One-Carbon Metabolism and Prostate Cancer Risk: Prospective Investigation of Seven Circulating B Vitamins and Metabolites

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Abstract

Purpose: **Components of one-carbon metabolism are believed to influence cancer development with suggested mechanisms, including DNA methylation and DNA repair mechanisms. However, few prospective studies have investigated one-carbon metabolism in relation to prostate cancer risk, and the results have been conflicting. The aim of this study was to do a comprehensive investigation of the components of one-carbon metabolism in relation to prostate cancer risk. A panel of seven circulating B vitamins and related metabolites was selected, most of which have not been studied before.**

Materials and Methods: **We analyzed plasma concentrations of betaine, choline, cysteine, methionine, methylmalonic acid (MMA), vitamin B2, and vitamin B6 in 561 cases and 1,034 controls matched for age and recruitment date, nested within the population-based Northern Sweden Health and Disease Cohort. Relative risks of prostate cancer were estimated by conditional logistic regression.**

Results: **Positive associations with prostate cancer risk were observed for choline and vitamin B2, and an inverse association was observed for MMA. The relative risks for a doubling in concentrations were 1.46 [95% confidence interval (95% CI), 1.04-2.05; P_{trend} = *0.03*] for choline, 1.11 (95% CI, 1.00-1.23; P_{trend} = *0.04*) for vitamin B2, and 0.78 (95% CI, 0.63-0.97; P_{trend} = *0.03*) for MMA. Concentrations of betaine, cysteine, methionine, and vitamin B6 were not associated with prostate cancer risk.**

Conclusion: **The results of this large prospective study suggest that elevated plasma concentrations of choline and vitamin B2 may be associated with an increased risk of prostate cancer. These novel findings support a role of one-carbon metabolism in prostate cancer etiology and warrant further investigation.** (Cancer Epidemiol Biomarkers Prev 2009;18(5):1538–43)

Introduction

One-carbon metabolism may play a role in cancer development (1). However, studies relating factors of one-carbon metabolism to prostate cancer risk are few, the results are inconsistent, and several B vitamins and metabolites have never been studied in a prospective setting before (1).

The pathways of one-carbon metabolism are illustrated in Fig. 1. Several components, including folate, vitamin B12, choline, betaine, methionine, cysteine, vitamin B6, and vitamin B2, are directly influenced by dietary intake.

The two primary mechanisms by which one-carbon metabolism has been proposed to influence cancer development are methylation and nucleotide synthesis (1). Genomic hypomethylation and gene-specific, CpG island promoter hypermethylation, causing silencing of gene expression, are often observed concurrently in tumor tissue (2). Promoter hypermethylation of the glutathione *S*-transferase π gene, which occurs in >90% of prostate cancer cases, is the most frequently reported epigenetic change in prostate cancer, suggesting that DNA hypermethylation may be particularly important in prostate carcinogenesis (3). However, although methyl availability seems to be a determinant of global DNA methylation (4, 5), evidence for an effect on gene-specific promoter methylation is sparse (6). Adequate methyl availability may also have a protective role in cancer development by ensuring sufficient nucleotide synthesis, in particular, that of thymidylate from uracil. Excessive uracil misincorporated into DNA can lead to double-strand breaks during the DNA repair process, possibly contributing to tumor initiation (7).

A dual role for one-carbon metabolism in cancer development has been suggested, by which factors increasing methyl group availability might prevent tumor initiation in healthy tissue but promote the progression of preneoplastic or neoplastic lesions (1). Such a dual role may be particularly important in prostate cancer given the high prevalence of subclinical prostate cancer in western populations (8).

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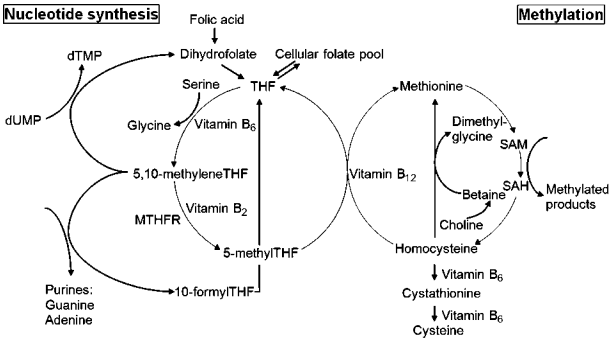

Figure 1. Diagram of the one-carbon metabolism pathway. Labile methyl groups are supplied by dietary serine, choline (via betaine), and methionine. One-carbon units are derived from serine through the activity of the vitamin B6–dependent serine hydroxymethyltransferase, which generates 5,10-methylenetetrahydrofolate. 5,10-Methylenetetrahydrofolate, in turn, is then reduced to 5-methyltetrahydrofolate, the predominant form of folate in the circulation, in an irreversible reaction catalyzed by vitamin B2–dependent methylenetetrahydrofolate reductase (33). 5-Methyltetrahydrofuran serves as a methyl donor in a reaction converting homocysteine to methionine, in which vitamin B12 serves a cofactor. Homocysteine can also be metabolized to cysteine through the sequential action of two vitamin B6–dependent enzymes [i.e., the transsulfuration pathway. The methionine derivative, S-adenosylmethionine, is the universal methyl donor for the methylation of a vast variety of molecules, including DNA (34)].

Previous prospective studies investigating one-carbon metabolism in relation to prostate cancer have primarily focused on folate and vitamin B12. Results to date suggest a positive or null association for vitamin B12 and a null association for folate (9-12). A null association for serum vitamin B6 concentrations and an inverse association for vitamin B6 intake have also been reported in relation to prostate cancer in a Finnish population (9, 10). In the same study population, a null or weakly positive association for methionine intake in relation to prostate cancer was reported (10). Circulating betaine, choline, cysteine, methionine, and vitamin B2 have, to our knowledge, never been studied in relation to prostate cancer risk.

The aim of this nested case-control study from the Northern Sweden Health and Disease Cohort (NSHDC) was to investigate plasma concentrations of betaine, choline, cysteine, methylmalonic acid (MMA), methionine, vitamin B2, and vitamin B6 in relation to prostate cancer risk.

Materials and Methods

Study Cohort. The NSHDC is an ongoing population-based study described in detail elsewhere (13). The NSHDC includes three cohorts, but only the largest of these, the Västerbotten Intervention Project, is included in the present study. In the Västerbotten Intervention Project, initiated in 1985, all residents of Västerbotten County are invited to a health survey at the age of 40, 50, and 60 y. As of 2006, a total of 36,344 men had been recruited to the cohort. The health examination includes measurement of weight, height, and blood pressure followed by a blood draw, an oral glucose tolerance test, and the completion of a self-administered lifestyle questionnaire (13, 14). The blood sample is separated into plasma, buffy coat, and erythrocyte fractions, and aliquoted and cryopreserved at -80°C.

All participants signed an informed consent form at the time of recruitment, and the study was approved by the Research Ethics Committee of Umeå University Hospital.

Follow Up and Selection of Case and Control Subjects. Incident cases of prostate cancer were identified through linkage with the Cancer Register of Northern Sweden. In January 2006, 641 cases were identified within the cohort. Plasma samples from 561 cases were available for this study. For each case, two male controls (1.9 on average) were selected at random from matched sets consisting of all cohort members alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the index case. The matching criteria were age at enrolment (±6 mo) and date of blood draw (±3 mo).

Data on tumor characteristics were obtained from the Northern Sweden section of the National Prostate Cancer Register, including date of diagnosis, local tumor stage, lymph node stage, metastasis at bone scan, tumor differentiation assessed by Gleason score, and serum prostate-specific antigen level at time of diagnosis (15). Before 2000, tumor differentiation was predominantly assessed in Sweden according to the WHO system. For cases that were diagnosed before 2000, biopsies were reanalyzed by a single pathologist and graded according to Gleason (16). In addition, the fraction of total core length occupied by cancer was assessed. We classified a case as high risk (i.e., prone to progression) if one or more of the following criteria were fulfilled: locally advanced stage (T_3 or T_4), lymph node metastasis (N_1), metastasis on bone scan (M_1), Gleason score ≥8, or a serum prostate-specific antigen level >50 ng/mL at the time of diagnosis. Low-risk cases were defined as the absence of all of these factors.

Biochemical Analyses. All biochemical analyses were done at Bevital AS (www.bevital.no). The study included measurements of plasma concentration of choline, betaine, methionine, cysteine, vitamin B6, and vitamin B2. We also included MMA, an inverse marker of vitamin B12 status.

Methionine was determined as the sum of methionine and methionine sulfoxide. Concentrations of methionine, cysteine, and MMA were determined with the use of an isotope dilution gas chromatography-mass spectrometry method (17). Concentrations of methionine sulfoxide, choline, and betaine were determined by liquid chromatography-tandem mass spectroscopy (18). Vitamins B6 and B2 were also determined by liquid chromatography-tandem mass spectroscopy (19). The coefficients of variations within run and between run at median concentrations of the controls were, respectively, 0.9% and 2.6% for methionine, 0.9% and 2.6% for cysteine, 1.9% and 2.6% for MMA, 2.1% and 3.5% for choline, 4.3% and 4.1% for betaine, 5.7% and 7.4% for vitamin B6 (pyridoxal phosphate), and 5.4% and 7.8% for vitamin B2 (riboflavin).

Samples from cases and their matched controls were analyzed together and positioned randomly within stratum triplets. All assays were done by laboratory personnel who were blinded to the case-control status of the blood samples.

Statistical Analyses. We used Wilcoxon signed rank tests to investigate the baseline differences between cases and controls for continuous variables and χ^2 tests for discrete variables.

Plasma concentrations of all analytes were categorized into quartiles with cut points based on the concentration distribution in the control subjects. Odds ratios for plasma concentrations were calculated as estimates of relative risks (RR) for prostate cancer with the use of conditional logistic regression models. The overall statistical significance for each analyte was assessed with the use of the RR trend estimate calculated by including the base 2 logarithm of the analyte concentrations in a separate conditional logistic regression model, thus achieving a trend RR for a doubling in concentration. The effects of potential confounders were examined by including dummy variables for additional covariates in the logistic regression models [i.e., smoking (never, past, current), alcohol intake (<8, 8-15, 16-39, and >40g/d), body mass index (BMI; kg/m^2; in quartiles), physical activity (index of combined recreational, household, and occupational physical activity: inactive, moderately inactive, active), marital status (married/cohabiting, not married/cohabiting), education level (primary school or none, secondary school or equivalent, university degree)]. We also considered plasma concentrations of folate (nmol/L; in quartiles) and vitamin B12 (pmol/L; in quartiles) as potential confounders because of their importance in the one-carbon metabolism pathway (1) and because of their previous implications in prostate cancer (9-12). For each of these variables, a small proportion of values were unknown, and these were included in the analyses as separate categories. For a covariate to be considered a confounder, we used the criterion of a change in variable estimate of >10% in adjusted analysis.

χ^2 tests were used to examine heterogeneity in RRs for prostate cancer between subgroups according to selected tumor and baseline characteristics, as well as plasma concentrations of folate and vitamin B12. Subgroups were defined according to T stage (T_1 or T_2 versus T_3 or T_4), lymph none metastasis (N_0 versus N_+), distant metastasis (M_0 versus M_+), Gleason score (Gleason <7 versus 7 versus >7), low-risk versus high-risk cancers (see Follow Up and Selection of Case and Control Subjects), BMI (BMI <25.6 versus BMI ≥25.6), age at recruitment (age <55 y versus age ≥55 y), smoking status (never versus ever versus current), plasma folate (folate <5.6 nmol/L versus ≥5.6 nmol/L), and plasma vitamin B12 (B12 <333 pmol/L versus B12 ≥333 pmol/L). All P values presented are two sided, and P values <0.05 were considered statistically significant. All statistical analyses were done with the use of the Statistical Analysis System software (SAS Institute).

Results

Baseline Characteristics. The baseline characteristics of cases and controls are shown in Table 1. The median age at blood collection was 59.8 years. Overall, we observed no major differences in baseline characteristics between cases and control, but cases were more likely to smoke ($P = 0.03$). The tumor characteristics are shown in Table 2. The median age at diagnosis was 64.6 years, and the median time from blood draw to diagnosis was 6.9 years. The baseline concentrations of all analyzed B vitamins and metabolites for cases and controls are shown in Table 3. The only analyte displaying a significant difference in baseline concentrations between cases and controls was MMA ($P = 0.002$), with mean concentrations of 0.161 and 0.195 μmol/L in cases and controls, respectively.

B Vitamins and Metabolites in Relation to Prostate Cancer Risk. The RRs of prostate cancer for quartiles of B vitamin and metabolite concentrations are shown in Table 4. Elevated concentrations of choline ($P_{trend} = 0.03$) and vitamin B2 ($P_{trend} = 0.04$) were associated with increased risk, whereas elevated concentrations of MMA was associated with decreased risk ($P_{trend} = 0.03$). RRs associated with a doubling in concentrations were 1.46 [95% confidence interval (95% CI), 1.04-2.05] for choline, 1.11 (95% CI, 1.00-1.23) for vitamin B2, and 0.78 (95% CI, 0.63-0.97) for MMA. The concentrations of betaine, methionine, vitamin B6, and cysteine were not associated with prostate cancer risk.

In logistic regression adjusted for vitamin B12, the association between MMA and prostate cancer risk was attenuated and not statistically significant ($RR_{trend} = 0.80$; 95% CI, 0.64-1.01; $P_{trend} = 0.06$). When adjusting for folate, the associations between choline and vitamin B2, and prostate cancer risk were also attenuated and not statistically significant (choline: $RR_{trend} = 1.36$; 95% CI, 0.96-1.93; $P_{trend} = 0.08$ and vitamin B2: $RR_{trend} = 1.09$; 95% CI, 0.98-1.21; $P_{trend} = 0.10$). Adjusting for other potential confounders had essentially no effect on the RR estimates (data not shown).

Investigating heterogeneity in RRs for subgroups defined by tumor characteristics revealed that the overall inverse association between MMA and prostate cancer risk was attributable to high-risk cases ($P_{heterogeneity} = 0.01$). The MMA RR trend estimates were 0.42 (95% CI, 0.25-0.72; $P_{trend} = 0.002$) for high-risk cases and 0.91 (95% CI, 0.72-1.16; $P_{trend} = 0.45$) for low-risk cases. In the subgroup of subjects >55 years at recruitment, both betaine and choline displayed significant associations with prostate cancer risk (betaine: $RR_{trend} = 1.46$; 95% CI, 1.05-2.03; $P_{trend} = 0.03$ and choline: $RR_{trend} = 1.69$; 95% CI, 1.14-2.51; $P_{trend} = 0.009$), whereas these relations were close to null in subjects <55 years at recruitment (betaine: $RR_{trend} = 0.93$; 95% CI, 0.53-1.63; $P_{trend} = 0.80$; $P_{heterogeneity} = 0.18$ and choline: $RR_{trend} = 0.96$; 95% CI, 0.48-1.90; $P_{trend} = 0.90$; $P_{heterogeneity} = 0.16$). In contrast, cysteine displayed a significant association in subjects <55 years at recruitment ($RR_{trend} = 0.25$; 95% CI, 0.07-0.93; $P_{trend} = 0.04$) but not in subjects >55 years at recruitment ($RR_{trend} = 0.97$; 95% CI, 0.40-2.33; $P_{trend} = 0.95$; $P_{heterogeneity} = 0.09$). When stratifying on vitamin B12 concentrations, the increased risk associated with elevated choline was primarily attributable to subjects with vitamin B12 concentrations below 333 pmol/L ($P_{heterogeneity} = 0.04$). The choline RR trend estimates were 2.04 (95% CI, 1.07-3.88; $P_{trend} = 0.03$) and 0.81 (95% CI, 0.44-1.50; $P_{trend} = 0.51$) for subjects with low and high vitamin B12 concentrations, respectively. No other analytes displayed heterogeneous associations when

Table 1. Baseline characteristics stratified by case-control status

Continuous variables		Cases	Controls	P*
Age at blood draw (y), median (5th-95th percentile)		59.8 (49.6-60.5)	59.8 (49.7-60.4)	0.37
BMI (kg/m^2), median (5th-95th percentile)		25.9 (21.6-31.3)	25.8 (21.4-32.5)	0.2
Height (cm), median (5th-95th percentile)		176 (167-190)	176 (166-186)	0.56
Discrete variables				P† test indicating the difference in frequencies between cases and controls.
Educational attainment, *n* (%)	Primary school	408 (75%)	805 (77%)	0.46
	Secondary school	65 (12%)	104 (10%)	
	University degree	73 (13%)	138 (13%)	
Marital status, *n* (%)	Married or cohabiting	489 (87%)	896 (84%)	0.09
	Not married nor cohabiting	70 (13%)	166 (16%)	
Smoking, *n* (%)	Never	242 (45%)	533 (52%)	0.03
	Ever	141 (26%)	236 (23%)	
	Current	153 (29%)	254 (25%)	
Physical activity, *n* (%)	Inactive	99 (21%)	200 (21%)	0.97
	Moderately inactive	208 (43%)	405 (43%)	
	Active	174 (36%)	344 (36%)	
Alcohol intake, *n* (%)	<8 g/d	249 (70%)	462 (70%)	0.44
	8-15 g/d	86 (24%)	147 (22%)	
	15-39 g/d	19 (5%)	52 (8%)	
	> 40 g/d	1 (0%)	1 (0%)	

*P values assessed by Wilcoxon signed rank test indicating the difference in characteristics between cases and controls.
†P values assessed by χ.

stratifying on tumor characteristics, baseline characteristics, or plasma concentrations of folate or vitamin B12.

Discussion

In this nested prostate cancer case-control study, we analyzed a panel of seven B vitamins and metabolites, most of which had never been studied in a prospective setting before. The main findings were novel associations between elevated concentrations of choline and vitamin B2, and increased prostate cancer risk.

Choline (via betaine) is an important methyl donor when folate levels are low (20-22). If methyl group availability is involved in tumor initiation and/or progression, then choline might be a stronger determinant in

Table 2. Tumor characteristics

Continuous variables, median (5th-95th percentile)		
Age at diagnosis (y)	64.6 (54.6-71.2)	
Time between blood draw and diagnosis (y)	6.9 (1.0-13.7)	
PSA at diagnosis (ng/mL)	11 (4-145)	
PSA at blood draw (ng/mL)	3.6 (1.1-20.6)	
Fraction of malign tissue in biopsy	7.0% (0%-60%)	
Discrete variables, *n* (%)		
Mode of diagnosis	Health checkup	125 (23%)
	Symptoms, other reasons	411 (77%)
	Missing	28
Stage	T_1	284 (52%)
	T_2	187 (34%)
	T_3	66 (12%)
	T_4	6 (1%)
	T_X	25
Lymph node metastasis	N_0	169 (92%)
	N_1	14 (4%)
	N_X	395
Distant metastasis	M_0	328 (88%)
	M_1	43 (12%)
	M_X	197
Gleason score	<7	307 (63%)
	7	135 (28%)
	>7	43 (9%)
	Missing	83
High-risk/low-risk cancer*	Low risk	436 (77%)
	High risk	132 (23%)

*High-risk cancer defined as T_3 or T_4, lymph node metastasis, distant metastasis, Gleason score ≥8, or PSA at diagnosis >50 ng/mL; low-risk cases defined as the absence of all of these factors.

Table 3. Baseline concentrations of B vitamins and metabolites stratified by case-control status

Analyte	Cases median (5th-95th percentile)	Controls median (5th-95th percentile)	*P**
Betaine (μmol/L)	32.7 (19.8-49.1)	31.4 (19.5-49.0)	0.18
Choline (μmol/L)	10.4 (6.7-14.7)	10.0 (6.8-14.4)	0.07
Cysteine (μmol/L)	277.5 (230.9-327.0)	278.6 (232.6-328.7)	0.16
MMA (μmol/L)	0.150 (0.095-0.269)	0.150 (0.104-0.274)	0.002
Methionine (μmol/L)	27.4 (21.0-35.1)	27.3 (21.1-35.8)	0.33
Vitamin B2 (Riboflavin; nmol/L)	11.3 (4.4-40.5)	10.3 (4.3-36.6)	0.82
Vitamin B6 (pyridoxal phosphate) (nmol/L)	36.5 (17.9-84.9)	36.4 (18.2-84.8)	0.13

*P values assessed by Wilcoxon signed rank test indicating the difference in concentrations between cases and controls.

low-folate populations, such as in northern Sweden. The similar, though nonstatistically significant, RR relationship for betaine supports this interpretation, whereas the null association for methionine does not. The comparable associations for choline and betaine also render the possibility of confounding by other dietary components unlikely because dietary choline is obtained primarily from animal sources, whereas betaine is largely derived from plant sources (23).

In addition to its role in one-carbon metabolism, choline is a precursor for cell membrane phospholipids and is thus required in increased amounts in proliferating cells, including cancer cells. A high dietary choline intake has also been related to reduction in markers of inflammation (24). Not surprisingly, given the complex biology of choline metabolism, epidemiologic studies of this pathway in other types of cancer have produced inconsistent results (25-27). Further research is warranted to elucidate the role of choline metabolism in prostate tumorigenesis.

Vitamin B2, acting as a cofactor in the methylenetetrahydrofolate reductase reaction, also contributes to methyl group availability, but prospective studies of vitamin B2 in cancer have produced equivocal results (28-30). The increased risk for subjects with higher vitamin B2 concentrations in the present study is in line with the results for choline and betaine and supports a role for methyl availability in prostate cancer development. As noted above, however, the lack of association between methionine and risk seems to contradict the methylation hypothesis in prostate cancer etiology. Null or weakly positive associations with prostate cancer risk have previously been reported for methionine (10, 31).

We observed null associations for circulating concentrations of cysteine and vitamin B6 in relation to prostate cancer risk. Previous studies of vitamin B6 and prostate cancer risk have yielded null or inverse associations (9, 10, 31, 32). To our knowledge, the role of cysteine in prostate cancer has not previously been studied in an epidemiologic study.

Previous prospective investigations of vitamin B12 in prostate cancer suggest a positive or null association with risk (9-12). Elevated plasma concentrations of MMA, an inverse marker of functional vitamin B12 status, were associated with a reduced risk of prostate cancer in the present study. Two of the previous studies of vitamin B12 were from the NSHDC (9, 11, 12), from which the study groups were combined for the present investigation. Parallel to the findings for vitamin B12, the association between MMA and prostate cancer risk in the present report was limited to the subjects from the first study period (diagnosed in 1985-2002; data not shown; refs. 11, 12). However, adjusting for vitamin B12 in the present study attenuated the risk estimate for MMA by only 11%, which is somewhat surprising given the strong correlation between the two analytes, but the association for MMA was no longer statistically significant.

The main strengths of this study include the extensive case characteristics acquired from the National Prostate Cancer Registry as well as the prospective design with two individually matched controls minimizing bias and reverse causality. Given that prostate cancer is a slowly developing disease with neoplastic transformations occurring many years before diagnosis (3), longer follow-up times and multiple plasma samples from each study subject would have been desirable. One particular feature of the present study population was the low concentrations of folate compared with other European populations (12). This provides a unique opportunity to investigate the role of one-carbon metabolism in relation

Table 4. RRs for quartiles of plasma concentrations of B vitamins and metabolites

Analyte	Cases/controls	RR*†			*P*‡
		2nd quartile	3rd quartile	4th quartile (high)	
Betaine	522/959	1.16 (0.84-1.61)	1.31 (0.94-1.81)	1.36 (0.98-1.88)	0.08
Choline	522/959	0.91 (0.65-1.28)	1.05 (0.74-1.47)	1.48 (1.07-2.04)	0.03
Cysteine	551/1035	0.84 (0.63-1.13)	0.91 (0.68-1.23)	0.73 (0.53-1.00)	0.20
MMA	548/1025	0.68 (0.50-0.92)	0.88 (0.66-1.18)	0.81 (0.60-1.09)	0.03
Methionine	551/1035	0.98 (0.73-1.31)	1.15 (0.85-1.55)	0.94 (0.69-1.29)	0.63
Vitamin B2	543/1014	1.06 (0.78-1.43)	1.03 (0.76-1.40)	1.36 (1.02-1.83)	0.04
Vitamin B6	543/1014	0.99 (0.74-1.33)	1.07 (0.80-1.43)	0.91 (0.67-1.23)	0.94

*Unadjusted RRs relative the 1st quintile. Adjusting for potential confounders had essentially no effect on the RR estimates (data not shown).
†Odds ratios were calculated as estimates of RRs with the use of conditional logistic regression.
‡*P* values were calculated by replacing the categorical quartile variables with the base 2 logarithm of the observed concentration in the logistic regression model.

to cancer in a study population of low folate status in which no mandatory folate fortification has been undertaken. However, the low folate concentrations must also be taken into consideration before extrapolating our findings to populations of higher folate status.

In conclusion, the results of this large, nested case-control study suggest that elevated concentrations of circulating choline and vitamin B2 may be associated with an increased risk of prostate cancer. These novel findings support a role of one-carbon metabolism in prostate cancer etiology and warrant investigation in further studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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