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Plasma methionine, choline, betaine, and dimethylglycine in relation to colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC)

M. Nitter[1,†], B. Norgård[1,†], S. de Vogel[1*], S. J. P. M. Eussen[1,2,3], K. Meyer[4], A. Ulvik[4], P. M. Ueland[2,5], O. Nygård[6,7], S. E. Vollset[1,8], T. Bjørge[1,8], A. Tjønneland[9], L. Hansen[9], M. Boutron-Ruault[10,11,12], A. Racine[10,11,12], V. Cottet[13], R. Kaaks[14], T. Kühn[14], A. Trichopoulou[15,16], C. Bamia[15,16], A. Naska[15,16], S. Grioni[17], D. Palli[18], S. Panico[19], R. Tumino[20], P. Vineis[21,22], H. B. Bueno-de-Mesquita[22,23,24], H. van Kranen[23], P. H. Peeters[22,25], E. Weiderpass[26,27,28,29], M. Dorronsoro[30], P. Jakszyn[31], M. Sánchez[32,33,34], M. Argüelles[35], J. M. Huerta[33,36], A. Barricarte[37,38], M. Johansson[39,40], I. Ljuslinder[41], K. Khaw[42], N. Wareham[43], H. Freisling[40], T. Duarte-Salles[40], M. Stepien[40], M. J. Gunter[22] & E. Riboli[22]

Departments of [1]Global Public Health and Primary Care; [2]Clinical Science, Section for Pharmacology, University of Bergen, Bergen, Norway; [3]Department of Epidemiology, School for Public Health and Primary Care, CAPHRI, Maastricht University, Maastricht, The Netherlands; [4]BEVITAL AS, Bergen; [5]Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen; Departments of [6]Clinical Science, Section of Cardiology, University of Bergen, Bergen; [7]Heart Disease, Haukeland University Hospital, Bergen; [8]Norwegian Institute of Public Health, Bergen, Norway; [9]Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark; [10]INSERM, Centre for Research in Epidemiology and Population Health (CESP), Nutrition, Hormones and Women's Health Team, Villejuif; [11]Paris South University, Villejuif; [12]IGR, Villejuif; [13]INSERM, Research Centre 'Lipids, Nutrition, Cancer', Dijon, France; [14]German Cancer Research Center (DKFZ), Heidelberg, Germany; [15]Hellenic Health Foundation, Athens; [16]WHO Collaborating Center for Food and Nutrition Policies, Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Athens, Greece; [17]Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano; [18]Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute—ISPO, Florence; [19]Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Naples; [20]Cancer Registry and Histopathology Unit, 'Civile - M.P. Arezzo' Hospital, ASP, Ragusa; [21]School of Public Health and HuGeF Foundation, Torino, Italy; [22]The School of Public Health, Imperial College London, London, UK; [23]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; [24]Department of Gastroenterology and Hepatology; [25]Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, The Netherlands; [26]Department of Community Medicine, Faculty of Health Sciences, UIT The Arctic University of Norway, Tromsø; [27]Department of Research, Cancer Registry of Norway, Oslo, Norway; [28]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; [29]Department of Genetic Epidemiology, Samfundet Folkhälsan, Helsinki, Finland; [30]Basque Regional Health Department, Public Health Direction and Biodonostia Research Institute (CIBERESP), San Sebastian; [31]Unit of Nutrition, Environment and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology, Barcelona; [32]Andalusian School of Public Health, Granada; [33]CIBER de Epidemiología y Salud Pública (CIBERESP), Madrid; [34]Instituto de Investigación Biosanitaria de Granada (Granada.ibs), Granada; [35]Public Health Directorate, Asturias, Oviedo; [36]Department of Epidemiology, Regional Health Council, Murcia; [37]Public Health Institute of Navarra, Pamplona; [38]Consortium for Biomedical Research in Epidemiology and Public Health, Madrid, Spain; [39]Department of Biobank Research, Umeå University, Umeå, Sweden; [40]International Agency for Research on Cancer (IARC-WHO), Lyon, France; [41]Department of Radiation Sciences, Oncology, University of Umeå, Umeå, Sweden; [42]University of Cambridge School of Clinical Medicine, Cambridge; [43]MRC Epidemiology Unit, University of Cambridge, Cambridge, UK

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Background: Disturbances in one carbon metabolism may contribute to carcinogenesis by affecting methylation and synthesis of DNA. Choline and its oxidation product betaine are involved in this metabolism and can serve as alternative methyl group donors when folate status is low.

Patients and methods: We conducted a case–control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC), to investigate plasma concentrations of the methyl donors methionine, choline, betaine (trimethylglycine), and dimethylglycine (DMG) in relation to colorectal cancer (CRC) risk. Our study included 1367 incident

*Correspondence to: Dr Stefan de Vogel, Department of Global Public Health and Primary Care, University of Bergen, PO Box 7804, 5018 Bergen, Norway. Tel: +47-55586100; E-mail: stefan.vogel@isf.uib.no

†M.N. and B.N. contributed equally to this study.

CRC cases (965 colon and 402 rectum) and 2323 controls matched by gender, age group, and study center. Multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for CRC risk were estimated by conditional logistic regression, comparing the fifth to the first quintile of plasma concentrations.

Results: Overall, methionine (OR: 0.79, 95% CI: 0.63–0.99, *P*-trend = 0.05), choline (OR: 0.77, 95% CI: 0.60–0.99, *P*-trend = 0.07), and betaine (OR: 0.85, 95% CI: 0.66–1.09, *P*-trend = 0.06) concentrations were inversely associated with CRC risk of borderline significance. In participants with folate concentration below the median of 11.3 nmol/l, high betaine concentration was associated with reduced CRC risk (OR: 0.71, 95% CI: 0.50–1.00, *P*-trend = 0.02), which was not observed for those having a higher folate status. Among women, but not men, high choline concentration was associated with decreased CRC risk (OR: 0.62, 95% CI: 0.43–0.88, *P*-trend = 0.01). Plasma DMG was not associated with CRC risk.

Conclusions: Individuals with high plasma concentrations of methionine, choline, and betaine may be at reduced risk of CRC.

Key words: methionine, choline, betaine, dimethylglycine, colorectal cancer risk, population-based case–control study

introduction

Colorectal cancer (CRC) is the second most commonly diagnosed cancer in Europe with 447 000 estimated new cases in the year 2012 [1]. In 2011, the World Cancer Research Fund indicated convincing evidence of physical activity and fiber intake to protect against CRC, whereas red meat, processed meat, intake of ethanol from alcoholic drinks, as well as body fatness and abdominal fatness are associated with increased risk [2].

One-carbon metabolism includes donors of methyl groups for DNA methylation and DNA synthesis, both of which are involved in carcinogenesis. The B-vitamin folate and related methyl group donors are hypothesized to affect DNA methylation status and thereby have the potential to prevent carcinogenesis [3]. Choline and its oxidation product betaine can serve as alternative methyl group donors during folate deficiency, whereas during choline deprivation, methyl groups from the methyl donor folate are used [4–6].

Few studies with inconclusive results reported on choline and betaine status in relation to CRC [7–9], and the majority of observational studies on dietary methionine and CRC do not suggest an association [8]. The association between plasma folate and CRC risk has previously been investigated in the European Prospective Investigation into Cancer and Nutrition (EPIC), but no association of plasma folate with CRC risk was observed [10].

We conducted a population-based case–control study nested within the EPIC, investigating associations between plasma methionine, choline, betaine (trimethylglycine), and dimethylglycine (DMG; the product of the enzymatic conversion from betaine), in relation to CRC risk. In view of the hypothesis that these alternative methyl group donors become particularly important when folate status is low, we evaluated whether the associations were different among individuals with a high or low folate status.

patients and methods

study population

The EPIC is a population-based prospective study to investigate associations between diet, nutritional and metabolic characteristics, lifestyle factors, and cancer risk [11]. The cohort comprises participants from 23 collaborating centers in 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and UK). Data were collected between 1992 and 1998 and included baseline questionnaires capturing diet, lifestyle factors, personal history, and anthropometric data. Anthropometric measurements were taken on the majority of subjects [12].

blood sample collection

Baseline blood samples were collected from 80% of participants depending on invitation in some countries or willingness to donate. Following a strict protocol, samples of each center were stored at 5–10°C while protected from light and transported to local laboratories in the same country for processing and aliquoting [12]. Exceptions from this procedure were the EPIC-Oxford and EPIC-Norway centers, where whole blood samples were transported to a central laboratory via mail. Since these samples were exposed to ambient temperatures for up to 48 h, and some B-vitamins and related metabolites are unstable under such conditions [13], all EPIC-Oxford (55 cases and 107 controls) and EPIC-Norway (5 cases and 9 controls) samples were excluded from the present analyses.

sample storage

Separation of blood into fractions of 0.5 ml (serum, plasma, erythrocytes, and buffy coat for DNA extraction) was done in all countries except Denmark and Sweden (constituting 38.4% of all participants), because the collection in these countries was initiated many years before the common EPIC protocol [14]. The fractions were placed into heat-sealed straws and stored in liquid nitrogen at −196°C. Half of the samples were stored at local study centers and the other half at the EPIC biorepository at the International Agency for Research on Cancer. Storage conditions in Denmark and Sweden are described elsewhere [12, 15].

cancer incidence follow-up

Follow-up in the EPIC is mainly based on national population-based cancer registries (Denmark, Italy, the Netherlands, Norway, Spain, Sweden, and UK). Other sources were health insurance records, pathology registries, or through self-reporting (France, Germany, and Greece). Self-reported cancer cases were verified through pathology reports and physicians, available for at least 95% of the cases. In our study, time between inclusion and diagnosis of CRC varied from 3 days to 11.5 years (mean 3.7 years).

selection of study subjects

We conducted a nested case–control study within the EPIC cohort. Colon cancer was defined by ICD-10 codes C18.0–C18.9, which includes tumors that

were overlapping or unspecified, and diagnosis codes C19 or C20 for rectal tumors. CRC is defined as a combination of colon and rectal cancer cases. The present study included 1367 CRC cases (colon $n = 965$ and rectum $n = 402$).

For each identified cancer case, 1–2 controls were randomly selected from all cohort members with available blood samples who were alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the index case. Controls were matched by gender, age group (±2.5 years), and study center, except for the Danish cases, which were post hoc matched [15].

laboratory measurements

Plasma methionine, choline, betaine, and DMG were determined by a method based on normal-phase liquid chromatography and tandem mass spectrometry [16]. Plasma folate was determined by a *Lactobacillus casei* microbiological assay (Hamilton Bonaduz AG, [17]). Between-day coefficients of variation (CV) were up to 7% (methionine), 7% (choline), 10% (betaine), 5% (DMG), and 5% (folate). Intra-class correlation coefficients were 0.33 (methionine), 0.36 (choline), 0.65 (betaine), 0.64 (DMG), and 0.56 (folate).

Single nucleotide polymorphisms (SNPs) of genes related to one-carbon metabolism were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [18]. These included *methylene-tetrahydrofolate reductase (MTHFR) 677C → T*, *MTHFR 1298A → C*, and *betaine-homocysteine methyltransferase (BHMT) 742G → A*. All laboratory analyses were carried out at BEVITAL AS, Bergen, Norway.

statistical methods

Differences in baseline characteristics between cases and controls were assessed non-parametrically by Kruskal–Wallis tests for variables with skewed distributions, and χ^2 tests and ANOVA where appropriate. Multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated by conditional logistic regression for CRC risk across quintiles of methionine, choline, betaine, and DMG concentrations, taking the lowest quintiles as reference. Quintile cutoff values were based on the distributions among controls. Tests for linear trend over quintiles were carried out by fitting the ordinal exposure variables as continuous variables. As epigenetic alterations, which possibly are affected by one-carbon balance, are associated with proximal tumor location in CRC [19], analyses were also conducted for colon and rectum cancer separately. We excluded cases ($N = 163$; 11.2%) diagnosed within the first year of follow-up to address potential reverse causation. In addition, we stratified analyses by time from blood donation to cancer diagnosis (below and above the median of 3.6 years). We adjusted for potential confounders; body mass index (BMI; kg/m^2), smoking status (never, former, and current), physical activity (inactive, moderately inactive, moderately active, and active), alcohol consumption (abstainers, >0 to <30 g/day, and ≥30 g/day), and dietary intake of fiber, red meat, processed meat, and total energy.

Because methyl group donors are believed to collectively contribute to one-carbon balance, and as B-vitamin status was associated with sex and age (data now shown), stratified analyses were conducted according to median folate concentration among controls (<11.3 and ≥11.3 nmol/l), and according to sex, and age at recruitment (<60 versus ≥60 years).

It could be expected that serum concentrations are differentially associated with cancer risk across the genotypes due to the influence on enzymatic activity. Therefore, in addition to previously reported associations of one-carbon genetic variants with CRC risk [10, 20], we analyzed the associations between *BHMT* (742G → A) genotypes and CRC risk, and between serum concentrations with CRC risk across *BHMT* (742G → A) as well as *MTHFR* (677C → T and 1298A → C) genotypes with CRC risk.

All statistical analyses were conducted using STATA, version 11.

results

Altogether, 1367 cases and 2323 controls were included. Mean age at blood donation and CRC diagnosis was 59.0 and 62.7 years, respectively (Table 1). BMI, current smoking, alcohol consumption, and meat intake were significantly higher among cases than controls, whereas fiber intake and the level of physical activity were lower among cases. Plasma concentrations of methionine, choline, betaine, and folate were lower among cases than among controls. Genotype frequencies were similar.

Adjusted analyses revealed that high methionine (OR: 0.79, 95% CI: 0.63–0.99, P-trend = 0.05), choline (OR: 0.77, 95% CI: 0.60–0.99, P-trend = 0.07), and betaine (OR: 0.85, 95% CI: 0.66–1.09, P-trend = 0.06) concentrations were associated with lower CRC risk of borderline significance (Table 2). Exclusion of cases diagnosed within the first year of follow-up modestly attenuated the associations of methionine and betaine, whereas choline remained associated with reduced CRC risk (data not shown).

Choline was inversely associated with colon cancer risk, while we did not observe significant associations between methionine, betaine, and DMG concentrations and risk of colon cancer or rectum cancer separately (supplementary Table S1, available at *Annals of Oncology* online). Plasma methionine was associated with reduced CRC risk exclusively in those cases who were diagnosed with CRC within 3.6 years after blood collection, and not in those diagnosed at a later time. Choline, betaine, and DMG were not significantly associated with CRC risk in either of these groups (data not shown).

Plasma betaine was inversely associated with CRC risk among subjects with folate concentration below the median of 11.3 nmol/l, but not among those with folate concentration above the median (Table 3). A similar, though borderline significant inverse association was observed for high choline concentrations among individuals with a lower folate status. Neither methionine nor DMG were differentially associated with CRC risk across the categories of folate status.

Subgroup analysis (supplementary Table S2, available at *Annals of Oncology* online) revealed an inverse association between choline and CRC risk in women (OR: 0.62, 95% CI: 0.43–0.88, P-trend = 0.01), but not in men (OR: 1.03, 95% CI: 0.71–1.50, P-trend = 0.87). The inverse associations of methionine and betaine were observed among individuals <60 years of age, but not among those ≥60 years. However, the tests for interaction, based on unconditional logistic regression models, were not significant for these associations.

No associations between the genotypes and CRC risk or of serum concentrations across genotypes were observed (data not shown).

discussion

In this large-scale population-based European nested case–control study, plasma methionine, choline, and betaine status were modestly inversely associated with CRC risk. Higher betaine concentration was associated with reduced CRC risk among individuals with folate concentration below the median of 11.3 nmol/l, but not among those with a higher folate status. Plasma choline was associated with reduced CRC risk among women, but not among men. The inverse associations of methionine and betaine were confined to individuals <60 years at recruitment.

Table 1. Baseline characteristics of colorectal cancer cases and matched controls in the European Prospective Investigation into Cancer and Nutrition

	Cases	Controls	*P*-difference
Number of individuals	1367	2323	
Sex, female, *n* (%)	700 (51.2)	1213 (52.2)	*0.55*[a]
Age at recruitment [years; mean (SD)]	58.9 (7.1)	58.7 (7.5)	*0.38*[b]
Body mass index [kg/m^2; mean(SD)]	26.8 (4.3)	26.4 (3.9)	*0.005*[b]
Smoking status, *n* (%)			
Never	561 (41.0)	1025 (44.1)	*0.05*[a]
Former	451 (33.0)	775 (33.4)	
Current	346 (25.3)	510 (22.0)	
Unknown	9 (0.7)	13 (0.6)	
Physical activity, *n* (%)			
Active	123 (9.0)	242 (10.4)	*0.05*[a]
Moderately active	574 (42.0)	1019 (43.9)	
Moderately inactive	423 (30.9)	697 (30.0)	
Inactive	219 (16.0)	304 (13.1)	
Unknown	28 (2.1)	61 (2.6)	
Alcohol consumption, *n* (%)			
Abstainers	172 (12.6)	347 (14.9)	*0.001*[a]
>0 g/day and <30 g/day	908 (66.5)	1597 (68.8)	
≥30 g/day	285 (20.9)	379 (16.3)	
Dietary intakes [mean (SD)]			
Energy (kcal/day)	2176 (710)	2136 (643)	*0.08*[b]
Total meat (g/day)	118.3 (69.8)	109.7 (56.4)	*<0.001*[b]
Red meat (g/day)	53.9 (39.5)	47.5 (35.5)	*<0.001*[b]
Processed meat (g/day)	38.2 (47.8)	35.3 (32.2)	*0.03*[b]
Fiber, mean (g/day)	22.2 (8.2)	22.9 (7.9)	*0.003*[b]
Plasma concentrations [median (5th–95th percentile)]			
Methionine (μmol/l)	23.7 (16.6–37.0)	24.2 (17.0–37.4)	*0.009*[c]
Choline (μmol/l)	9.3 (6.2–14.2)	9.4 (6.3–14.4)	*0.02*[c]
Betaine (μmol/l)	31.5 (18.4–52.6)	33.0 (18.5–53.8)	*0.005*[c]
Dimethylglycine (μmol/l)	3.6 (2.4–5.9)	3.6 (2.3–6.1)	*0.86*[c]
Folate (nmol/l)	10.9 (5.1–32.1)	11.3 (4.9–34.0)	*0.03*[c]
MTHFR 677C → T, %			
CC	41.8	42.7	*0.86*[a]
CT	46.1	45.8	
TT	12.1	11.6	
MTHFR 1298A → C, %			
AA	45.9	45.8	*0.96*[a]
AC	43.0	43.4	
CC	11.1	10.8	
BHMT 742G → A, %			
GG	50.8	48.6	*0.45*[a]
GA	41.0	42.5	
AA	8.2	8.8	

Italic values are defined as statistical significance at $P < 0.05$.
[a]χ^2 test, unknown category not included.
[b]ANOVA.
[c]Kruskal–Wallis test.

This study is the largest prospective study on plasma methionine and the first on plasma DMG, choline, and betaine concentrations in relation to CRC risk to date. The large sample size and extensive data collection on modifiable risk factors for CRC allowed subgroup analyses. The strength of this study is its prospective design, where blood samples were taken before cancer diagnosis. The mean time between inclusion and cancer diagnosis was relatively short (median 3.6 years), which may have led to reverse causality if undiagnosed (preclinical) cancer has affected exposure status. Although possibly resulting from reduced power to demonstrate an underlying true association, the inverse associations of methionine and betaine with CRC

Table 2. Conditional logistic regression analyses with corresponding odds ratios and 95% confidence intervals for colorectal cancer, according to quintiles of methionine, betaine, choline, and dimethylglycine concentrations

Plasma concentration	Quintiles (range)[a]	Cases/controls	OR[b]	
			Crude analyses	Adjusted analyses[c]
Methionine (μmol/l)	1 (<20.2)	335/465	Reference	Reference
	2 (20.2–<22.9)	264/465	0.79 (0.63–0.97)	0.79 (0.63–0.98)
	3 (22.9–<25.6)	260/464	0.81 (0.65–1.00)	0.83 (0.66–1.03)
	4 (25.6–<29.6)	249/465	0.77 (0.61–0.96)	0.77 (0.61–0.97)
	5 (≥29.6)	259/464	0.78 (0.62–0.98)	0.79 (0.63–0.99)
			P-trend = *0.04*	*P*-trend = *0.05*
Choline (μmol/l)	1 (<7.7)	317/460	Reference	Reference
	2 (7.7–<8.9)	268/472	0.84 (0.68–1.04)	0.83 (0.66–1.03)
	3 (8.9–<10.1)	276/462	0.92 (0.73–1.15)	0.91 (0.73–1.15)
	4 (10.1–<11.7)	254/457	0.86 (0.68–1.09)	0.82 (0.64–1.04)
	5 (≥11.7)	250/472	0.84 (0.66–1.07)	0.77 (0.60–0.99)
			P-trend = *0.26*	*P*-trend = *0.07*
Betaine (μmol/l)	1 (<24.8)	297/461	Reference	Reference
	2 (24.8–<30.4)	304/469	1.01 (0.81–1.25)	1.03 (0.82–1.28)
	3 (30.4–<35.3)	282/459	0.94 (0.76–1.17)	0.98 (0.79–1.22)
	4 (35.3–<42.1)	246/468	0.79 (0.63–1.00)	0.84 (0.66–1.06)
	5 (≥42.1)	236/466	0.78 (0.62–1.00)	0.85 (0.66–1.09)
			P-trend = *0.01*	*P*-trend = *0.06*
DMG (μmol/l)	1 (<2.9)	271/464	Reference	Reference
	2 (2.9–<3.3)	267/452	1.09 (0.87–1.36)	1.05 (0.84–1.31)
	3 (3.3–<3.9)	288/473	1.15 (0.92–1.44)	1.12 (0.89–1.41)
	4 (3.9–<4.6)	277/467	1.21 (0.96–1.51)	1.13 (0.89–1.42)
	5 (≥4.6)	262/467	1.18 (0.93–1.50)	1.10 (0.86–1.40)
			P-trend = *0.12*	*P*-trend = *0.33*

Italic values are defined as statistical significance at $P < 0.05$.

[a]Quintiles are based on the distribution of serum concentrations among controls.

[b]Case–control matching factors included sex, age, and study center.

[c]Adjusted for BMI, smoking status, physical activity and alcohol consumption, intakes of energy, fiber, red meat, and processed meat.

risk tended to attenuate after exclusion of cases diagnosed within the first year of follow-up. Nevertheless, the possibility cannot be excluded that reverse causation has biased the estimated associations to some extent. Another advantage of our study was that the main exposure variables were measured in blood rather than obtained from dietary questionnaires [7, 9, 21, 22], which rely on subjects' memory or ability in recording dietary intake [11].

Furthermore, blood samples were collected according to a standardized protocol [11] at each study center and all the biochemical analysis were conducted at one laboratory, thereby eliminating variability in sampling procedures and assay methods. However, a single blood sample may not represent lifetime exposure as variations in plasma concentrations over time may occur due to lifestyle changes and diet variation. Furthermore, the measured blood levels may not directly reflect the dietary intake or body stores of nutrients.

Inverse associations between plasma levels or dietary intake of methionine and betaine with the risk of colorectal adenomas or CRC were previously reported [8, 22]. Conversely, a positive association was observed between choline intake and colorectal adenoma (CRA) risk [7]. However, one would expect high choline to be protective against neoplasia, as choline deficiency has the potential to induce DNA damage by uracil misincorporation and to alter DNA methylation patterns [23]. In this respect, a population-based case–control study in China suggested that dietary choline and betaine intake was associated with reduced lung cancer risk [24].

B-vitamins are components of a network with major effects on the transfer of one-carbon units [25]. Similarly, choline and betaine may serve as alternative methyl group donors when folate status is low [6]. Our observation of a possible protective role of choline and betaine among individuals with lower folate status may support this possibility. Although in this EPIC cohort plasma folate and vitamin B12 were not associated with CRC risk [10, 15], inverse associations were observed of plasma concentrations of vitamins B2 and B6 with CRC risk [15]. In addition, dietary intake of vitamins B2 and B6 were associated with reduced CRC risk in the Women's Health Initiative Observational Study [26]. Although not associated with overall CRC risk, we observed that high plasma choline may protect against CRC in women. In addition to the dietary source, choline is synthesized endogenously from phosphatidylethanolamine by phosphatidylethanolamine-*N*-methyltransferase (PEMT), the activity of which is increased by estrogen [27]. This

Table 3. Logistic regression analyses with corresponding odds ratios and 95% confidence intervals for colorectal cancer, according to quintiles of methionine, betaine, choline, and dimethylglycine concentrations, for low (under median of 11.3 nmol/l) and high (above median) folate concentrations

Plasma concentration	Quintiles (range)[a]	Folate <1.3 nmol/l		Folate ≥11.3 nmol/l	
		Cases/controls	OR[a]	Cases/controls	OR[a]
Methionine (μmol/l)	1 (<20.2)	170/218	Reference	165/245	Reference
	2 (20.2–<22.9)	139/228	0.76 (0.57–1.03)	125/235	0.81 (0.60–1.09)
	3 (22.9–<25.6)	132/239	0.79 (0.55–1.01)	128/225	0.87 (0.64–1.17)
	4 (25.6–<29.6)	133/239	0.73 (0.54–0.98)	116/225	0.79 (0.58–1.08)
	5 (≥29.6)	147/232	0.76 (0.56–1.02)	112/232	0.76 (0.56–1.04)
			P-trend = *0.07*		*P*-trend = *0.11*
		P-interaction = 0.85			
Choline (μmol/l)	1 (<7.7)	195/245	Reference	122/213	Reference
	2 (7.7–<8.9)	129/250	0.64 (0.48–0.86)	139/221	1.08 (0.79–1.48)
	3 (8.9–<10.1)	148/227	0.80 (0.60–1.08)	128/234	0.95 (0.69–1.31)
	4 (10.1–<11.7)	125/219	0.71 (0.52–0.96)	129/237	0.91 (0.66–1.26)
	5 (≥11.7)	124/215	0.71 (0.52–0.96)	126/257	0.81 (0.58–1.13)
			P-trend = *0.07*		*P*-trend = *0.12*
		P-interaction = *0.13*			
Betaine (μmol/l)	1 (<24.8)	182/266	Reference	115/193	Reference
	2 (24.8–<30.4)	176/256	0.98 (0.75–1.30)	128/213	1.03 (0.74–1.42)
	3 (30.4–<35.3)	147/232	0.89 (0.66–1.20)	135/225	1.01 (0.73–1.40)
	4 (35.3–<42.1)	123/214	0.80 (0.59–1.09)	123/254	0.83 (0.60–1.16)
	5 (≥42.1)	93/188	0.71 (0.50–1.00)	143/277	0.99 (0.67–1.30)
			P-trend = *0.02*		*P*-trend = *0.37*
		P-interaction = *0.86*			
DMG (μmol/l)	1 (<2.9)	141/243	Reference	130/219	Reference
	2 (2.9–<3.3)	133/213	1.06 (0.78–1.44)	134/238	0.93 (0.68–1.27)
	3 (3.3–<3.9)	155/241	1.10 (0.81–1.48)	133/231	0.98 (0.71–1.34)
	4 (3.9–<4.6)	151/208	1.25 (0.92–1.71)	126/259	0.78 (0.57–1.07)
	5 (≥4.6)	141/251	0.93 (0.68–1.28)	121/215	0.90 (0.65–1.25)
			P-trend = *0.94*		*P*-trend = *0.29*
		P-interaction = *0.28*			

Italic values are defined as statistical significance at $P < 0.05$.

[a]Quintiles are based on the distribution of serum concentrations among controls.

[b]Unconditional logistic regression with case–control matching factors sex, age, and study center modeled as co-variates, adjusted for BMI, smoking status, physical activity, alcohol consumption, intakes of energy, fiber, red meat, and processed meat.

may explain why postmenopausal women tend to be less resistant against choline deficiency compared with premenopausal women [28]. Interestingly, betaine and choline intakes were associated with decreased all-cause and breast cancer-specific mortality [29], and rare variants of the *PEMT* rs12325817 SNP, which is associated with decreased choline biosynthesis, were associated with an increased risk of breast cancer [29]. In our study, the majority of the women were postmenopausal, as mean age at inclusion was 58.8 and 58.6 years among cases and controls, respectively. We also observed that mean choline concentration was lower among women than among men in our study (data not shown), and women may therefore have benefited more from a high choline status.

Although there was no significant interaction with age, the inverse associations of methionine and betaine were observed exclusively among participants <60 years at baseline, whereas DMG was associated with increased CRC risk in this age group. These differences could not be explained by an underlying difference with respect to the time between cohort inclusion and cancer diagnosis or large differences of the main exposure variables between the age groups (data not shown). Nevertheless, individuals ≥60 years may have benefited less from higher methyl group concentrations, possibly partly because they more often had undiagnosed colorectal adenomas compared with the younger age group. In this respect, a screening study of individuals 50–64 years of the general Norwegian population revealed that 17.1% of participants had at least one distal CRA [8], and this proportion is likely to increase with increasing age.

Finally, the number of CRC cases identified may have been insufficient to demonstrate an association, if any, of *BHMT* genotypes in the current study, and of other related one-carbon genetic variants with CRC risk [10, 20].

This study suggests that individuals with high plasma methionine, choline, or betaine concentrations may be at reduced risk

of CRC, and that these methyl group donors should be investigated further with respect to CRC risk. Repeated blood samples could help reflect lifetime exposure more accurately and could be an important focus for future research. A longer follow-up period is also recommendable to exclude the potential problem of reverse causality.

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disclosure

The authors have declared no conflicts of interest.

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