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Sarcosine and other metabolites along the choline oxidation pathway in relation to prostate cancer—A large nested case–control study within the JANUS cohort in Norway

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Methyl group donors and intermediates of one-carbon metabolism affect DNA synthesis and DNA methylation, and may thereby affect prostate carcinogenesis. Choline, the precursor of betaine, and the one-carbon metabolite sarcosine have been associated with increased prostate cancer risk. Within JANUS, a prospective cohort in Norway ($n = 317{,}000$) with baseline serum samples, we conducted a nested case–control study among 3,000 prostate cancer cases and 3,000 controls. Using conditional logistic regression, odds ratios (ORs) and 95% confidence intervals (CIs) for prostate cancer risk were estimated according to quintiles of circulating betaine, dimethylglycine (DMG), sarcosine, glycine and serine. High sarcosine and glycine concentrations were associated with reduced prostate cancer risk of borderline significance (sarcosine: highest *vs.* lowest quintile OR = 0.86, CI = 0.72–1.01, $p_{trend} = 0.03$; glycine: OR = 0.83, CI = 0.70–1.00, $p_{trend} = 0.07$). Serum betaine, DMG and serine were not associated with prostate cancer risk. However, individuals with a high glycine/serine ratio were at decreased prostate cancer risk (OR = 0.74, CI = 0.69–0.85, $p_{trend} < 0.001$). This population-based study suggested that men with high serum sarcosine or glycine concentrations have modestly reduced prostate cancer risk. Ratios of metabolites reflecting one-carbon balance may be associated with prostate cancer risk, as demonstrated for the glycine/serine ratio, and should be explored in future studies.

The folate-mediated one-carbon metabolism, which involves B-vitamins as enzymatic cofactors, is characterized by the transfer of methyl groups and may affect carcinogenesis by influencing methylation and synthesis of DNA.[1] Betaine and its precursor choline may act as methyl group sources,[2] whereas intermediates involved in the transfer of one-carbon units may be hypothesized to reflect bioavailability of methyl groups.[3] These factors may therefore affect the development and growth of cancer and should be investigated when studying the role of one-carbon metabolism in carcinogenesis. Interestingly, a high *S*-adenosylmethionine/*S*-adenosylhomocysteine ratio, which facilitates transmethylation reactions, has been associated with carcinogenesis.[4] Similarly, it may be hypothesized that ratios of one-carbon metabolites, which might reflect one-carbon balance, are related to carcinogenesis.

Prostate cancer is the second most frequently diagnosed cancer among men globally.[5] Results from observational studies suggest that high concentrations of circulating folate or high vitamin B12 status are associated with increased prostate cancer risk, although reported associations were modest and sometimes inconsistent.[6–8] Although increased prostate cancer risk was suggested in one trial among subjects receiving folic acid supplements,[9] recent meta-analyses did not suggest an association with increased prostate cancer risk.[10,11] Nevertheless, an observational population-based study previously showed

Key words: sarcosine, choline oxidation pathway, prostate cancer risk, population-based, case-control study

Abbreviations: BHMT: betaine-homocysteine *S*-methyltransferase; BMI: body mass index; CI: confidence interval; DD: dimethylglycine dehydrogenase; DMG: dimethylglycine; GAM: generalized additive models; GNMT: glycine *N*-methyltransferase; MTHFR: methylenetetrahydrofolate reductase; OR: odds ratio; pABG: *p*-aminobenzoylglutamate; SD: sarcosine dehydrogenase; SHMT: serine hydroxymethyltransferase.

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What's new?

The one-carbon metabolic pathway plays a role in carcinogenesis, possibly through involvement with DNA methylation. Circulating choline and vitamin B2 may up the risk of prostate cancer, according to some studies. The urine concentration of sarcosine, a product of choline oxidation, has also been associated with progression of prostate cancer, although subsequent analyses suggest that urine detection may not be good predictor of disease aggressiveness. This study compared serum concentration of sarcosine and various other metabolites with prostate cancer risk. They found that high sarcosine and glycine concentration correlate with modestly reduced risk of prostate cancer, in constrast with previous reports.

positive associations of prostate cancer risk with circulating choline and vitamin B2, and a nonsignificant positive association with plasma betaine concentration.[12] These observations may support the hypothesis that high methyl group bioavailability plays a role in cancer development.

Sarcosine in urine, as an intermediate along the choline oxidation pathway (Fig. 1), has been associated with prostate cancer progression in a metabolomics screening study.[13] However, subsequent validation suggested urinary sarcosine levels to have inadequate diagnostic power for detection of prostate cancer or as predictor for disease aggressiveness or disease progression.[14–17] Nevertheless, although based on relatively small and selected study populations, observations in these validation studies suggested a potential role of the choline metabolite sarcosine in prostate cancer development.

To further investigate the possible role of metabolites along the choline oxidation pathway, including sarcosine, in the etiology of prostate cancer, we conducted a large-scale population-based case–control study of 3,000 prostate cancer cases and 3,000 matched controls nested within the JANUS serum bank, a Norwegian prospective cohort of 317,000 individuals with baseline serum samples. We comprehensively investigated associations of prostate cancer risk with serum concentrations of sarcosine and related metabolites, and with ratios of metabolites that are substrate–product pairs of enzymes involved in the metabolism of choline to serine.

Subjects and 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 blood donors in Norway between 1973 and 2004.[18] Cancer incidence data were obtained from The Cancer Registry of Norway, with follow-up available until June 2007. The Regional Committee for Medical Research Ethics Review and the Data Inspectorate 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 ($n = 3{,}000$) were matched to male controls ($n = 3{,}000$) by age (± 6 months) and 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 nonmelanoma skin cancer.

Sample handling, biochemical analyses and genotyping

Biochemical and genotyping analyses were conducted at BEVITAL AS, Bergen, Norway.[19] Serum samples were stored at -25°C. Routines for sample collection and processing have been described previously.[20] Serum betaine, dimethylglycine (DMG) and creatinine were analyzed by liquid chromatography–mass spectrometry.[19] Sarcosine, glycine and serine concentrations were analyzed by gas chromatography–mass spectrometry.[19,21] To correct for degradation during sample storage, folate concentration was measured as

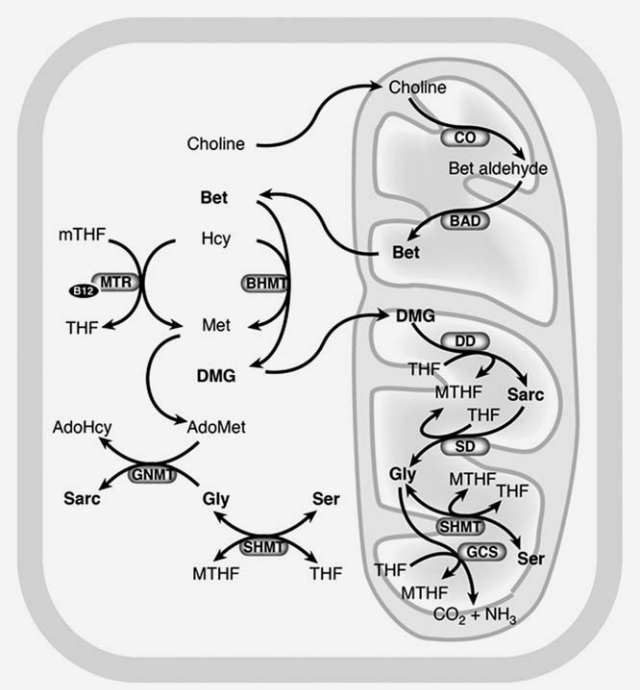

Figure 1. Choline oxidation pathway and its relation to sarcosine metabolism. The scheme encompasses reactions known to take place in the mammalian liver. Mitochondrial reactions are depicted at the right-hand side of the figure. AdoHcy: *S*-adenosylhomocysteine; AdoMet: *S*-adenosylmethionine; BAD: betaine aldehyde dehydrogenase; Bet: betaine; BHMT: betaine-homocysteine *S*-methyltransferase; CO: choline oxidase; DD: dimethylglycine dehydrogenase; DMG: dimethylglycine; GCS: glycine cleavage system; Gly: glycine; GNMT: glycine *N*-methyltransferase; Hcy: homocysteine; Met: methionine; mTHF: 5-methyltetrahydrofolate; MTHF: methylenetetrahydroflate; MTR: methionine synthase; Sarc: sarcosine (monomethylglycine); SD: sarcosine dehydrogenase; Ser: serine; SHMT: serine hydroxymethyltransferase; THF: tetrahydrofolate.

Epidemiology

p-aminobenzoylglutamate (pABG) equivalents.[22] pABG and the above mentioned biochemical markers were stable during sample storage,[23,24] except serine concentration which slightly increased over time.[24]

Methylene-tetrahydrofolate reductase (*MTHFR*) 677C→T (rs1801133) genotypes were determined using matrix-assisted laser-desorption/ionization-time-of-flight mass spectrometry.[25] Because of insufficient amounts of DNA, genotyping could not be performed in 770 (13.2%) of the samples.

Potential confounders

Smoking behavior, physical activity, body mass index (BMI) and educational level were considered as potential confounders. Our study exclusively included cohort members with available health survey data. Information on smoking habits (never, former, current), physical activity (sedentary, $\geq$4 hr/week moderate, frequent vigorous) and BMI ($<$25, 25–$<$30, $\geq$30 kg/m^2) was available 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

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 and differences assessed nonparametrically by Kruskal–Wallis tests.

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 (ORs) and 95% confidence intervals (CIs) 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. Generalized additive models (GAMs) were used to explore potential nonlinear dose-response relations. In these models, the top and bottom 1 percentile of the distributions of exposure variables were excluded. The continuous variables were then log-transformed and results are presented after back-transformation.

In addition to individual serum concentrations and prostate cancer risk, we modeled ratios between biomarkers that are substrate–product pairs of enzymes involved in the choline oxidation pathway. Such ratios may reflect activities of the enzymes involved. In this respect, we calculated ratios of betaine/DMG to reflect enzymatic flux through the betaine-homocysteine *S*-methyltransferase (BHMT) reaction, DMG/sarcosine [DMG dehydrogenase (DD) reaction], sarcosine/glycine [sarcosine dehydrogenase (SD) and glycine *N*-methyltransferase (GNMT) reactions] and of glycine/serine [serine hydroxymethyltransferase (SHMT) reaction] (Fig. 1).

Crude associations are 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, all models included serum creatinine as a marker of renal function.

To examine whether associations between the serum markers and cancer risk were modified by folate status, the analyses were stratified according to folate concentration (*i.e.*, below and above the median folate concentration among controls of 13.7 nmol/L), and tested for interaction by including a product term of the serum marker variable with the folate status variable. In addition, MTHFR is a key enzyme in one-carbon metabolism, and the 677C$>$T polymorphism affects the distribution of folate species.[26] Therefore, analyses were also stratified by *MTHFR* 677C→T genotypes. In these stratified analyses, unconditional logistic regression was performed in which the matching variables were used as covariates.

Statistical analyses were performed using SAS (version 9.2) and R (version 2.14.1).

Results

Characteristics of the study population

Mean age of prostate cancer cases and matched controls was 49.1 (standard deviation 8.7) years at inclusion, with mean time to diagnosis being 15.6 (5.1) years (Table 1). On average, samples were stored for 24.2 (3.3) years until biochemical analyses were conducted. High education and BMI below 25 were more common among cases than controls, whereas smoking behavior and physical activity levels were similar for cases and controls. Glycine concentrations were somewhat higher among controls compared to cases, whereas betaine, DMG, sarcosine and serine concentrations were similar.

Prostate cancer risk

Logistic regression analyses adjusted for serum creatinine, highest education, smoking habits, physical activity and BMI revealed an inverse trend between sarcosine concentration and prostate cancer risk (highest *vs.* lowest quintile OR = 0.86, CI = 0.72–1.01, $p_{trend} = 0.03$; Table 2). In addition, high glycine concentration was borderline-significantly associated with reduced prostate cancer risk (OR = 0.83, CI = 0.70–1.00, $p_{trend} = 0.07$). Serum betaine, DMG and serine were not associated with prostate cancer risk. Unadjusted analyses showed risk estimates comparable to the multivariate-adjusted models. GAM analyses (Fig. 2) revealed similar associations compared to those based on quintiles of exposure estimated by logistic regression analyses.

Metabolite ratios

Individuals with a high glycine/serine ratio were at markedly decreased prostate cancer risk (OR = 0.74, CI = 0.69–0.85, $p_{trend} < 0.001$; Table 3). However, the betaine/DMG, DMG/sarcosine and sarcosine/glycine ratios were not associated with prostate cancer risk.

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

	Prostate cancer cases	Matched controls	*p*-value[1]
N	3,000	3,000	
Matching criteria and patient characteristics [mean (SD)]			
Age at blood sampling (years)	49.1 (8.7)	49.1 (8.7)	
Serum sample storage time (years)	24.2 (3.3)	24.2 (3.3)	
Age at prostate cancer diagnosis (years)	64.8 (8.0)		
Time between blood sampling and diagnosis (years)	15.6 (5.1)		
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 $\geq$4 hr/week	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)	
$\geq$30	233 (7.8)	257 (8.6)	0.05
No information	26	18	
Serum concentrations [median (p5–p95)]			
Betaine (μmol/L)	43.50 (29.9–63.2)	43.30 (30.1–61.40)	0.59
DMG (μmol/L)	4.98 (3.14–8.85)	5.02 (3.12–8.88)	0.35
Sarcosine (nmol/L)	1.81 (1.09–3.24)	1.83 (1.10–3.23)	0.12
Glycine (μmol/L)	339.9 (269.3–443.1)	343.1 (270.5–449.2)	0.06
Serine (μmol/L)	216.6 (172.2–277.6)	217.0 (170.6–282.3)	0.97
Folate as *p*-aminobenzoylglutamate (nmol/L)[22]	13.98 (8.67– 23.60)	13.70 (8.65–23.40)	0.08
Creatinine (μmol/L)	77.70 (61.00–101.00)	77.60 (60.50–101.00)	0.59
***MTHFR 677C>T* genotypes (*n*, %)**			
CC	1407 (55.8)	1334 (51.2)	
CT	820 (32.5)	929 (35.6)	
TT	295 (11.7)	344 (13.2)	0.004
No data available	478	393	

[1] *p*-value for differences between cases and controls. Chi-square test for categorical variables, Kruskal–Wallis test for continuous variables.
Abbreviation: MTHFR: methylene tetrahydrofolate reductase.

Table 2. Conditional logistic regression analyses with corresponding odds ratios and 95% confidence intervals for prostate cancer, according to quintiles of serum concentrations

Serum concentration	Quintiles (range)[1]	Number of cases/controls	Crude analyses[2]	Adjusted analyses[3]
Betaine (μmol/L)	1 (<36.1)	565/587	1.00	1.00
	2 (36.1–<41.1)	590/592	1.05 (0.90–1.24)	1.04 (0.88–1.22)
	3 (41.1–<45.6)	584/583	1.05 (0.89–1.24)	1.02 (0.87–1.20)
	4 (45.6–<52.0)	624/594	1.09 (0.93–1.29)	1.05 (0.89–1.24)
	5 (≥52.0)	578/595	1.03 (0.87–1.22)	0.98 (0.83–1.16)
	p-trend		0.67	0.81
DMG (μmol/L)	1 (<3.94)	606/590	1.00	1.00
	2 (3.94–<4.63)	550/585	0.92 (0.78–1.09)	0.94 (0.80–1.11)
	3 (4.63–<5.43)	676/592	1.10 (0.93–1.29)	1.12 (0.95–1.32)
	4 (5.43–<6.48)	536/590	0.88 (0.75–1.05)	0.90 (0.76–1.07)
	5 (≥6.48)	573/594	0.93 (0.78–1.10)	0.95 (0.79–1.13)
	p-trend		0.29	0.40
Sarcosine (nmol/L)	1 (<1.40)	602/586	1.00	1.00
	2 (1.40–<1.69)	638/605	1.00 (0.84–1.17)	1.00 (0.85–1.18)
	3 (1.69–<1.98)	614/595	1.00 (0.85–1.18)	0.99 (0.84–1.17)
	4 (1.98–<2.40)	578/598	0.92 (0.78–1.08)	0.91 (0.77–1.08)
	5 (≥2.40)	555/603	0.86 (0.73–1.02)	0.86 (0.72–1.01)
	p-trend		0.03	0.03
Glycine (μmol/L)	1 (<303)	634/597	1.00	1.00
	2 (303–<329)	597/597	0.95 (0.81–1.11)	0.95 (0.81–1.12)
	3 (329–<355)	607/598	0.95 (0.81–1.12)	0.95 (0.80–1.12)
	4 (355–<391)	609/597	0.96 (0.82–1.14)	0.97 (0.82–1.15)
	5 (≥391)	540/598	0.84 (0.70–1.00)	0.83 (0.70–1.00)
	p-trend		0.07	0.07
Serine (μmol/L)	1 (<192)	571/597	1.00	1.00
	2 (192–<209)	638/597	1.13 (0.96–1.33)	1.16 (0.99–1.37)
	3 (209–<225)	580/598	1.02 (0.87–1.21)	1.05 (0.89–1.24)
	4 (225–<244)	589/597	1.05 (0.88–1.24)	1.10 (0.92–1.31)
	5 (≥244)	608/598	1.07 (0.90–1.28)	1.13 (0.94–1.35)
	p-trend		0.75	0.35

[1]Quintiles are based on the distribution of serum concentrations among controls.
[2]Adjusted for serum creatinine concentration.
[3]Adjusted for serum creatinine concentration, education, smoking, physical activity and body mass index.

Stratification by folate status and MTHFR 677C>T genotype

High concentrations of sarcosine and glycine were associated with reduced prostate cancer risk in combination with folate concentration above 13.7 nmol/L, whereas no associations were observed among participants with folate concentration below this threshold (Table 4). However, these interactions of folate concentration with sarcosine and glycine were not statistically significant. Conversely, although not significantly associated in overall analyses, we observed that a high serine concentration was associated with increased risk among individuals with low folate concentration ($p_{interaction} = 0.05$). High concentrations of sarcosine and glycine, and of betaine, were associated with reduced risk among individuals carrying the CC genotype of the *MTHFR* 677C→T polymorphism, whereas no associations were observed for the CT or TT genotypes.

Finally, the metabolite ratios were not differentially associated with prostate cancer risk across strata of folate status or *MTHFR* genotypes (data not shown).

Discussion

Principal findings

In this large-scale Norwegian population-based nested case–control study, we comprehensively investigated associations

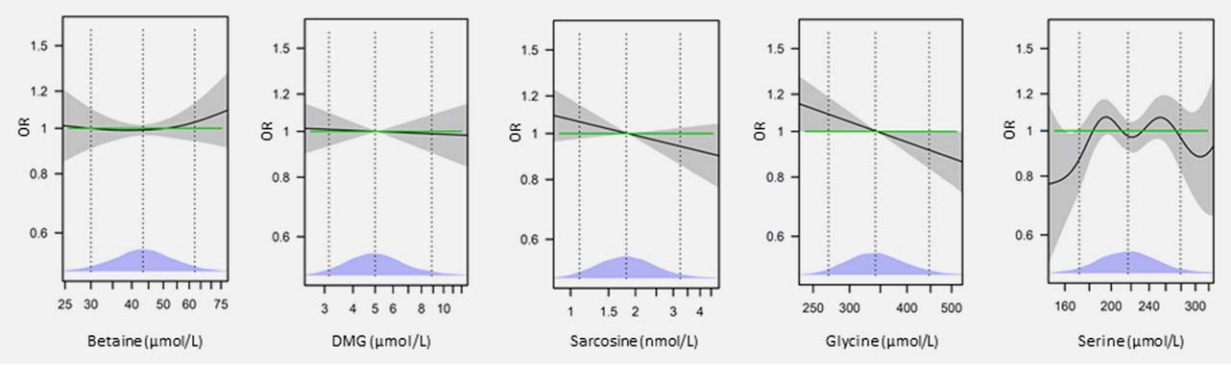

Figure 2. Generalized additive regression curves for the associations between biochemical parameters and prostate cancer risk. OR: odds ratio with 95% confidence interval; distributions of exposure variables are presented at the bottom of each figure; dotted lines: 5th, 50th and 95th percentiles of exposure variables. Analyses are adjusted for serum creatinine concentration, education, smoking, physical activity and body mass index. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 3. Conditional logistic regression analyses with corresponding odds ratios and 95% confidence intervals for prostate cancer, according to quintiles of ratios of metabolites

Ratio	Quintiles[1]	Number of cases/controls	Odds ratio[2]
Betaine/DMG	1	576/591	1.00
	2	523/590	0.93 (0.79–1.10)
	3	586/589	1.04 (0.88–1.23)
	4	598/591	1.05 (0.89–1.24)
	5	658/590	1.13 (0.95–1.34)
	p-trend		0.07
DMG/sarcosine	1	539/590	1.00
	2	602/589	1.14 (0.97–1.35)
	3	632/590	1.19 (1.00–1.41)
	4	567/589	1.09 (0.92–1.29)
	5	601/589	1.15 (0.97–1.36)
	p-trend		0.28
Sarcosine/glycine	1	650/598	1.00
	2	577/597	0.85 (0.73–1.01)
	3	555/597	0.83 (0.70–0.98)
	4	630/597	0.93 (0.79–1.10)
	5	575/598	0.83 (0.70–0.98)
	p-trend		0.14
Glycine/serine	1	657/598	1.00
	2	622/597	0.91 (0.78–1.08)
	3	592/598	0.86 (0.72–1.02)
	4	604/597	0.85 (0.72–1.00)
	5	511/597	0.71 (0.59–0.85)
	p-trend		<0.001

[1]Quintiles are based on the distribution of calculated ratios among controls.
[2]Adjusted for serum creatinine concentration, education, smoking, physical activity and body mass index.

of metabolites along the choline oxidation pathway with prostate cancer risk. We observed that high baseline serum concentrations of sarcosine and glycine were associated with modestly reduced prostate cancer risk. However, betaine, DMG and serine concentrations were not significantly associated. We observed that a high glycine/serine ratio, which

Table 4. Associations of serum concentrations with prostate cancer risk, stratified for folate status and *MTHFR* 677C→T genotypes

Serum concentration	Quintiles (range)[1]	Folate concentration[2]					MTHFR 677C→T						
		<13.7 nmol/L		≥13.7 nmol/L			CC		CT		TT		
		ca/co[3]	OR (95% CI)[4]	ca/co[3]	OR (95% CI)[4]	*p* interaction	ca/co[3]	OR (95% CI)[4]	ca/co[3]	OR (95% CI)[4]	ca/co[3]	OR (95% CI)[4]	*p* interaction
Betaine (μmol/L)	1 (<36.1)	352/372	1.00	213/215	1.00		275/256	1.00	129/174	1.00	67/75	1.00	
	2 (36.1–<41.1)	302/332	0.95 (0.77–1.18)	288/260	1.10 (0.85–1.42)		280/234	1.10 (0.86–1.40)	178/180	1.32 (0.97–1.80)	49/75	0.77 (0.46–1.29)	
	3 (41.1–<45.6)	270/296	0.96 (0.77–1.20)	314/287	1.08 (0.84–1.38)	0.60	265/262	0.91 (0.71–1.16)	161/184	1.18 (0.86–1.61)	61/65	1.07 (0.65–1.76)	0.21
	4 (45.6–<52.0)	268/257	1.08 (0.86–1.36)	356/337	1.03 (0.80–1.31)		305/270	1.02 (0.80–1.29)	173/196	1.17 (0.86–1.59)	55/68	0.92 (0.56–1.53)	
	5 (≥52.0)	193/198	1.00 (0.78–1.29)	385/397	0.92 (0.72–1.16)		256/290	0.77 (0.61–0.99)	167/186	1.16 (0.85–1.59)	58/56	1.12 (0.66–1.88)	
	p-trend		0.69		0.20			0.03		0.70		0.52	
DMG (μmol/L)	1 (<3.94)	286/323	1.00	320/267	1.00		301/263	1.00	172/185	1.00	58/59	1.00	
	2 (3.94–<4.63)	281/289	1.10 (0.88–1.39)	269/296	0.77 (0.61–0.97)		253/263	0.84 (0.66–1.07)	130/191	0.74 (0.54–1.01)	60/71	0.87 (0.52–1.45)	
	3 (4.63–<5.43)	305/294	1.17 (0.93–1.47)	371/298	1.07 (0.85–1.34)	0.09	315/261	1.03 (0.82–1.31)	189/194	1.05 (0.78–1.40)	69/64	1.07 (0.64–1.78)	0.76
	4 (5.43–<6.48)	233/271	0.97 (0.76–1.23)	303/319	0.80 (0.64–1.01)		257/275	0.81 (0.63–1.03)	147/160	1.00 (0.73–1.37)	48/72	0.65 (0.38–1.11)	
	5 (≥6.48)	280/278	1.13 (0.89–1.44)	293/316	0.79 (0.62–1.00)		255/250	0.88 (0.68–1.13)	170/190	0.96 (0.71–1.31)	55/73	0.77 (0.45–1.31)	
	p-trend		0.60		0.08			0.32		0.65		0.21	
Sarcosine (nmol/L)	1 (<1.40)	299/315	1.00	303/271	1.00		291/259	1.00	174/182	1.00	53/64	1.00	
	2 (1.40–<1.69)	306/311	0.98 (0.78–1.24)	332/294	1.03 (0.82–1.30)		297/280	0.93 (0.73–1.18)	178/176	1.05 (0.78–1.42)	65/67	1.32 (0.79–2.22)	
	3 (1.69–<1.98)	282/311	0.92 (0.73–1.16)	332/284	1.04 (0.82–1.31)	0.23	307/256	1.03 (0.81–1.31)	136/198	0.70 (0.52–0.96)	64/79	0.94 (0.56–1.58)	0.20
	4 (1.98–<2.40)	267/286	0.95 (0.75–1.20)	311/312	0.92 (0.73–1.16)		264/263	0.85 (0.66–1.09)	181/198	0.97 (0.72–1.31)	55/60	1.20 (0.70–2.06)	
	5 (≥2.40)	251/252	0.98 (0.77–1.24)	304/351	0.78 (0.62–0.99)		243/272	0.75 (0.59–0.97)	150/172	0.89 (0.65–1.22)	58/73	0.97 (0.57–1.64)	
	p-trend		0.81		0.009			0.02		0.47		0.68	
Glycine (μmol/L)	1 (<303)	241/258	1.00	293/339	1.00		314/259	1.00	164/194	1.00	52/57	1.00	
	2 (303–<329)	271/286	1.01 (0.79–1.29)	326/311	0.93 (0.75–1.15)		266/276	0.82 (0.65–1.05)	175/182	1.12 (0.83–1.52)	55/68	0.99 (0.58–1.71)	
	3 (329–<355)	288/299	1.00 (0.78–1.28)	319/299	0.94 (0.75–1.17)	0.84	295/270	0.91 (0.71–1.15)	161/172	1.08 (0.80–1.47)	51/84	0.71 (0.42–1.22)	0.03
	4 (355–<391)	323/317	1.08 (0.85–1.38)	286/280	0.89 (0.71–1.11)		305/251	1.02 (0.80–1.29)	149/191	0.90 (0.66–1.23)	76/69	1.41 (0.83–2.40)	
	5 (≥391)	282/315	0.94 (0.73–1.21)	258/283	0.78 (0.62–0.98)		222/274	0.68 (0.53–0.87)	170/187	1.02 (0.75–1.39)	61/65	1.12 (0.64–1.93)	
	p-trend		0.73		0.04			0.02		0.69		0.32	
Serine (μmol/L)	1 (<192)	231/276	1.00	340/321	1.00		256/235	1.00	167/188	1.00	67/86	1.00	
	2 (192–<209)	267/283	1.13 (0.88–1.45)	371/314	1.19 (0.96–1.49)		309/264	1.14 (0.89–1.46)	157/189	0.97 (0.72–1.32)	59/73	1.14 (0.69–1.86)	
	3 (209–<225)	248/280	1.07 (0.83–1.37)	332/318	1.05 (0.84–1.31)	0.05	277/268	0.99 (0.77–1.27)	162/191	0.98 (0.73–1.33)	56/68	1.20 (0.73–1.98)	0.64
	4 (225–<244)	314/327	1.17 (0.92–1.49)	275/270	1.04 (0.83–1.32)		283/290	0.93 (0.73–1.20)	169/177	1.11 (0.81–1.50)	55/56	1.48 (0.88–2.50)	
	5 (≥244)	344/309	1.38 (1.08–1.75)	264/289	0.92 (0.73–1.16)		276/273	1.00 (0.78–1.29)	164/181	1.02 (0.75–1.40)	58/60	1.43 (0.86–2.39)	
	p-trend		0.01		0.24			0.53		0.67		0.10	

[1]Quintiles are based on the distribution of serum concentrations among controls.
[2]Folate as *p*-aminobenzoylglutamate.[22] Median serum folate concentration among controls was 13.7 nmol/L.
[3]Number of cases and controls within categories of exposure.
[4]Adjusted for serum creatinine concentration, education, smoking, physical activity and body mass index.

may reflect one-carbon balance, was associated with markedly decreased prostate cancer risk.

Strengths and limitations

Strength of this study involves the fact that consecutive metabolites of the choline oxidation pathway, *i.e.*, from betaine down to serine, have been analyzed. This complementary information on closely related metabolites may allow assessment of potential mechanisms involved in the etiology of prostate cancer. Another advantage of the study is that betaine, DMG, sarcosine and glycine concentrations are stable during storage at -25°C as used for samples in the JANUS serum bank,[24] causing minimal preanalytical bias. In addition, the measured metabolites have high test–retest reliability with intraclass correlation coefficients in the range of 0.55–0.84 (unpublished data). Such good to excellent reproducibility minimizes regression dilution bias. It is a limitation that choline was not measured, but this was not feasible because of pronounced increases of choline concentrations during storage,[24] most likely as a result of degradation of phosphatidylcholine into choline.

The study comprised a large sample size, thereby allowing for subgroup analyses. Although carcinogenesis of prostate cancer is a long-term process and subclinical prostate cancer is not uncommon in Western populations,[27] 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. An advantage in this respect is the nested prospective design, with exposure measurement before clinical manifestation of the disease. In addition, misclassification is probably low as the majority of the cases (*i.e.*, >99%) were diagnosed in the period after PSA tests became commercially available, which is likely to have contributed to earlier detection of prostate cancer among cohort members. A limitation of our study is that data on disease grading, as indicated by Gleason scores for prostate cancer, were not available. Disease staging information was available in 61% of the cases. However, analyses limited to these individuals did not reveal substantially different associations between the serum markers and prostate cancer risk across disease stages compared to overall analyses.

Sarcosine and prostate cancer

A metabolomics screening study by Sreekumar *et al.* suggested that urinary sarcosine may be a marker for prostate cancer progression.[13] Moreover, although urinary sarcosine concentrations were higher among prostate cancer cases than in controls in one[14] but not in another validation study,[16] high sarcosine concentration was associated with prostate cancer aggressiveness when measured in prostatic tissue,[15] and with prostate cancer recurrence when measured in serum at prostatectomy.[17]

Although the above mentioned observations were based on relatively small and selected study populations, we investigated the role of sarcosine prospectively in a large population-based nested case–control study. Our study showed that high serum sarcosine levels were associated with reduced prostate cancer risk when sarcosine was measured in presumptively disease-free individuals. These apparently opposing findings could be explained by differences in study design, or by the different types of biospecimen in which sarcosine has been measured. However, to our knowledge, no studies have been conducted to investigate the association between sarcosine concentrations in serum and urine. Nevertheless, we do have unpublished data from a cohort of 3,419 individuals with stable angina pectoris among whom plasma sarcosine and the urinary sarcosine/creatinine ratio were modestly associated (Pearson's $r = 0.20$, $p < 0.001$), suggesting that low serum concentrations of sarcosine are more likely consistent with lower (rather than with higher) urinary sarcosine concentrations. Furthermore, the concern has been raised that in the initial study by Sreekumar *et al.*, the analytical method used may not have been able to differentiate sarcosine from alanine in GC-MS analyses, and that in the absence of internal analytical validation sarcosine levels may have been overestimated.[28] For the method used in our study, based on methylchloroformate derivatization followed by GC-MS/MS, sarcosine was separated from alanine (with a difference in retention time of 0.15 min), ruling out interference from alanine (unpublished data). In contrast to previous observations, our study suggested that high serum sarcosine may play a protective role in etiology of prostate cancer.

One-carbon balance and prostate cancer

The hypothesis of methyl group donor availability affecting DNA methylation is complicated by the fact that gene-specific promoter hypermethylation occurs concomitantly with genome-wide hypomethylation.[29] Nevertheless, blood levels of one-carbon metabolites modify DNA methylation levels in blood cells, which could serve as a surrogate tissue to study mechanisms of carcinogenesis in general.[30]

To our knowledge, this is the first investigation on the association between serum concentrations of glycine and serine with prostate cancer risk. We observed that a high ratio of glycine/serine was associated with markedly decreased prostate cancer risk. This ratio comprises the substrate and product of the reversible SHMT enzymatic transmethylation reaction. A shift in the bioavailability of methyl groups, or one-carbon balance, may be hypothesized to affect purine and pyrimidine synthesis and DNA methylation reactions that are involved in carcinogenesis. However, SHMT flux appeared relatively insensitive to changes in folate status, as suggested by a study showing that total remethylation flux was not significantly affected by folate restriction.[31] In addition, mathematical modeling of the one-carbon metabolism revealed that SHMT velocity remained relatively unchanged when decreasing the folate pool.[32] Nevertheless, the rare variant of the *SHMT1* (rs1979277) polymorphism may be associated with higher folate status[33] and increased prostate cancer

risk,[34] and *SHMT* polymorphisms were associated with increased risk of lung cancer[35] and ovarian cancer.[36]

In addition to a likely role of SHMT activity, a probably much stronger determinant of plasma and tissue glycine concentration and flux is the activity of the mitochondrial glycine cleavage system. Reduced activity of the glycine cleavage system, as would occur during vitamin B6 deficiency, causes accumulation of glycine.[37] In this way, plasma glycine is a functional biomarker of vitamin B6 status. The glycine cleavage system is a major contributor to cellular 5,10-methylene THF, which is needed for thymidylate synthesis. Further, plasma cystathionine is also increased in vitamin B6 deficiency because of the sensitivity of cystathionine gamma-lyase to loss of its PLP coenzyme. Unfortunately, we were unable to measure vitamin B6 or its markers owing to considerable degradation of vitamin B6 after storage.[24]

In the choline oxidation pathway, sarcosine donates a methyl group to THF during the conversion into glycine and MTHF by SD (Fig. 1). Interestingly, *GNMT*, which encodes the enzyme that catalyzes the conversion of glycine to sarcosine (Fig. 1), has been suggested to be a tumor susceptibility gene for prostate cancer.[38,39] However, although we hypothesized that the combination of sarcosine and glycine can therefore reflect methyl balance, the sarcosine/glycine ratio was not associated with prostate cancer risk in our study.

In a previous population-based case–control study, prostate cancer risk was associated with high concentrations of circulating choline, vitamin B2 and nonsignificantly with high betaine concentrations.[12] In our study population, high serum folate concentration was associated with modestly increased prostate cancer risk.[40] In addition, here we observed that high serine was associated with increased prostate cancer risk when folate status is low, and that for sarcosine and glycine, the inverse associations were only observed in combination with high folate status. Although some caution is warranted in interpreting the observations of the various comparisons made, such potential dependence on folate status, as well as ratios of metabolites reflecting one-carbon balance, underscore the advantage of simultaneous assessment of related metabolites involved in the one-carbon network, rather than investigating one metabolite at the time when estimating prostate cancer risk.

Amino acid profiles in prostate cancer

Epidemiological studies suggested that obesity, a component of the metabolic syndrome, was associated with increased prostate cancer risk.[41] One plausible mechanism linking obesity to prostate cancer is chronic inflammation, which has been suggested to contribute to prostate carcinogenesis.[41] Interestingly, inflammatory markers were associated with lower vitamin B6 status both at baseline and after vitamin B6 treatment.[42] Moreover, vitamin B6 restriction has been demonstrated to lead to an increase of plasma glycine concentration.[37] In addition, in a cross-sectional study, plasma concentrations of glycine and serine were lower in individuals with metabolic syndrome compared to those without.[43] We observed that betaine, DMG, sarcosine and glycine concentrations were significantly lower among men with a high BMI. However, adjustment for BMI did not materially change the estimated associations between serum metabolite concentrations and prostate cancer risk (data not shown).

Conclusions

Serum sarcosine and glycine concentrations were associated with modestly reduced prostate cancer risk. Combinations of metabolites involved in one-carbon metabolism, including ratios reflecting one-carbon balance such as the glycine/serine ratio, may be useful to estimate prostate cancer risk and should be explored in future studies.

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