Research Article

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Biomarkers Related to One-Carbon Metabolism as Potential Risk Factors for Distal Colorectal Adenomas

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Abstract

Background: Efficient one-carbon metabolism, which requires adequate supply of methyl group donors and B-vitamins, may protect against colorectal carcinogenesis. However, plasma folate and vitamins B2 and B12 have inconsistently been associated with colorectal cancer risk, and there have been no previous studies relating plasma concentrations of methionine, choline, and betaine to this outcome.

Methods: This study comprised 10,601 individuals, 50 to 64 years of age, participating in the Norwegian Colorectal Cancer Prevention (NORCCAP) screening study. Using logistic regression analyses, we cross-sectionally investigated associations between distal colorectal adenoma occurrence–potential precursor lesions of colorectal carcinomas–and plasma concentrations of methyl group donors and B-vitamins, and polymorphisms of genes related to one-carbon metabolism.

Results: Screening revealed 1,809 subjects (17.1%) with at least one adenoma. The occurrence of high-risk adenomas (observed in 421 subjects) was inversely associated with plasma concentrations of methionine (highest *versus* lowest quartile: odds ratio (OR) = 0.61; 95% confidence interval (CI) = 0.45–0.83), betaine: OR = 0.74; 95% CI = 0.54–1.02, the vitamin B2 form flavin-mononucleotide (FMN): OR = 0.65; 95% CI = 0.49–0.88, and the vitamin B6 form pyridoxal 5′-phosphate (PLP): OR = 0.69; 95% CI = 0.51–0.95, but not with folate, choline, vitamin B12 concentrations, or with the studied polymorphisms. High methionine concentration in combination with high vitamin B2 or B6 concentrations was associated with lower occurrence of high-risk adenomas compared with these factors individually.

Conclusions: High plasma concentrations of methionine and betaine, and vitamins B2 and B6 may reduce risk of developing colorectal adenomas.

Impact: In addition to B-vitamins, methyl group donors such as methionine and betaine may play a role in colorectal carcinogenesis. *Cancer Epidemiol Biomarkers Prev; 20(8); 1726–35. ©2011 AACR.*

Introduction

Efficient one-carbon metabolism requires adequate supply of methyl group donors and B-vitamins, and may protect against colorectal cancer by reducing DNA instability and by affecting DNA methylation patterns (1). Using methyl-tetrahydrofolate (CH_3THF) as cosubstrate, homocysteine is remethylated to methionine, a reaction that is either catalyzed by the enzyme methionine synthase (MTR) that requires vitamin B12 as cofactor, or by betaine homocysteine methyltransferase (BHMT) that uses betaine as a methyl donor. Methionine is subsequently converted to S-adenosylmethionine, which is the universal methyl group donor (Fig. 1).

Similar to low folate status, a low status of the methyl group donors methionine, choline, and betaine is potentially involved in carcinogenesis (2). Vitamins B2, B6, and B12 function as enzymatic cofactors in the one-carbon metabolism, and an adequate supply of these vitamins is hypothesized to protect against cancer. In addition, single nucleotide polymorphisms (SNP) in genes encoding one-carbon metabolism enzymes may affect enzymatic activity and thereby the bioavailability of methyl groups, and subsequent cancer risk.

Little epidemiological data exists on the associations of choline and its metabolite betaine with colorectal cancer

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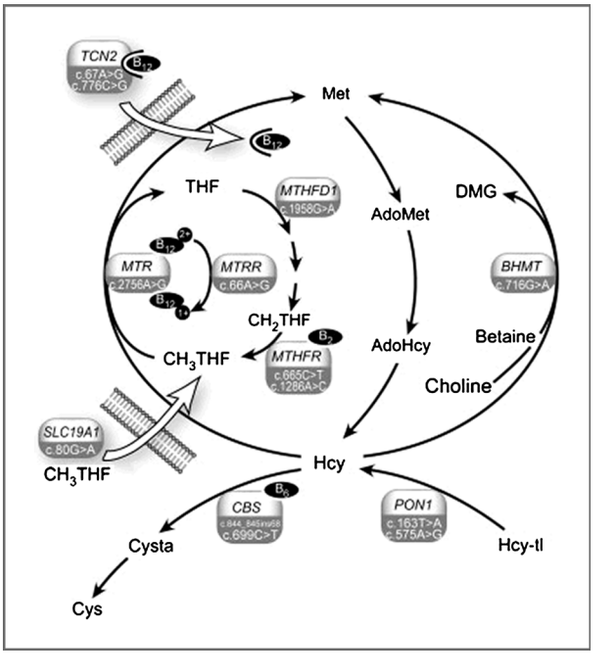

Figure 1. One-carbon metabolism and related gene polymorphisms. The figure shows the components of the one-carbon metabolism that have been measured in the study population, and the enzymes and transports involved. Homocysteine (Hcy) is formed from S-adenosylhomocysteine (AdoHcy), which is a product of S-adenosylmethionine (AdoMet)-dependent transmethylation reactions. It is remethylated to methionine (Met), a reaction that is either catalyzed by the enzyme, methionine synthase (MTR), that requires vitamin B12 as cofactor and methyltetrahydrofolate (CH3THF) as cosubstrate or by betaine homocysteine methyltransferase (BHMT) that uses betaine as a methyl donor. Hcy is converted to cysteine (Cys) in the transsulfuration pathway, the first step of which is formation of cystathionine (Cysta) catalyzed by the vitamin B6-dependent enzyme, cystathionine beta-synthase (CBS). (CH_2THF, methylenetetrahydrofolate; DMG, dimethylglycine; Hcy-tl, homocysteine thiolactone; MTHFD1, trifunctional enzymemethylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase; MTHFR, methylenetetrahydrofolate reductase; MTRR, methionine synthase reductase; PON1, paraoxonase-1; SLC19A1, reduced folate carrier-1;TCN2, transcobalamin-II; and THF, tetrahydrofolate).

risk. Cho and colleagues (3) previously reported a positive association of choline intake and an inverse association of betaine intake with distal colorectal adenoma risk among women, whereas no associations were observed with colorectal cancer among men (4). Methionine intake was not associated with colorectal cancer risk in several studies (5–13). However, the associations of plasma concentrations of methionine, choline, and betaine with risk of colorectal adenomas or carcinomas have not been studied so far.

There was a weak inverse association between dietary folate intake and colorectal cancer risk (14), and blood folate levels were inversely associated in some (15–18), but not all (10, 19–24) studies. Vitamin B12 status was not associated with colorectal cancer in the majority of the studies (21, 24–26). In addition, plasma vitamin B2 has been reported to be not associated (24) or inversely associated (25) with colorectal cancer risk. On the other hand, vitamin B6 has consistently been associated with reduced colorectal cancer risk, as indicated by a recent meta-analysis on prospective studies (27).

It is important to identify potential risk factors for high-risk colorectal adenomas, which are the assumed precursors of most colorectal carcinomas (28). NORCCAP (Norwegian Colorectal Cancer Prevention) is a large-scale population-based trial in which presumptively healthy individuals underwent flexible sigmoidoscopy screening. All neoplastic lesions were removed and ascertained for size and histology. Here we report on associations between plasma or serum concentrations of folate, methionine, choline, betaine, vitamins B2, B6, and B12, related one-carbon metabolism genetic variants, and the occurrence of distal colorectal adenoma in the NORCCAP trial.

Subjects and Methods

Study population and end-points

Detailed information about the study design, inclusion criteria, response rates, and measurements of NORCCAP has been published previously (29). In brief, 12,960 individuals 50 to 64 years of age living in the city of Oslo or Telemark County in Norway were randomly drawn from the population registry, and underwent flexible sigmoidoscopy screening for colorectal cancer between 1999 and 2001. Any adenoma detected at the screening prompted work-up colonoscopy. Adenoma status for individual participants was defined irrespective of localization, by the most advanced adenoma found at sigmoidoscopy screening and colonoscopy work-up. Colorectal adenomas were categorized into high-risk adenomas (i.e., adenomas $\geq$10 mm in diameter and/or those showing villous components and/or severe dysplasia) or alternatively low-risk adenomas. Individuals with screen-detected cancer ($n = 34$) were excluded. The present study includes 10,601 NORCCAP participants where blood samples were available. Written informed consent was obtained from all participants, and the study was approved by the Regional Ethics Committee and the Norwegian Data Inspectorate.

Biochemical analyses

Nonfasting blood samples were collected at screening and centrifuged at 1100 *g* for 10 minutes at 23°C. Serum and EDTA plasma were separated and subsequently stored at −80°C until further analysis. Serum concentrations of folate and vitamin B12 (cobalamin) were measured by microbiological assays (30, 31), whereas methionine, choline, betaine, vitamin B2 species [riboflavin and flavin-mononucleotide (FMN)], and vitamin B6 species [pyridoxal 5′-phosphate (PLP), pyridoxal (PL), and 4-pyridoxic acid (PA)] were measured in plasma using liquid chromatography-tandem mass spectrometry (32, 33). Samples were analyzed in batches of 86 and quality control included six calibration samples, two

control samples and one blank sample in each batch. Coefficients of variation were 4% (folate), 2.6% (methionine), 4% (betaine), 3% (choline), 3% to 6% (vitamin B2 species), 3% to 9% (vitamin B6 species), and 4% (cobalamin). All samples were kept at −80°C and analyzed in random order. The laboratory staff was blinded to the clinical outcome status in both the main analyses as well as in quality control analyses. All biomarkers were analyzed in the laboratory of BEVITAL AS (34).

Genotyping

SNPs in genes encoding one-carbon metabolism enzymes were determined using real-time PCR for *methylenetetrahydrofolate reductase (MTHFR) 677C > T* (35), or by matrix-assisted laser-desorption/ionization-time-of-flight mass spectrometry (MALDITOF-MS; ref. 36) for *MTHFR 1298A > C, methionine synthase (MTR) 2756A > G, methionine synthase reductase (MTRR) 66A > G, methylenetetrahydrofolate dehydrogenase (MTHDF1) 1958G > A, reduced folate carrier-1 (SLC19A1) 80G > A, transcobalamin-II (TCN2) 67A > G, TCN2 776C > G, betaine homocysteine methyltransferase (BHMT) 742G > A, cystathionine β-synthase (CBS) 699C > T, CBS 844ins68, paraoxonase-1 (PON1) 163T > A,* and *PON1 575A > G*, as has been described in detail previously (37). Four blank DNA samples were added to each set of 92 samples to monitor contaminations during (real-time) PCR or primer extension reaction. Hardy–Weinberg equilibrium was estimated by Chi-square tests and equilibrium was defined as $P > 0.05$. Among the 13 polymorphisms, three (*MTHFR 677C > T*, *MTR 2756A > G*, and *MTDF1 1958G > A*) were in Hardy–Weinberg equilibrium, whereas the 10 remaining SNPs showed deviation from equilibrium due to the large size of the study population (37).

Other characteristics

Trained medical staff interviewed participants on attendance to register age at screening, weight, and height reported by the participant, smoking habits, alcohol consumption, hormone replacement therapy (HRT) among women, and the use of nonsteroidal antiinflammatory drugs (NSAIDS) including acetylsalicylic acid (ASA).

Statistical analyses

Mean (SD) age and body mass index (BMI, kg/m^2), as well as frequencies of categories of the remaining characteristics were calculated among participants without neoplasia, low-risk colorectal adenoma, and high-risk colorectal adenoma, and tested for differences across these three categories using linear regression analyses or Chi-square tests, respectively. Least-square mean concentrations and 95% confidence intervals (CI) were estimated across the three groups for folate, methionine, betaine, choline, vitamin B2 (riboflavin and FMN), vitamin B6 (PLP, PL, PA), and vitamin B12 (cobalamin). The mean values were adjusted for age, sex, study center, smoking habits, and alcohol consumption, and analyzed for differences by type III sums of squares hypothesis testing.

Genotype frequencies were calculated for common homozygotes, heterozygotes, and variant homozygotes across subjects without neoplasia, low-risk colorectal adenoma, and high-risk colorectal adenoma. We used logistic regression analyses to estimate odds ratios (OR) and corresponding 95% CIs as an approximation of prevalence ratios. As such, we estimated age–, sex–, and study center–adjusted ORs for low-risk and high-risk colorectal adenoma taking common homozygotes as reference categories.

In addition to age, sex, and study center, the associations of methyl group donors and B-vitamin concentrations with colorectal adenoma occurrence were adjusted for smoking habits and alcohol consumption. These factors were associated with adenoma occurrence (as indicated by logistic regression analyses), with the majority of the biochemical variables (as indicated by partial correlation coefficients), and inclusion of either smoking or alcohol in the logistic regression models altered at least one of the estimated associations by more than 10%.

The biochemical variables were categorized into quartiles based on the distribution among participants without neoplasia at screening, and the concentrations among participants with neoplasia were subsequently categorized according to these quartiles. Multivariate-adjusted odds ratios and 95% CIs were estimated for low-risk colorectal adenoma and high-risk colorectal adenoma taking the lowest quartiles as reference categories. Tests for linear trend over quartiles were carried out by fitting the ordinal exposure variables as continuous variables. Associations of the methyl donors and B-vitamins were evaluated across genotypes and tested for interaction.

Because it may be hypothesized that methyl group donors and B-vitamins affect carcinogenesis by collectively affecting the bioavailability of methyl groups, we investigated two-way interactions between the methyl group donors (folate, methionine, betaine, and choline) on the one hand, and B-vitamins (B2, B6, and B12) on the other. Based on the distribution among individuals free of neoplasia at screening, the median concentration for each methyl group donor or B-vitamin was used as cutoff value to define low and high concentrations. These interactions were tested by modeling continuous variables. In addition, we calculated interactions and carried out stratified analyses across categories of smoking habits and alcohol consumption.

All statistical analyses were conducted with version 9.2 of the SAS statistical software package.

Results

Among the 10,601 individuals participating in NORCCAP, 1,388 had low-risk colorectal adenomas whereas 421 individuals had high-risk adenomas (Table 1). Participants with low-risk and high-risk colorectal adenomas were more often men, were generally older, had a higher BMI, consumed more alcohol, and were more likely current smokers compared with those without neoplasia.

Table 1. Baseline characteristics of participants with available blood samples in the Norwegian colorectal cancer prevention (NORCCAP) screening study

	No neoplasia	Low-risk colorectal adenoma	High-risk colorectal adenoma	*P*-value[a]
N (%)[b]	8792 (82.9)	1388 (13.1)	421 (4.0)	
Sex [men (*n*,%)]	4131 (47.0)	816 (58.8)	271 (64.4)	<0.001
Age [mean, (SD)]	56.1 (3.8)	56.7 (3.7)	57.2 (3.6)	<0.001
Body mass index [kg/m^2; mean, (SD)]	25.7 (3.8)	26.0 (3.8)	25.9 (4.0)	0.001
Smoking habits (*n*,%)				
Never smoker	3630 (41.5)	451 (32.7)	108 (25.7)	
Ex smoker	2551 (29.2)	405 (29.3)	110 (26.1)	
Current smoker	2568 (29.3)	524 (38.0)	203 (48.2)	<0.001
Alcohol consumption (*n*,%)				
Abstainer	3326 (37.9)	478 (34.5)	120 (28.5)	
1–29 units per month	4464 (50.9)	702 (50.6)	220 (52.3)	
30–59 units per month	800 (9.1)	149 (10.8)	64 (15.2)	
$\geq$ 60 units per month	178 (2.0)	57 (4.1)	17 (4.0)	<0.001
Use of NSAIDs and/or ASA (*n*,%)				
Yes	868 (9.9)	119 (8.6)	37 (8.8)	0.24
Use of HRT (*n*,%)				
Never	3405 (73.7)	443 (77.7)	115 (77.2)	
<5 years	766 (16.6)	77 (13.5)	21 (14.1)	
$\geq$5 years	449 (9.7)	50 (8.8)	13 (8.7)	0.26
MTHFR C677T genotypes (*n*,%)				
CC	4463 (51.1)	747 (54.1)	200 (47.8)	
CT	3563 (40.8)	531 (38.5)	183 (43.8)	
TT	708 (8.1)	100 (7.3)	35 (8.4)	0.14
Plasma and serum concentrations[c]				
Folate (nmol/L)	18.35 (17.93–18.78)	18.08 (17.40–18.76)	18.27 (17.15–19.39)	0.72
Methionine (μmol/L)	24.00 (23.79–24.21)	23.87 (23.54–24.20)	23.09 (22.54–23.63)	0.003
Betaine (μmol/L)	37.30 (36.86–37.74)	36.76 (36.06–37.47)	35.80 (34.63–36.97)	0.02
Choline (μmol/L)	9.15 (9.07–9.23)	9.16 (9.03–9.28)	9.08 (8.87–9.28)	0.78
Riboflavin (nmol/L)	17.88 (16.85–18.90)	18.44 (16.80–20.08)	17.46 (14.75–20.17)	0.72
FMN (nmol/L)	13.30 (13.03–13.56)	13.43 (13.01–13.86)	13.01 (12.31–13.71)	0.54
PLP (nmol/L)	71.33 (69.31–73.34)	69.62 (66.37–72.87)	64.58 (59.20–69.96)	0.03
PL (nmol/L)	23.30 (18.19–28.41)	23.38 (15.20–31.56)	17.96 (4.43–31.50)	0.73
PA (nmol/L)	44.29 (38.31–50.28)	43.04 (33.46–52.63)	37.18 (21.32–53.04)	0.66
Cobalamin (pmol/L)	334.3 (326.6–342.0)	335.8 (323.5–348.1)	341.2 (320.8–361.6)	0.78

[a]*P*-value for differences between participants without neoplasia, low-risk adenoma, or high-risk adenoma. Chi-square tests for categorical variables, ANOVA for the continuous variables age and BMI, or type III sums of squares hypothesis tests for the plasma and serum concentrations.

[b]Summarized frequencies of subject characteristics may be lower than the total number of subjects due to missing values.

[c]Least-square mean concentrations (95% confidence interval) adjusted for age, sex, study centre, smoking habits, and alcohol consumption. Folate and cobalamin concentrations were measured in serum; all other biochemical variables were measured in plasma.

Concentrations of methionine, betaine, and PLP were lower in participants with high-risk colorectal adenoma compared with individuals free of neoplasia. However, folate, choline, vitamin B2, and vitamin B12 concentrations did not differ significantly between the groups. None of the genotype frequencies differed across the three groups, and we did not observe associations between the genotypes and colorectal adenoma occurrence (Supplementary Tables S1 and S2).

In analyses adjusted for age, sex, study center, smoking habits and alcohol intake, we observed that high methionine concentration was associated with lower occurrence of high-risk colorectal adenoma (highest *versus* lowest quartile: OR = 0.61; 95% CI 0.45–0.83, $P_{trend} < 0.001$, Table 2). Plasma betaine concentration was also inversely associated with high-risk colorectal adenoma ($OR_{highest\ vs\ lowest\ quartile} = 0.74$; 95% CI 0.54–1.02, $P_{trend} = 0.03$), but plasma choline did not show an association.

Table 2. Multivariate-adjusted logistic regression analyses with corresponding odds ratios and 95% confidence intervals for colorectal adenoma, according to quartiles of methyl group donors and B-vitamin concentrations

		Odds ratios for colorectal adenoma occurrence[a]				
		Overall analyses		High-risk adenomas, across *MTHFR C677T* genotypes		$P_{interaction}$[b]
Concentration	Quartiles (range)[c]	Low-risk Adenomas (n = 1380)	High-risk Adenomas (n = 421)	*677CC* (n = 200)	*677CT+TT* (n = 218)	
Methyl group donors						
Folate (nmol/L)	1 (<10.10)	1.00	1.00	1.00	1.00	
	2 (10.10–<13.74)	1.01 (0.86–1.19)	1.00 (0.76–1.32)	1.38 (0.89–2.15)	0.85 (0.59–1.24)	
	3 (13.74–<20.33)	1.05 (0.89–1.23)	0.98 (0.74–1.30)	1.37 (0.88–2.13)	0.80 (0.53–1.19)	0.49
	4 (≥20.33)	0.94 (0.79–1.11)	1.04 (0.78–1.39)	1.27 (0.81–2.00)	1.00 (0.68–1.47)	
	P-trend	0.60	0.82	0.40	0.80	
Methionine (μmol/L)	1 (<19.53)	1.00	1.00	1.00	1.00	
	2 (19.53–<22.51)	0.89 (0.76–1.05)	0.88 (0.67–1.15)	0.86 (0.58–1.28)	0.87 (0.60–1.25)	
	3 (22.51–<26.35)	0.91 (0.77–1.08)	0.66 (0.50–0.89)	0.77 (0.52–1.16)	0.56 (0.37–0.84)	0.90
	4 (≥26.35)	0.90 (0.76–1.07)	0.61 (0.45–0.83)	0.65 (0.42–1.01)	0.52 (0.33–0.80)	
	P-trend	0.31	<0.001	0.05	<0.001	
Betaine (μmol/L)	1 (<28.00)	1.00	1.00	1.00	1.00	
	2 (28.00–<35.30)	1.08 (0.91–1.28)	1.10 (0.82–1.47)	0.98 (0.65–1.50)	1.17 (0.78–1.76)	
	3 (35.30–<43.50)	1.09 (0.92–1.29)	0.94 (0.70–1.28)	0.96 (0.63–1.47)	0.93 (0.60–1.43)	0.14
	4 (≥43.50)	0.86 (0.72–1.03)	0.74 (0.54–1.02)	0.66 (0.42–1.05)	0.82 (0.53–1.29)	
	P-trend	0.09	0.03	0.07	0.19	
Choline (μmol/L)	1 (<7.30)	1.00	1.00	1.00	1.00	
	2 (7.30–<8.62)	1.23 (1.04–1.45)	0.86 (0.65–1.14)	0.53 (0.35–0.81)	1.35 (0.91–2.01)	
	3 (8.62–<10.18)	1.10 (0.92–1.30)	0.79 (0.59–1.06)	0.63 (0.42–0.95)	1.05 (0.68–1.60)	0.11
	4 (≥10.18)	1.11 (0.92–1.33)	0.80 (0.59–1.09)	0.74 (0.48–1.14)	0.92 (0.59–1.44)	
	P-trend	0.58	0.14	0.24	0.43	
Vitamin B2						
Riboflavin (nmol/L)	1 (<6.91)	1.00	1.00	1.00	1.00	
	2 (6.91–<10.64)	0.99 (0.85–1.15)	0.80 (0.61–1.04)	0.83 (0.57–1.22)	0.78 (0.54–1.13)	
	3 (10.64–<18.35)	0.87 (0.74–1.02)	0.67 (0.51–0.89)	0.70 (0.47–1.06)	0.63 (0.42–0.93)	0.04
	4 (≥18.35)	0.88 (0.75–1.04)	0.81 (0.61–1.07)	0.87 (0.59–1.30)	0.74 (0.50–1.09)	
	P-trend	0.06	0.05	0.33	0.06	
FMN (nmol/L)	1 (<8.57)	1.00	1.00	1.00	1.00	
	2 (8.57–<11.60)	1.00 (0.86–1.18)	0.82 (0.62–1.07)	1.17 (0.79–1.72)	0.55 (0.37–0.82)	
	3 (11.60–<15.50)	0.89 (0.76–1.06)	0.83 (0.63–1.09)	0.97 (0.64–1.46)	0.73 (0.50–1.06)	0.03
	4 (≥15.50)	0.94 (0.80–1.12)	0.65 (0.49–0.88)	0.72 (0.46–1.12)	0.60 (0.40–0.90)	
	P-trend	0.29	0.009	0.11	0.03	
Vitamin B6						
PLP (nmol/L)	1 (<32.80)	1.00	1.00	1.00	1.00	
	2 (32.80–<48.50)	1.09 (0.93–1.28)	0.94 (0.72–1.23)	0.95 (0.65–1.40)	0.94 (0.65–1.38)	
	3 (48.50–<73.85)	0.98 (0.82–1.16)	0.65 (0.48–0.88)	0.66 (0.42–1.03)	0.66 (0.43–1.01)	0.67
	4 (≥73.85)	0.94 (0.78–1.12)	0.69 (0.51–0.95)	0.68 (0.43–1.06)	0.74 (0.48–1.14)	
	P-trend	0.29	0.004	0.04	0.07	
Vitamin B12						
Cobalamin (pmol/L)	1 (<245.4)	1.00	1.00	1.00	1.00	
	2 (245.4–<307.7)	1.15 (0.98–1.35)	0.95 (0.73–1.25)	0.85 (0.57–1.28)	1.05 (0.72–1.53)	
	3 (307.7–<381.3)	1.04 (0.89–1.23)	0.90 (0.68–1.18)	0.94 (0.63–1.39)	0.90 (0.61–1.33)	0.11
	4 (≥381.3)	1.03 (0.87–1.21)	0.88 (0.66–1.16)	0.87 (0.58–1.31)	0.91 (0.61–1.34)	
	P-trend	0.92	0.31	0.63	0.48	

[a]Adjusted for age, sex, study centre, smoking habits, and alcohol consumption.
[b]Interaction between quartiles of the biochemical variable and *MTHFR C677T* genotpyes.
[c]Based on the distribution of concentrations among individuals without neoplasia at screening.

About vitamin B2, we observed inverse associations for riboflavin and FMN, being most pronounced for FMN (OR = 0.65; 95% CI 0.49–0.88, $P_{trend} = 0.009$). The vitamin B6 form PLP was inversely associated with high-risk colorectal adenoma occurrence (OR = 0.69; 95% CI 0.51–0.95, $P_{trend} = 0.004$), but the vitamin B6 forms PL and PA were not associated (data not shown). Folate and vitamin B12 concentrations were neither associated with high-risk nor with low-risk colorectal adenoma.

An inverse association of FMN concentration with high-risk colorectal adenoma occurrence could be observed among individuals carrying the variant T allele of the *MTHFR C677T* polymorphism (OR = 0.60; 95% CI 0.40–0.90, $P_{interaction} = 0.03$, Table 2), but not among those having the *MTHFR 677CC* genotype. FMN was the only plasma marker to be differentially associated with colorectal adenoma across *MTHFR C677T* genotypes. We did not observe genotype dependence of associations with colorectal adenomas of the remaining methyl group donors or B-vitamins.

High methionine concentration in combination with either high riboflavin, FMN, or PLP concentrations tended to be more strongly inversely associated with high-risk adenomas than these factors individually (Table 3). However, only weak interaction was observed between methionine and riboflavin ($P = 0.10$) and between methionine and FMN ($P = 0.09$), whereas there was no interaction between methionine and PLP concentrations ($P = 0.74$, Table 3). There were no interactions between the remaining methyl group donors folate, betaine, and choline with any of the B-vitamin concentrations (data not shown).

When stratifying by smoking category, we observed that FMN concentration was inversely associated with high-risk colorectal adenoma among current smokers only ($P_{interaction} = 0.06$, Table 4). Moreover, methionine and vitamin B6 appeared protective among former smokers and current smokers, and not among those who never smoked, although interactions of these concentrations with smoking were not statistically significant. Associations of folate, betaine, choline, riboflavin, and vitamin B12 with high-risk adenoma did not differ across smoking categories. Finally, we observed that alcohol consumption did not modify the associations of methyl donors or B-vitamins with high-risk adenoma occurrence (data not shown).

Discussion

Principal findings

In this Norwegian colorectal adenoma screening population, plasma concentrations of both methionine and

Table 3. Multivariate-adjusted logistic regression analyses with corresponding odds ratios and 95% confidence intervals for high-risk colorectal adenoma: interactions between plasma concentrations of methionine and vitamins B2, B6, and B12

Plasma concentration	Without neoplasia (*n*)	High-risk CRA (*n*)	Odds ratio[a] high-risk adenoma	*P*-interaction high-risk adenoma[b]
Methionine and vitamin B2				
Low methionine, low riboflavin[c]	2379	145	1.00	
Low methionine, high riboflavin	2013	96	0.87 (0.67–1.15)	
High methionine, low riboflavin	2013	99	0.72 (0.55–0.95)	0.10
High methionine, high riboflavin	2380	81	0.57 (0.42–0.76)	
Low methionine, low FMN	2405	146	1.00	
Low methionine, high FMN	1987	95	0.84 (0.64–1.11)	
High methionine, low FMN	1973	95	0.70 (0.53–0.92)	0.09
High methionine, high FMN	2420	85	0.56 (0.42–0.76)	
Methionine and vitamin B6				
Low methionine, low PLP	2619	158	1.00	
Low methionine, high PLP	1773	83	0.74 (0.56–0.99)	
High methionine, low PLP	1772	89	0.73 (0.55–0.96)	0.74
High methionine, high PLP	2621	91	0.50 (0.37–0.67)	
Methionine and vitamin B12				
Low methionine, low cobalamin	2333	122	1.00	
Low methionine, high cobalamin	2051	119	1.13 (0.87–1.47)	
High methionine, low cobalamin	2042	99	0.85 (0.64–1.12)	0.62
High methionine, high cobalamin	2336	79	0.60 (0.44–0.81)	

[a]Adjusted for age, sex, study centre, smoking, alcohol.
[b]Interaction terms are tested by modelling continuous variables (e.g., methionine[a]riboflavin).
[c]Low and high concentrations with median concentration among controls are used as cut-off values.

Table 4. Multivariate-adjusted logistic regression analyses with corresponding odds ratios and 95% confidence intervals for high-risk colorectal adenoma across smoking categories, according to quartiles of folate, methionine, and vitamins B2, B6, and B12 concentrations

	Quartile	Odds ratios for high-risk adenoma[a]			
		Never smokers ($n = 108$)[b]	Ex-smokers ($n = 110$)	Current smokers ($n = 203$)	$P_{interaction}$
Methyl group donors					
Folate	1	1.00	1.00	1.00	
	2	0.95 (0.53–1.71)	0.78 (0.42–1.42)	1.16 (0.70–1.93)	
	3	0.91 (0.50–1.65)	0.87 (0.49–1.56)	1.24 (0.76–2.02)	0.25
	4	1.29 (0.73–2.27)	1.02 (0.57–1.81)	1.48 (0.93–2.34)	
	P-trend	0.35	0.75	0.07	
Methionine	1	1.00	1.00	1.00	
	2	1.36 (0.81–2.29)	0.81 (0.47–1.38)	0.72 (0.49–1.06)	
	3	0.65 (0.35–1.20)	0.74 (0.43–1.27)	0.62 (0.41–0.94)	0.45
	4	0.89 (0.49–1.59)	0.39 (0.21–0.72)	0.64 (0.41–1.00)	
	P-trend	0.23	0.003	0.03	
Betaine	1	1.00	1.00	1.00	
	2	0.73 (0.41–0.30)	1.06 (0.59–1.94)	1.37 (0.91–2.08)	
	3	0.80 (0.45–1.41)	1.08 (0.60–1.96)	0.95 (0.61–1.49)	0.68
	4	0.81 (0.45–1.44)	0.63 (0.33–1.21)	0.79 (0.50–1.26)	
	P-trend	0.61	0.13	0.10	
Choline	1	1.00	1.00	1.00	
	2	0.63 (0.35–1.12)	1.15 (0.63–2.11)	0.88 (0.60–1.30)	
	3	0.81 (0.46–1.41)	1.08 (0.58–1.98)	0.68 (0.44–1.04)	0.38
	4	0.86 (0.49–1.51)	0.83 (0.43–1.58)	0.79 (0.50–1.24)	
	P-trend	0.81	0.43	0.17	
Vitamin B2					
Riboflavin	1	1.00	1.00	1.00	
	2	1.12 (0.66–1.90)	0.88 (0.51–1.51)	0.65 (0.45–0.95)	
	3	0.78 (0.44–1.39)	0.80 (0.45–1.40)	0.60 (0.40–0.90)	0.48
	4	0.92 (0.53–1.59)	0.84 (0.49–1.44)	0.76 (0.50–1.14)	
	P-trend	0.50	0.50	0.06	
FMN	1	1.00	1.00	1.00	
	2	1.12 (0.65–1.96)	0.93 (0.51–1.66)	0.68 (0.47–0.99)	
	3	0.91 (0.51–1.64)	1.34 (0.78–1.28)	0.41 (0.41–0.93)	0.06
	4	0.98 (0.55–1.75)	0.85 (0.47–1.52)	0.28 (0.28–0.72)	
	P-trend	0.77	0.89	<0.001	
Vitamin B6					
PLP	1	1.00	1.00	1.00	
	2	1.22 (0.68–2.17)	0.70 (0.41–1.20)	1.01 (0.70–1.46)	
	3	0.89 (0.48–1.66)	0.59 (0.33–1.04)	0.58 (0.36–0.92)	0.69
	4	1.08 (0.58–2.02)	0.48 (0.26–0.88)	0.69 (0.44–1.10)	
	P-trend	0.92	0.02	0.03	
Vitamin B12					
Cobalamin	1	1.00	1.00	1.00	
	2	0.61 (0.35–1.07)	1.02 (0.60–1.72)	1.17 (0.78–1.74)	
	3	0.64 (0.36–1.11)	0.73 (0.42–1.26)	1.21 (0.81–1.80)	0.59
	4	1.03 (0.63–1.68)	0.81 (0.47–1.41)	0.81 (0.53–1.26)	
	P-trend	0.92	0.27	0.48	

[a]Odds ratio for high-risk colorectal adenoma, adjusted for age, sex, study centre, and alcohol intake.
[b]Number of subjects with high-risk adenomas.

betaine were inversely associated with the occurrence of high-risk colorectal adenoma, which may suggest that these methyl group donors inhibit or prevent colorectal carcinogenesis. We also observed that vitamin B2 and B6 concentrations were inversely associated with high-risk adenomas. However, neither folate, choline, and vitamin B12, nor genotypes of related one-carbon metabolism enzymes were associated with colorectal adenoma occurrence.

Strengths and weaknesses

This is a cross-sectional study based on sigmoidoscopy findings and plasma/serum concentrations of several biomarkers related to one-carbon metabolism in a large population of 10,601 healthy subjects. Although reverse causality is a potential bias in a cross-sectional setting, colorectal adenoma is a preclinical disorder, which most likely has no systemic effects influencing one-carbon metabolism and related B-vitamins. Moreover, the inverse associations of vitamins B2 and B6 with colorectal adenoma are in line with observed inverse associations with colorectal cancer in prospective studies (25, 27).

Sigmoidoscopy allows visualization of the rectum and the distal part of the colon. This may have led to misclassification of participants as being free of neoplasia as proximal adenomas may have been overlooked. However, the risk of having proximal advanced neoplasia after a negative sigmoidoscopy examination has been estimated to be 1.5% to 4.8% (38–40), indicating that a marked bias as a result of misclassification is not likely to have occurred. In addition, in our study, participants with any distal neoplasia (i.e., among 20% of the screenees) qualified for a work-up colonoscopy, of which the compliance was high (96%) (29). Although false-negative findings were thereby not eliminated, this has at least provided the opportunity to detect proximal high-risk adenomas among subjects who also had a distal low-risk adenoma. It was observed that only 3.7% had high-risk adenoma proximal to the reach of flexible sigmoidoscopy at subsequent work-up colonoscopy.

Another drawback of our study is that a single measurement of plasma concentrations of methyl donors and B-vitamins may not be fully representative for life-time exposure. Variation in plasma concentrations may for example occur due to changes in diet and life-style. However, rather than to predict future colorectal adenoma incidence, we attempted to identify determinants for the occurrence of colorectal adenoma at the time of blood sampling, as investigated in a cross-sectional setting.

Methyl group donors and B-vitamins in relation to colorectal adenoma

This is the first population-based study investigating plasma concentrations of the methyl group donors methionine, choline, and betaine in relation to colorectal adenoma. The observed inverse associations of methionine and betaine as well as plasma concentrations of vitamins B2 and B6 with colorectal adenoma underscore the importance of one-carbon metabolism in colorectal carcinogenesis, especially because associations were present with high-risk colorectal adenoma, which are more likely to develop into colorectal carcinomas compared with low-risk ones. Our observations also underline the need to concurrently investigate both methyl group donor and B-vitamin status. This is underscored by the observed interactions of methionine with vitamin B2 and B6, which are biologically plausible because the transfer of methyl groups depends on the efficiency of enzymatic conversions. Investigating such interactions has not been common practice in epidemiological studies on colorectal carcinogenesis, although an interaction between serum levels of methionine and vitamin B6 has recently been reported in relation to lung cancer (41).

Smoking as potential effect modifier

We observed that methionine and vitamins B2 and B6 were inversely associated with high-risk adenomas among current or former smokers only. Because smoking is known to be associated with compromised folate status, this suggests an increased importance of alternative methyl group donors under folate deficient conditions, which for example has been shown for betaine (42, 43) and may also apply to methionine and choline. In addition, smoking has been associated with lower circulating concentrations of vitamin B2 and B6 (44).

Progression of colorectal adenomas into cancer

It may be debated whether colorectal adenoma is the optimal endpoint to study determinants for colorectal cancer, because the majority of colorectal adenomas do not progress to colorectal cancer, as indicated by one retrospective study which estimated that the cumulative risk of developing cancer at a diagnosed polyp site is 24% after 20 years (45). However, a recently published 11-year follow-up of a British screening study showed a 23% incidence reduction of colorectal cancer after sigmoidoscopy screening, where advanced distal neoplasia qualified for colonoscopic work-up (28), which strongly suggests the potential of adenomas to progress into carcinomas.

Null findings

In the present study, serum folate and B12 concentrations and genetic polymorphisms of enzymes involved in one-carbon metabolism were not associated with colorectal adenoma occurrence, which may partly have been due to limited power of the 421 high-risk adenoma cases to show such associations. With regard to folate, another reason may be that a certain proportion of the folate supply in the distal colonic mucosa stems from luminal absorption of folate produced *in situ* by colonic bacteria and not from folate delivered by blood supply (46, 47). This may have attenuated the associations of blood folate levels with adenoma risk. In addition, DNA methylation patterns have been reported to be different in distal

adenomas than in proximal ones. In this respect, the CpG island methylator phenotype (CIMP), a distinct subgroup of colorectal cancers harboring frequent hypermethylation of gene promoter regions, occurs predominantly in the proximal colon (48). It may be hypothesized that methyl groups and genetic variants of one-carbon metabolism play a larger role in the etiology of proximal neoplasms as compared with those without CIMP occurring more often distally. For example, it has been observed that folate concentration in normal colonic mucosa was inversely associated with prevalence of proximal adenomas but not distal ones, suggesting that folate is aetiologically more important in proximal colorectal carcinogenesis (49). Moreover, while a recent meta-analysis of 25 studies showed that the variant allele of the *MTHFR C677T* polymorphism was associated with reduced colorectal cancer risk in general (50), this association was especially strong in proximal colon carcinomas harboring promoter methylation (51) and with CIMP (52). Nonetheless, we did observe a modest interaction between FMN concentration and the *MTHFR C677T* polymorphism in association with high-risk distal adenomas.

Conclusions

This study suggests that high-plasma concentrations of the methyl group sources methionine and betaine, and vitamins B2 and B6 may reduce risk of developing colorectal adenomas. The methyl group sources methionine and betaine may also have a potential role in colorectal carcinogenesis and should be investigated further.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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