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Components of One-carbon Metabolism Other than Folate and Colorectal Cancer Risk

Robin Myte,[a] *Björn Gylling,*[b] *Jörn Schneede,*[c] *Per Magne Ueland,*[d] *Jenny Häggström,*[e] *Johan Hultdin,*[f] *Göran Hallmans,*[g] *Ingegerd Johansson,*[h] *Richard Palmqvist,*[b] *and Bethany Van Guelpen*[a]

Background: Despite extensive study, the role of folate in colorectal cancer remains unclear. Research has therefore begun to address the role of other elements of the folate-methionine metabolic cycles. This study investigated factors other than folate involved in one-carbon metabolism, i.e., choline, betaine, dimethylglycine, sarcosine, and methionine and relevant polymorphisms, in relation to the risk of colorectal cancer in a population with low intakes and circulating levels of folate.

Methods: This was a prospective case–control study of 613 case subjects and 1,190 matched control subjects nested within the population-based Northern Sweden Health and Disease Study. We estimated odds ratios (OR) by conditional logistic regression, and marginal risk differences with weighted maximum likelihood estimation using incidence data from the study cohort.

Results: Higher plasma concentrations of methionine and betaine were associated with modest colorectal cancer risk reductions (OR [95% confidence interval {CI}] for highest versus lowest tertile: 0.76 [0.57, 0.99] and 0.72 [0.55, 0.94], respectively). Estimated marginal risk differences corresponded to approximately 200 fewer colorectal cancer cases per 100,000 individuals on average. We observed no clear associations between choline, dimethylglycine, or sarcosine and colorectal cancer risk. The inverse association of methionine was modified by plasma folate concentrations (OR [95% CI] for highest/lowest versus lowest/lowest tertile of plasma methionine/folate concentrations 0.39 [0.24, 0.64], $P_{\text{interaction}} = 0.06$).

Conclusions: In this population-based, nested case–control study with a long follow-up time from baseline to diagnosis (median: 8.2 years), higher plasma concentrations of methionine and betaine were associated with lower colorectal cancer risk.

See Video Abstract at http://links.lww.com/EDE/B83.

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One-carbon metabolism is an intracellular network involving the folate and methionine cycles, in which methyl groups and other one-carbon units are transferred to acceptor molecules (Figure 1). A central role for one-carbon metabolism in cancer development is biologically plausible, as related nucleotide synthesis and methylation reactions including DNA methylation are vital for genome stability and function.

The potential role of one-carbon metabolism in colorectal cancer development has been extensively studied, with most research to date focusing on folate. Diets rich in natural folates are associated with reduced risk of colorectal cancer.[1] However, a dual role of folate has been proposed, both preventing and promoting cancer, depending on the dose and timing of exposure.[2,3] Folate is not a one-carbon unit donor per se, but an enzymatic co-factor important for maintenance of the substrate flux in one-carbon metabolism. It might therefore be expected to stabilize the genome of normal mucosa, protecting against cancer, while accelerating the progression of precancerous or malignant lesions.[2] Although evidence from animal studies and some observational studies support the dual role of folate in cancer development,[4–7] confirmation of the hypothesis has been challenging, due in part to very large differences in folate status between populations.[2]

For this reason, research increasingly addresses other components of one-carbon metabolism that serve as alternative sources of one-carbon units (Figure 1).[8,9] Choline, an essential nutrient obtained largely from egg, is oxidized to betaine, a

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From the [a]Department of Radiation Sciences, Oncology, [b]Department of Medical Biosciences, Pathology, [c]Department of Clinical Pharmacology, Pharmacology and Clinical Neurosciences, Umeå University, Umeå, Sweden; [d]Department of Clinical Science, University of Bergen and Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway; [e]Department of Statistics, Umeå School of Business and Economics, [f]Department of Medical Biosciences, Clinical Chemistry, [g]Department of Biobank Research, Public Health and Clinical Medicine, and [h]Department of Odontology, Cariology, Umeå University, Umeå, Sweden.

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Correspondence: Bethany Van Guelpen, Department of Radiation Sciences, Oncology Umeå University, SE-901 87 Umeå, Sweden. E-mail: bethany.vanguelpen@umu.se.

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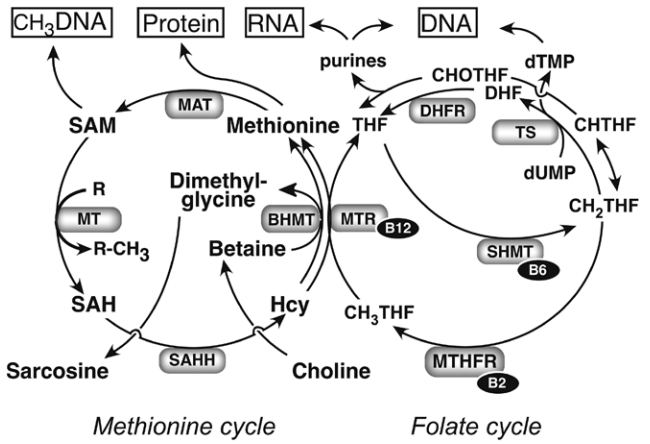

FIGURE 1. The folate and methionine cycles. Homocysteine is remethylated to methionine by the ubiquitous MTR using 5-methyltetrahydrofolate (CH3THF) as the methyl donor. Choline is oxidized to betaine, which donates a methyl group in an alternative remethylation of homocysteine to methionine catalyzed by BHMT in the liver and kidney. The other product of the BHMT reaction is dimethylglycine, which is further metabolized to sarcosine. Sarcosine is recycled to choline. Methionine is converted to the universal methyl group donor SAM. During the SAM-dependent transmethylation reaction, SAH is formed, which is then hydrolyzed to homocysteine. CBS indicates cystathionine β-synthase; CH2THF, 5,10-methylenetetrahydrofolate; CH3DNA, methylated DNA; CHOTHF, formyltetrahydrofolate; CHTHF, methenyltetrahydrofolate; CL, cystathionine lyase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxythymidine 5′-monophosphate; dUMP, deoxyuridine 5′-monophosphate; MTHFR, 5,10-methylenetetrahydrofolate reductase; MAT, methionine adenosyltransferase; MT, methyltransferase; MTR, methionine synthase; SAH, S-adenosylhomocysteine; SAHH, S-adenosylhomocysteine hydrolase; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; TS, thymidylate synthase.

methyl group donor in the betaine homocysteine S-methyltransferase (BHMT) reaction in the liver and kidney. This is an alternative route to methionine formation, in addition to the ubiquitous 5-methyltetrahydrofolate-dependent remethylation of homocysteine. The other product of the BHMT reaction besides methionine, dimethylglycine, is further metabolized to sarcosine. Both dimethylglycine and sarcosine serve as one-carbon unit donors in reactions forming methylenetetrahydrofolate.[10] While folate is involved in both nucleotide synthesis and methylation, other components of one-carbon metabolism are mainly sources of methyl groups.[4,7] A greater understanding of the relative contribution of components in the one-carbon metabolism, including methionine and factors in the choline oxidation pathway, to colorectal cancer risk may therefore provide new insight into the mechanistic role of one-carbon metabolism in carcinogenesis.[11]

To the best of our knowledge, only two previous prospective studies of colorectal cancer, nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort and the Women's Health Initiative (WHI),[12,13] and one cross-sectional study of colorectal adenoma,[14] have addressed the role of circulating one-carbon metabolites other than folate in colorectal cancer development. Inverse risk associations for betaine and null results for choline were observed in all three studies,[12–14] and an inverse association to colorectal cancer for methionine was reported in the EPIC study.[13]

The aim of this study was to investigate whether plasma concentrations of components of one-carbon metabolism, i.e., choline, betaine, dimethylglycine, sarcosine, and methionine, were related to colorectal cancer risk in a large, population-based, nested case–control study with long follow-up time. The population studied was characterized by low intake and circulating levels of folate. A secondary aim was to employ methods of marginal absolute risk estimation, in addition to more conventional statistical analyses and relative risks.

METHODS

Study Design and Cohorts

This is a nested case–control study within the Northern Sweden Health and Disease Study (NSHDS). Two population-based cohorts within the NSHDS were included: the Västerbotten Intervention Programme (VIP, 78 % of the study subjects) and the Mammography Screening Project in Västerbotten (MSP, 22 % of the study subjects), both of which have been described in detail elsewhere.[15] In the VIP, established in 1985, residents of Västerbotten County are invited to take part in a health survey upon turning 30 (years 1990–1996), 40, 50, and 60 years of age. The participants undergo laboratory tests and a medical examination, and may complete an extensive participant-administered lifestyle questionnaire as well as donate a fasting blood sample. As of March 31, 2009, the final date for case identification for the present study, the VIP included 83,621 individuals and 114,793 blood samples. Selection bias has been found to be low,[16] and the population-based nature of the VIP cohort is supported by comparisons of cancer incidence rates.[17] In the MSP, established in 1995 and concluded in 2006, women residing in Västerbotten County, approximately 50–70 years of age, were invited to donate a blood sample and completed a lifestyle questionnaire while attending mammography screening (54,787 blood samples from 28 802 women).

Study Subjects

Colorectal cancer case subjects diagnosed between October 17, 1986 and March 31, 2009 were identified by linkage with the Cancer Registry of Northern Sweden (ICD-10 18.0 and 18.2–18.9 for colon, 19.9 and 20.9 for rectum), with complete inclusion. All cases, as well as tumor stage data, were histopathologically verified by a single pathologist specialized in gastrointestinal pathology. We used patient records to verify tumor site. After case exclusions, we selected two control subjects for each case, matched by sex, birth year, cohort, fasting

status, and year of blood sampling and data collection. Exclusion criteria were previous cancer diagnosis other than nonmelanoma skin cancer, insufficient volume of plasma sample available (46 cases, nine controls), prioritizing to other studies (eight cases, 18 controls), location of primary tumor outside the colorectum (18 cases), sample infected by contagious disease (one case), no matching control obtainable (three cases), and no diagnosed cancer other than nonmelanoma skin cancer for controls at the time of diagnosis of their index cases. In addition, in the plasma analyses, five cases had high levels of methionine sulfoxide, indicating sample degradation.[7] We excluded these case sets (five cases and 10 controls) from the data analysis. After exclusions, a total of 613 cases and 1,190 controls were in included for the data analyses.

The subjects in the present study have previously been analyzed for plasma folate, vitamin B12, and homocysteine concentrations,[4,7,18] and for subjects with index case diagnosis in 1986–2003, also *MTHFR* polymorphisms,[4] in relation to colorectal cancer risk. A total of 17 cases and 33 controls in the present study were also included in previous EPIC studies.[13,19]

The study protocol was approved by the Research Ethics Committee of Umeå University, Umeå, Sweden. At the time of recruitment to the VIP or MSP cohort, all participants gave a written informed consent.

Blood Sampling and Analyses

In the VIP cohort, venous blood samples were collected in the morning, and only 34 of 1,409 study subjects (2 %) had fasted <4 hours, and 295 (21 %) <8 hours. In the MSP cohort, venous blood samples were collected during the course of the day, and 379 of 393 study subjects (96 %) had fasted for less than 4 hours at the time of blood sampling. Blood samples used in the present study were collected in EDTA tubes, separated into plasma, buffy coat, and erythrocyte fractions, aliquoted and cryopreserved at −80°C within 1 hour of collection, or at −20°C for at most 1 week before storage at −80°C. A large panel of plasma metabolites and polymorphisms involved in or relevant for one-carbon metabolism were analyzed at Bevital AS (Bergen, Norway).[20] Several of these were included in the present study, either as primary exposures of interest or as potential confounders or effect modifiers. Concentrations of riboflavin, pyridoxal 5′-phosphate, methionine, choline, betaine, dimethylglycine, sarcosine, and creatinine were measured with liquid chromatography–mass spectrometry methods.[21,22] Total plasma homocysteine concentrations were measured using an isotope dilution gas chromatography–mass spectrometry method.[23] Folate and vitamin B12 concentrations were determined with a microbiological method using *Lactobacillus casei* and *Lactobacillus leichmannii*, respectively, and adapted to a microtiter plate format and carried out by a robotic workstation.[24,25] In addition, three single nucleotide polymorphisms (*BHMT* 742G>A, *MTR* 2756A>G, and *MTHFR* 677C>T) were determined using MALDI-TOF mass spectrometry.[22] To prevent batch effects, samples were analyzed in matched case–control sets, with the position of the case randomized within each set. The investigators and laboratory staff were blinded to case and control status. Inter- and intra-assay coefficients of variation for the plasma analyses ranged from 2% to 12%.[26]

Statistical Analyses

We divided plasma concentrations of the metabolites into tertiles, with cut-off values based on the distribution of the controls. Plasma concentrations of metabolites between men and women did not vary by sex, so tertile cut-offs were not sex specific. For the polymorphisms, the allele frequencies of the minor alleles were low, so to maintain statistical power, we compared the common type genotype to the variant genotypes (heterozygous and homozygous pooled).

We calculated measures of relative risks for colorectal cancer in relation to the different levels of exposure variables as odds ratios (ORs) with 95% confidence intervals (CIs) by conditional logistic regression. We conducted tests for linear trends of the associations by evaluating the regression coefficient tests in conditional logistic regression models including quintiles of the plasma concentration variables as continuous variables, labelled with discrete numbers 1–5, with cut-offs based on the distribution of the controls. Quintiles were chosen over tertiles to account for the dose–response relationship while still taking into account the effect of potential high leverage observations.

To improve the validity and interpretability of the analyses, we computed absolute risk estimates in the form of marginal risk differences with the weighted maximum likelihood estimator using cumulative incidence data from the study cohort at large, and within groups defined by sampling year, age, sex, and cohort.[27] The cumulative incidence of colorectal cancer in the study cohort was approximately 830 per 100,000 over the period 1987–2009, and the average cumulative incidence over sampling year, age, sex, and cohort was approximately 660 per 100,000 individuals. We calculated cross validation 95% CIs for the risk differences by normal approximation based on 1,000 nonparametric bootstrap replications resampled from within the matched case sets.[28]

To adjust for potential confounders, we considered several variables for both relative and absolute risk estimation. Life-style-related variables included smoking status (current, not current smoker), body mass index (<25, 25–30, ≥30 kg/m^2), alcohol intake (zero intake, above/below sex-specific median of self-reported grams of alcohol/day), and recreational and occupational physical activity (self-reported on a scale from 1 to 5). We also evaluated glomerular filtration rates by the Cockcroft-Gault formula (calculated using plasma creatinine levels, age, sex, and body weight) as a possible confounder.[29] Since one-carbon unit donors and B-vitamins could jointly affect carcinogenesis,[2,14] we evaluated plasma concentrations of vitamins B2 (riboflavin), B6 (pyroxidal 5′-phosphate), and B12 (cobalamin) and methyl group donors (folate, methionine,

betaine, and choline) as potential confounders. We evaluated plasma total homocysteine concentrations, as a marker of the overall folate and cobalamin-dependent remethylation of homocysteine to methionine. As the *BHMT* 742G>A, *MTR* 2756A>G, and *MTHFR* 677C>T polymorphisms may affect enzymatic activity in one-carbon pathways, which in turn can affect both levels of circulating metabolites and influence the risk of carcinogenesis,[30–32] we also evaluated these polymorphisms as confounders. Finally, the metabolic syndrome may be related to colorectal cancer risk and plasma levels of choline and betaine.[33,34] Therefore, we evaluated factors related to the metabolic syndrome: diastolic and systolic blood pressure, plasma glucose and total cholesterol divided in tertiles based on the distribution of the controls, as potential confounders. Variables were included as covariates in the models if they altered any of the risk estimates by more than 10%.

We studied heterogeneity of the risk estimates between subgroups of colorectal cancer cases by stratification according to cancer site (right colon, left colon and rectum), cancer stage (I & II and III & IV), sex, age, and follow-up time from blood sampling to diagnosis. We evaluated two-way interactions between tertile groups of the one-carbon metabolites and relevant polymorphisms or folate tertiles in separate conditional logistic regression interaction models. We only tested for interactions with clear biological plausibility, i.e., exhibiting a close relation to folate-dependent and folate-independent pathways of remethylation (Figure 1).[35] Overall statistical significance for the interaction effects were determined by the regression coefficient tests corresponding to the multiplicative interaction term when modeling quintile metabolite and polymorphism variables as continuous variables. The significance level for the six interaction tests were adjusted for multiple testing with Bonferroni correction.[36]

All computations were conducted in R v.3.0.2 (R Foundation for Statistical Computing, Vienna, Austria.) All tests were two sided, and P values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics of cases and controls and tumor characteristics are presented in Table 1. Due to inclusion of the MSP cohort, females were slightly overrepresented. Subjects were slightly older at recruitment in the MSP compared with the VIP (median age 63.5 years [25th–75th percentile: 57.8–67.7 years] and 59.6 years [25th–75th percentile: 50.0–60.0 years], respectively). The median age at diagnosis was 65.2 years (25th–75th percentile: 59.3–70.2 years). Body mass index, physical activity (occupational and recreational), alcohol intake, and smoking habits were similar for cases and controls. No seasonal variation by date of blood sampling was observed for any of the plasma metabolites considered. Chi-square tests for the Hardy-Weinberg equilibrium showed that the genotype frequencies of the *MTR* 2756A>G polymorphism were not in equilibrium for the cases. Plasma concentrations of betaine and methionine were slightly lower in colorectal cancer cases compared with controls. None of the other metabolites or genotype distributions differed between cases and controls.

Risk estimates for the plasma metabolites are presented in Figure 2. Inverse associations were observed between colorectal cancer risk and plasma concentrations of betaine (highest versus lowest tertile OR: 0.76, 95% CI: 0.59, 0.99, P_{trend} = 0.05) and methionine (highest versus lowest tertile OR: 0.72, 95% CI: 0.55, 0.94, P_{trend} = 0.08). For dimethylglycine, a similar, although weaker, inverse relation with colorectal cancer risk was observed for the middle tertile, with no overall linear trend (middle versus lowest tertile OR: 0.80, 95% CI: 0.62, 1.0, P_{trend} = 0.93). In the marginal risk difference analyses, concentrations of betaine and methionine in the highest tertiles were associated with an estimated average absolute colorectal cancer risk reduction of 215 (95% CI: 9, 401) and 201 (95% CI: 26, 372) cases per 100,000 respectively, compared with the lowest tertiles (Figure 2). Plasma concentrations of choline or sarcosine showed no association with colorectal cancer risk in either analysis. In the risk difference analyses, adjusting for potential confounders, among which folate and riboflavin were strongest, slightly enhanced the estimated average effect of dimethylglycine and reduced the effect of methionine. Estimated risk parameters for choline and betaine were not altered by factors related to the metabolic syndrome.

An inverse relationship between betaine and colorectal cancer risk was only apparent in the subgroup of cases with follow-up times above the median of 8.2 years, whereas the association for methionine was only observed for the subgroup with follow-up times ≤8.2 years (Table 2). Furthermore, a suggestion of an inverse association between choline and colorectal cancer risk was found for subjects with the longer follow-up. Subtype analysis by cancer site showed that the association between betaine and colorectal cancer occurrence was present primarily in colon cancers (eAppendix, eTable 1, http://links.lww.com/EDE/B75), specifically the left colon. Separate analyses for stages I, II and stages III, IV tumors revealed no clear patterns, with the exception of methionine, for which an inverse association with colorectal cancer risk was observed in stages I, II tumors only (eAppendix, eTable 1, http://links.lww.com/EDE/B75). In the age-stratified analyses, we only observed associations for betaine among younger subjects (≤59 years at recruitment), whereas associations for methionine were confined to older subjects (>59 years; eAppendix, eTable 2, http://links.lww.com/EDE/B75). There were no clear sex differences in the associations between one-carbon metabolites and colorectal cancer risk (eAppendix, eTable 2, http://links.lww.com/EDE/B75).

The variant genotypes of the *MTHFR* 677C>T and *MTR* 2756A>G polymorphisms were weakly associated with a lower colorectal cancer risk (*MTHFR* 677 CT/TT versus CC OR: 0.85, 95% CI: 0.70, 1.0, *MTR* 2756 AG/GG versus AA OR:

TABLE 1. Baseline Characteristics of Colorectal Cancer Cases and Their Matched Controls, and Tumor Characteristics of Cases

	Cases		Controls	
	N	**Median (quartiles) or %**	**N**	**Median (quartiles) or %**
Sex, women	360	59%	703	59%
Age[a] (y)	613	59.8 (50.1–60.1)	1,190	59.6 (50.1–60.1)
Follow-up time[a] (y)	613	8.2 (4.7–11.9)		
Tumor site[b]				
Right colon	183	30%		
Left Colon	215	35%		
Rectum	214	35%		
Stage[c]				
I–II	308	53%		
III–IV	276	47%		
Plasma concentrations[a]				
Choline (μmol/L)	606	8.62 (7.62–9.68)	1,189	8.64 (7.62–9.75)
Betaine (μmol/L)	606	30.0 (25.7–34.4)	1,189	30.8 (26.1–23.5)
Dimethylglycine (μmol/L)	606	3.6 (2.9–4.4)	1,189	3.6 (3.0–4.4)
Sarcosine (μmol/L)	613	1.5 (1.1–2.0)	1,190	1.5 (1.2–2.1)
Methionine (μmol/L)	613	25.9 (23.2–29.1)	1,190	26.4 (23.4–29.9)
MTHFR 677C>T[d]				
CC	326	49%	580	53%
CT	232	41%	489	38%
TT	53	10%	112	9%
MTR 2756A>G				
AA	375	59%	685	62%
AG	192	35%	413	31%
GG	42	6%	75	7%
BHMT 742G>A				
GG	297	50%	580	49%
GA	264	42%	494	43%
AA	47	8%	94	8%

[a]Median and 25th–75th percentile.
[b]Site could not be determined for 1 case.
[c]Stage could not be determined for 29 cases.
[d]Pooled results for cases diagnosed 1986–2003 and their matched controls (previously published[16]) and for cases diagnosed 2003–2009. Genotype frequencies were essentially the same for the two time periods.

0.87, 95% CI: 0.71, 1.1, Figure 3). For the *BHMT* 742G>A polymorphism, there was no association with colorectal cancer risk. The results for *MTHFR* 677C>T in case sets with case diagnosis in the period 2003–2009, for which data have not previously been published, did not differ markedly from the pooled data presented above (*MTHFR* 677 CT/TT versus CC OR: 0.92, 95% CI: 0.70–1.3).

In the interaction analyses between folate or one-carbon metabolism-related polymorphisms and the plasma metabolites, we observed an inverse association between methionine and colorectal cancer risk in subjects with the variant *MTR* 2756 AG/GG genotypes ($P_{interaction}$ = 0.02, Table 3), whereas there were essentially no associations in subjects with the common *MTR* 2756 AA genotype. Similarly, an inverse association with betaine seemed to be restricted to subjects with the *BHMT* 742 GA/AA genotype. High methionine concentrations in combination with *MTHFR* 677 CT/TT genotypes appeared to be more strongly related to colorectal cancer risk. Yet, none of the gene–metabolite interaction tests were significant in the overall test after adjustment for multiple testing (corrected significance level in the six tests: $P < 0.008$). We observed a reduced colorectal cancer risk for high plasma methionine concentrations in subjects with low plasma folate concentrations (highest/lowest versus lowest/lowest methionine/folate tertiles OR: 0.39, 95%CI: 0.24, 0.64, $P_{interaction}$ = 0.06). There were no other clear interactions between the metabolites or with folate or polymorphisms.

DISCUSSION

The main finding of this population-based, prospective, case–control study nested within the NSHDS was a reduced colorectal cancer risk in subjects with higher plasma

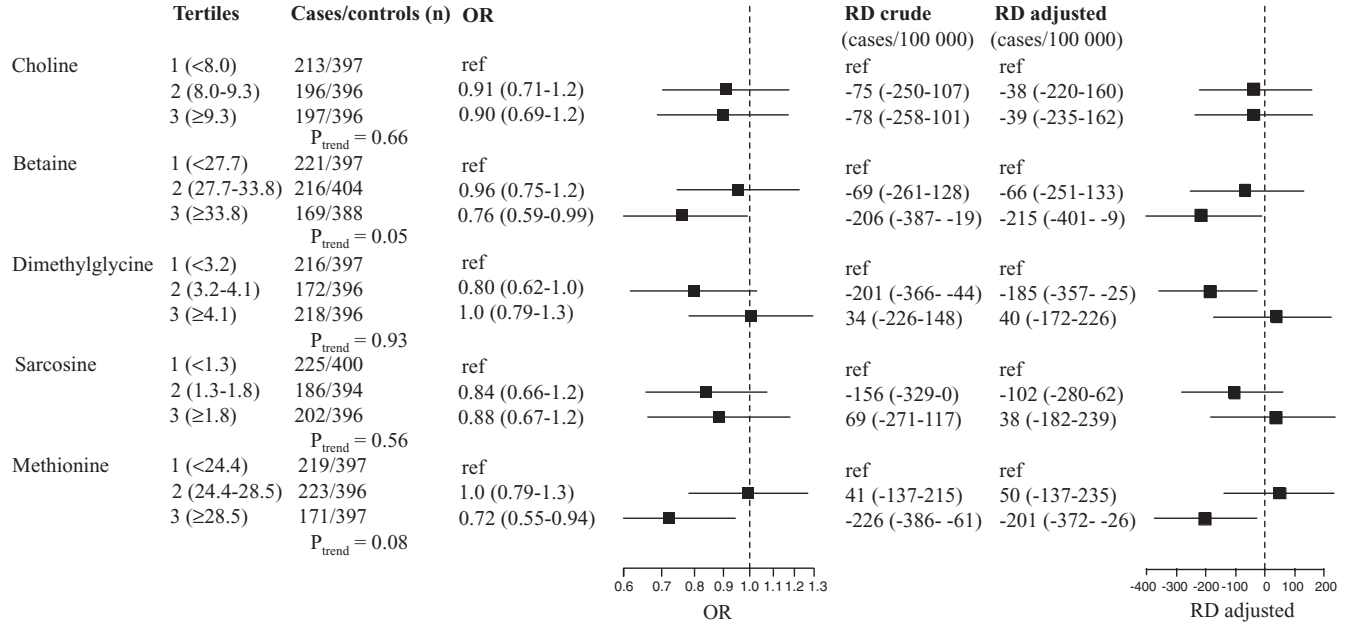

FIGURE 2. ORs and marginal RDs for colorectal cancer risk for the middle and highest versus lowest tertiles of plasma concentrations of one-carbon metabolites (μmol/L), with tertile cut-offs based on the distribution of the controls. Unadjusted ORs are presented, since adjusting for potential confounders had no effect on OR estimates. P_{trend} was calculated by modeling quintiles as continuous variables (labelled 1–5) in conditional logistic regression models. RDs were defined as the risk for the middle and highest tertile group minus the risk for the lowest tertile group. Crude RDs are adjusted only for the matching variables. Adjusted RDs were adjusted for folate, riboflavin (vitamin B2), cobalamin (vitamin B12), methionine, BMI, occupational physical activity, smoking status, and the matching variables. Other potential confounders had essentially no effect on RD estimates. BMI indicates body mass index; RD, indicates risk differences.

concentrations of methionine and betaine. Notably, this is consistent with earlier studies.[12–14] Absolute risk calculations in terms of marginal risk differences for high versus low plasma levels of betaine and methionine yielded estimated average reductions of colorectal cancer occurrence of approximately 200 cases per 100,000 individuals in the study cohort. Given the population-based nature of the cohort,[16,17] the potential effects of higher methionine and betaine concentrations on colorectal cancer risk may be substantial.

These results were in line with the two previous prospective studies of circulating levels of the same one-carbon metabolites,[12,13] despite a considerably lower folate status in our study cohort.[7,12,19] There is no mandatory folate fortification of food products in Sweden, and the use of supplements and natural food folate intake is generally low in the Northern Swedish population.[37] This might be expected to allow the discrimination of associations otherwise masked or compensated by higher folate status. Circulating levels of components of one-carbon metabolism need not necessarily reflect intracellular conditions in all types of cells, and even less relative distributions between intracellular compartments, such as the cytoplasm and mitochondria.[11] Rather, plasma levels are related to nutritional intake and cellular supply. It is thus conceivable that the relative contribution of methionine and betaine as one-carbon unit donors may be more pronounced in populations with low natural folate intake. Although the consistency of the inverse risk relationships for betaine and methionine and the null associations for choline support the validity of our findings across a wide range of folate levels, population differences should still be taken into account in future investigations.

We observed, in the present study, a rather strong (but imprecise) inverse relationship between methionine and colorectal cancer risk in subjects with low folate concentrations, which contrasts with the findings of the EPIC study.[13] This discrepancy may be due to the higher overall folate status in the EPIC cohort. Furthermore, no associations between plasma folate levels and colorectal cancer risk have been observed in the EPIC cohort.[19] The inverse association between plasma methionine and colorectal cancer risk in our study tended to be confined to cases with variant AG/GG *MTR* 2756 genotypes. Overall, the associations between the studied metabolites and colorectal cancer risk were essentially not modified by sex, median age at screening, tumor site, or tumor stage, with the possible exception of tumor site which modified the associations for of betaine and methionine.

Our results may support facilitated nucleotide synthesis, rather than DNA methylation, as the primary mechanism behind the putative accelerating effect of folate on premalignant and malignant lesions. Folate, choline, betaine, and

TABLE 2. ORs (95% CIs) for Colorectal Cancer Risk for Tertiles of Plasma Concentrations of One-carbon Metabolites in Subgroups Based on the Median Follow-up Time Between Blood Sampling and Diagnosis

		Median Follow-up <8.2 y		Median Follow-up ≥8.2 y	
	Tertiles[a]	Cases/Controls	OR (95% CI)[b]	Cases/Controls	OR (95% CI)[b]
Choline[a]	1 (<8.0)	99/205	Ref	114/192	Ref
	2 (8.0–9.3)	105/191	1.1 (0.80, 1.6)	91/205	0.72 (0.50, 1.0)
	3 (≥9.3)	96/187	1.0 (0.71, 1.5)	101/209	0.78 (0.54, 1.1)
	P_{trend}[c]		0.61		0.27
Betaine[a]	1 (<27.7)	111/196	Ref	110/201	Ref
	2 (27.7–33.8)	100/212	0.84 (0.59, 1.2)	116/192	1.1 (0.78, 1.6)
	3 (≥33.8)	89/175	0.89 (0.62, 1.3)	80/213	0.66 (0.45, 0.95)
	P_{trend}[c]		0.72		0.02
Dimethylglycine[a]	1 (<3.2)	100/185	Ref	116/212	Ref
	2 (3.2–4.1)	89/210	0.79 (0.55, 1.1)	83/186	0.81 (0.57, 1.2)
	3 (≥4.1)	111/188	1.1 (0.75, 1.5)	107/208	0.93 (0.66, 1.3)
	P_{trend}[c]		0.38		0.35
Sarcosine[a]	1 (<1.3)	96/176	Ref	129/224	Ref
	2 (1.3–1.8)	76/152	0.92 (0.62, 1.4)	110/242	0.80 (0.58, 1.1)
	3 (≥1.8)	134/256	0.97 (0.64, 1.5)	68/140	0.82 (0.56, 1.2)
	P_{trend}[c]		0.88		0.37
Methionine[a]	1 (<24.4)	112/166	Ref	107/231	Ref
	2 (24.4–28.5)	103/201	0.74 (0.52, 1.0)	120/195	1.3 (0.94, 1.8)
	3 (≥28.5)	91/217	0.56 (0.39, 0.82)	80/180	0.92 (0.63, 1.3)
	P_{trend}[c]		0.04		0.69

[a]Tertile cut-offs based on the distribution of the controls (concentrations in µmol/L).
[b]Unadjusted ORs calculated by conditional logistic regression. Adjusting for potential confounders had essentially no effect on parameter estimates.
[c]P_{trend} values were calculated by modeling quintiles of plasma concentrations (labelled 1–5), with cut-offs based on the distribution of the controls, as continuous variables in conditional logistic regression models.

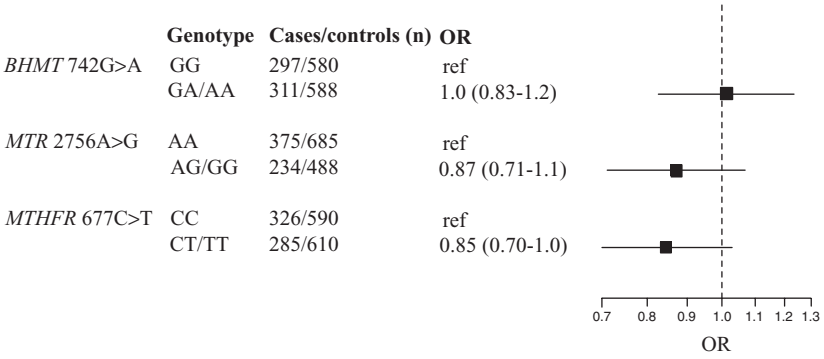

FIGURE 3. ORs for colorectal cancer risk according to genotypes of single nucleotide polymorphisms involved in one-carbon metabolism. Unadjusted ORs are presented because adjusting for potential confounders had no effect on OR estimates. MTR indicates methionine synthase; MTHFR, 5,10-methylenetetrahydrofolate reductase.

methionine all contribute to the availability of one-carbon units.[9,11] However, whereas folate is important for both nucleotide synthesis and methylation reactions, the role of choline, betaine, and methionine is limited to folate-independent remethylation pathways, where they may gain importance in the presence of low folate status.[38] Still, betaine is a relatively weak determinant of total homocysteine concentrations.[39] Thus, the findings in this study contradict neither the null association for plasma total homocysteine concentrations nor the positive association for plasma folate concentrations in relation to colorectal cancer risk previously observed in our study population.[4,7] Conceivably, lower folate supply may decrease synthesis of purine and pyrimidine in rapidly dividing hyperplastic and dysplastic cells, hampering colorectal cancer development.[2]

Given the relatively slow development of colorectal cancer, roughly 10 years, cases with shorter follow-up time between baseline and diagnosis are more likely to harbor undiagnosed precancerous lesions or occult colorectal cancer at the time of sampling. Methionine, but not betaine, showed stronger associations with colorectal cancer risk in cases with shorter follow-up times (Table 2). Methionine and betaine are

TABLE 3. Biologically Plausible Interactions Between One-carbon Metabolites and Related Single Nucleotide Polymorphisms for Colorectal Cancer Risk

	First Tertile		Second Tertile		Third Tertile	
Variable	**Cases/Controls (n)**	**OR (95%CI)[a]**	**Cases/Controls (n)**	**OR (95%CI)[a]**	**Cases/Controls (n)**	**OR (95%CI)[a]**
Betaine[b]	<27.7 µmol/L		27.7–33.8 µmol/L		≥33.8 µmol/L	
BHMT 742G>A						
GG	100/192	Ref	99/202	0.93 (0.65, 1.3)	96/186	0.97 (0.68, 1.4)
GA/AA	118/199	1.1 (0.81, 1.6)	96/186	1.1 (0.80, 1.6)	73/197	0.68 (0.47, 0.99)
$P_{interaction}$[c] = 0.34						
Dimethylglycine[b]	<3.2 µmol/L		3.2–4.1 µmol/L		≥4.1 µmol/L	
BHMT 742G>A						
GG	96/192	Ref	85/181	0.94 (0.65, 1.4)	114/207	1.1 (0.77, 1.6)
GA/AA	119/199	1.2 (0.85, 1.6)	84/206	0.79 (0.55, 1.1)	103/182	1.1 (0.77, 1.6)
$P_{interaction}$ = 0.77						
Methionine[b]	<24.4 µmol/L		24.4–28.5 µmol/L		≥28.5 µmol/L	
BHMT 742G>A						
GG	110/199	Ref	113/183	1.1 (0.79, 1.5)	74/198	0.64 (0.44, 0.92)
GA/AA	106/193	0.98 (0.70, 1.4)	108/204	0.92 (0.66, 1.3)	97/191	0.81 (0.57, 1.2)
$P_{interaction}$ = 0.26						
MTR 2756A>G						
AA	122/244	Ref	145/235	1.2 (0.90, 1.7)	108/206	0.96 (0.69, 1.4)
AG/GG	94/150	1.3 (0.90, 1.8)	77/152	0.98 (0.69, 1.4)	63/186	0.62 (0.43, 0.91)
$P_{interaction}$ = 0.02						
MTHFR 677C>T						
CC	117/185	Ref	111/200	0.85 (0.61, 1.2)	98/195	0.73 (0.51, 1.0)
CT/TT	100/211	0.74 (0.53, 1.0)	112/191	0.91 (0.65, 1.3)	73/199	0.53 (0.37, 0.77)
$P_{interaction}$ = 0.93						
Folate tertiles[b]						
1 (<5.4 nmol/L)	78/135	Ref	64/121	0.85 (0.56, 1.3)	38/141	0.39 (0.24, 0.64)
2 (5.4–9.1 nmol/L)	76/136	1.0 (0.67, 1.6)	85/144	1.1 (0.71, 1.6)	68/116	1.0 (0.65, 1.6)
3 (≥9.1 nmol/L)	65/126	0.96 (0.61, 1.5)	74/131	1.1 (0.68, 1.6)	65/140	0.82 (0.52, 1.3)
$P_{interaction}$ = 0.06						

[a]Unadjusted ORs calculated by conditional logistic regression. Adjusting for potential confounders had essentially no effect on parameter estimates.
[b]Tertile cut-offs based on the distribution of the controls.
[c]*P* values were calculated by modeling quintile plasma concentration variables (labelled 1–5) and genotype variables as continuous variables in multiplicative conditional logistic regression interaction models.
MTR indicates methionine synthase; MTHFR, 5,10-methylenetetrahydrofolate reductase.

essential for S-adenosylmethionine (SAM) synthesis, which provides methyl groups for DNA methylation, relevant for colorectal cancer in which global hypomethylation is common, but in which some tumors acquire increased promoter hypermethylation.[40] SAM also appears to induce apoptosis in colon cancer cells.[41] Choline also donates (via betaine) methyl moieties for SAM synthesis. However, the conversion of choline to betaine may be inhibited in the presence of metabolic syndrome,[34] which is also positively related to colorectal cancer risk.[33] However, factors related to the metabolic syndrome did not appear to represent confounders in the present study.

The main strengths of the present study were the population-based design in combination with the availability of prediagnostic plasma samples. The risk of reverse causation was reduced by the long follow-up time between baseline blood sampling and diagnosis of the colorectal cancer cases (median 8.2 years). The median follow-up times of the previously published studies were relatively short (median 3.6 and 5.2 years for EPIC and WHI, respectively),[12,13] and concerns about potential reverse causality were expressed.[13] A longer follow-up is particularly advantageous in studies of folate and related metabolites, for investigation of possible dual effects

in colorectal cancer prevention and progression. Another strength of this study was the calculation of marginal risk differences, which provides additional information on how colorectal cancer occurrence in the study cohort could have been affected by variation in the studied metabolites.[27] The main weakness of the study was that only a single baseline blood sample from each participant was analyzed, preventing assessment of longitudinal variability in metabolite status. However, we have previously observed a fair to good within-subject reproducibility over time for betaine, dimethylglycine, and sarcosine,[42] which minimizes the risk of regression dilution bias for these biomarkers. In contrast, reproducibility is poor for choline and methionine,[42] which may suggest that our findings for choline and methionine could be underestimations of the "true" associations. Another potential drawback of this study and other similar studies in which associations between candidate risk factors and colorectal cancer are sequentially tested in univariate models is that more complex interactions and joint effects of the studied genetic and environmental factors may not be discovered.

In conclusion, we observed an inverse association between plasma concentrations of methyl group donors betaine and methionine and colorectal cancer risk, for methionine particularly in subjects with shorter follow-up or in combination with low plasma folate concentrations. These results suggest that facilitated nucleotide synthesis, rather than changes in genomic DNA methylation, may represent the driving force behind the putative accelerating effect of folate on colorectal cancer development.

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