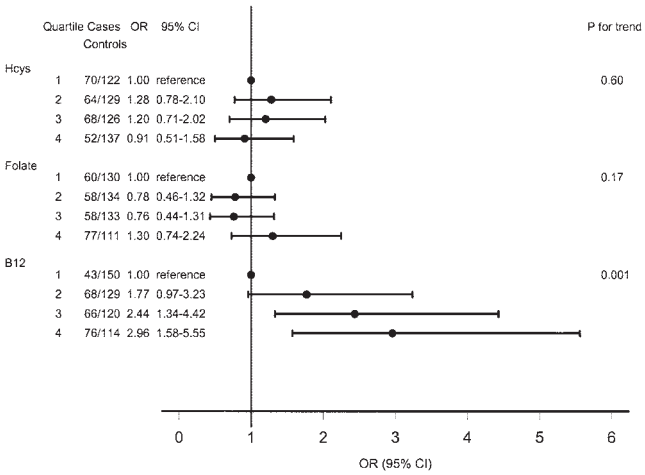

FIGURE 1 – Adjusted ORs and 95% CIs for prostate cancer by quartiles of folate, vitamin B12 and homocysteine. ORs and 95% CIs adjusted for the other 2 plasma variables, BMI and smoking. Smoking status categories were never smokers, ex-smokers and current smoker. BMI was divided into quartiles, and the lowest 3 quartiles, which yielded very similar ORs in univariate analysis, were combined. *p* for trend was determined by assigning quartiles their midpoint values and including them as a continuous variable in analysis by conditional logistic regression.

ability in plasma levels of vitamin B12, despite being the primary dietary source.[23] This observation, combined with the consistency of our results for folate, vitamin B12 and homocysteine, supports a biologic role for vitamin B12 in prostate cancer development in the present study without negating an involvement of vitamin B12 in the association between meat and risk.

For several cancer sites, and for colorectal cancer in particular, evidence has tended to support a protective effect for folate.[3,24] In contrast, our findings suggest a possible increased risk of prostate cancer at higher plasma folate and a strong increase in risk with increasing plasma vitamin B12 levels. Reports relating folate metabolism and prostate cancer development have been scarce. A recent prospective study of prostate cancer risk in smokers reported a null result for plasma folate, vitamin B12 and homocysteine,[4] while a case-control study based on dietary questionnaire data associated a higher vitamin B12 intake with increased risk.[25] Reports of a possible increased risk of prostate cancer in individuals homozygous for the MTHFR 677 C→T polymorphism seem to contradict our findings, but both were underpowered, having only 21 case subjects[5] or 132 case subjects with women constituting 84 of 150 control subjects.[6] Studies of the same polymorphism in other cancers have supported a protective effect in colon cancer (though not in combination with low folate or high alcohol intake)[7] and leukemia,[8] but a possible increased risk in esophageal and gastric cancer,[9–11] and mixed results in breast cancer.[12–15] Vitamin B12 has been implicated in genotoxicity in smokers,[26] and some preliminary evidence suggests a possible increased risk of lung cancer with increasing plasma vitamin B12 levels,[27,28] observations in line with our results.

Methylation of CpG islands in the promoter region of genes, which inactivates expression, may be especially pertinent in prostate cancer development.[2] Several genes, including the caretaker gene *glutathione S-transferase π* (*GSTP*1), *E-cadherin, endothelin B*, *insulin-like growth factor II* (*IGF-II*), certain steroid receptor genes and tumor suppressors *PTEN/MMAC1* and *CDKN2*, have been observed to be hypermethylated and inactivated in prostate cancer.[2,29] In mice, a methyl-supplemented diet (including vitamin B12) has been found to silence gene expression via increased CpG methylation.[30] One might therefore hypothesize that enhanced methyl group availability could also increase susceptibility gene-specific CpG methylation in the human prostate.

In conclusion, our results suggest that factors involved in maintaining an adequate folate status are not protective against prostate cancer, and that high plasma levels of folate and especially vitamin B12 may even have a detrimental effect. In fact, increasing plasma vitamin B12 was associated with an up to 3-fold increase in prostate cancer risk, which was independent of age at recruitment, follow-up time and disease state at diagnosis. These results are novel and should be explored further in future studies.

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