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Plasma Choline Metabolites and Colorectal Cancer Risk in the Women's Health Initiative Observational Study

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Abstract

Few studies have examined associations between plasma choline metabolites and risk of colorectal cancer. Therefore, we investigated associations between plasma biomarkers of choline metabolism [choline, betaine, dimethylglycine, and trimethylamine *N*-oxide (TMAO)] and colorectal cancer risk among postmenopausal women in a case–control study nested within the Women's Health Initiative Observational Study. We selected 835 matched case–control pairs, and cases were further stratified by tumor site (proximal, distal, or rectal) and stage (local/regional or metastatic). Colorectal cancer was assessed by self-report and confirmed by medical records over the mean of 5.2 years of follow-up. Baseline plasma choline metabolites were measured by LC/MS-MS. In multivariable-adjusted conditional logistic regression models, plasma choline tended to be positively associated with rectal cancer risk [OR (95% confidence interval, CI)$_{\text{highest vs. lowest quartile}}$ = 2.44 (0.93–6.40); *P* trend = 0.08], whereas plasma betaine was inversely associated with colorectal cancer overall [0.68 (0.47–0.99); *P* trend = 0.01] and with local/regional tumors [0.64 (0.42–0.99); *P* trend = 0.009]. Notably, the plasma betaine: choline ratio was inversely associated with colorectal cancer overall [0.56 (0.39–0.82); *P* trend = 0.004] as well as with proximal [0.66 (0.41–1.06); *P* trend = 0.049], rectal [0.27 (0.10–0.78); *P* trend = 0.02], and local/regional [0.50 (0.33–0.76); *P* trend = 0.001] tumors. Finally, plasma TMAO, an oxidative derivative of choline produced by intestinal bacteria, was positively associated with rectal cancer [3.38 (1.25–9.16); *P* trend = 0.02] and with overall colorectal cancer risk among women with lower (vs. higher) plasma vitamin B12 levels (*P* interaction = 0.003). Collectively, these data suggest that alterations in choline metabolism, which may arise early in disease development, may be associated with higher risk of colorectal cancer. The positive association between plasma TMAO and colorectal cancer risk is consistent with an involvement of the gut microbiome in colorectal cancer pathogenesis. *Cancer Res; 74(24); 7442–52. ©2014 AACR.*

Introduction

Colorectal cancer is the third most commonly diagnosed cancer in both men and women and a major cause of cancer deaths in the United States (1). Disturbances in one-carbon metabolism, which lead to genomic instability (e.g., aberrant DNA methylation and DNA damage), may contribute to colorectal cancer development (2, 3). Choline and folate are methyl nutrients involved in one-carbon metabolism and play a critical role in methylation reactions, including DNA methylation, as well as DNA stability and repair (4–6). Although low folate intake and low circulating levels of folate are associated with high risk of colorectal cancer (2, 7–9), less is known about the association between choline and colorectal cancer risk.

Choline participates in methylation reactions following its oxidation to betaine, which donates a methyl group for homocysteine remethylation, forming methionine and dimethylglycine (DMG). Betaine also serves as an osmolyte and plays a major role in protecting cells from hyperosmotic stress that can lead to chronic inflammation, a risk factor for colorectal cancer (1, 10, 11). To date, only a few studies have examined the association between plasma betaine and colorectal carcinogenesis. In a Norwegian population, plasma betaine was inversely associated with the occurrence of distal colorectal adenomas (12). A recent case–control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) also reported an inverse association between plasma

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betaine and colorectal cancer risk among participants with low plasma folate concentrations (13).

Choline can also undergo catabolism by the intestinal bacteria to form trimethylamine (TMA), which is further converted to trimethylamine *N*-oxide (TMAO) by the liver enzyme flavin monooxygenase (FMO; refs. 14, 15). Although intestinal microbiota have been implicated in the development of colorectal cancer (16–18), the association between gut microbiota–dependent choline metabolites and colorectal cancer risk is unknown.

In this report, we investigated the associations between plasma biomarkers of choline metabolism (choline, betaine, DMG, and TMAO) and colorectal cancer risk in a case–control study nested within the Women's Health Initiative Observational Study (WHI-OS) cohort. Because of the interdependence of choline and folate as well as other B vitamins (vitamin B6 and B12) in one-carbon metabolism (4, 5), we further explored their influence and that of folic acid (FA) fortification (19) on the associations between plasma choline metabolites and colorectal cancer risk.

Patients and Methods

Study population

The WHI-OS is a prospective cohort study designed to investigate the predictors and causes of morbidity and mortality in postmenopausal women (20, 21). The study enrolled 93,676 postmenopausal women, ages 50 to 79 years, at 40 centers throughout the United States between 1993 and 1998. Women were excluded if they had medical conditions with a predicted survival of <3 years; if they had adherence/retention issues; or if they were participating in another clinical trial.

For the present study, incident colorectal cancer cases were selected as of April 24, 2008, and the average time from baseline to colorectal cancer diagnosis was 5.2 ± 3.1 years (mean ± SD; refs. 11, 22). Women were excluded if they had a history of colorectal cancer or *in situ* colorectal cancer; if they had no available biospecimens; or if a death certificate provided the only report of colorectal cancer. Controls who were free of cancer at the time of case diagnosis were selected from the WHI-OS by using risk-set sampling. Cases and controls were matched on age (± 3 years), race/ethnicity, timing of baseline blood draw (± 6 months), enrollment date (± 1 year), and baseline hysterectomy status (11, 22). Thus, the present study included 835 incident colorectal cancer cases and 835 matched controls. Approval for conducting the study was obtained from human subject review committees at the Fred Hutchinson Cancer Research Center (WHI Clinical Coordinating Center), as well as at all 40 clinical centers. Written informed consent was obtained from all participants.

Data collection

Demographic and health-related characteristics were collected at baseline using standardized questionnaires (20). Height and weight were measured using a standardized protocol, and body mass index (BMI) was calculated as weight (kg)/height (m^2). Colorectal cancer was annually assessed using self-administered questionnaires collected from each participant by mail and during an in-person clinical follow-up visit at year 3 (23). All colorectal cancer cases were confirmed by physician adjudicators. The International Classification of Diseases for Oncology, second edition codes were used to identify colorectal cancer cases based on tumor site as previously described (11). The Surveillance Epidemiology and End Results (SEER) program guidelines of the NCI were used for classifications of cancer cases (23).

Analytic measurements

Blood samples were drawn at study baseline after at least 12 hours of fasting. Samples were kept at 4°C for up to 1 hour before centrifugation. Plasma and serum were collected and stored at −70°C until analysis (22). Plasma concentrations of choline and its metabolites (betaine, DMG, and TMAO) were measured in de-identified samples using LC/MS-MS methodology with modifications based on our instrumentation (24). Plasma and red blood cell (RBC) folate as well as plasma vitamin B12 were measured by radioassays (SimulTRAC; MP Biomedicals); plasma pyridoxal-5′-phosphate (PLP) was analyzed by high-pressure liquid chromatography (HPLC) with fluorescence detection (25); and total plasma homocysteine was determined by HPLC with postcolumn fluorescence detection (26). Interassay coefficients of variance of the blind duplicate control samples for each of the assays were as follows: choline, 7%; betaine, 5%; DMG, 9%; TMAO, 6%; plasma folate, 5%; RBC folate, 10%; vitamin B12, 6%; PLP, 6%; and homocysteine, 7%.

Statistical analysis

Baseline characteristics of colorectal cancer cases and controls were compared using (i) *t* tests for normally distributed continuous variables; (ii) Wilcoxon tests for non-normally distributed continuous variables; and (iii) χ^2 tests for categorical variables. Associations among plasma concentrations of choline metabolites were assessed using Spearman correlation analysis. Plasma choline metabolites were divided into quartiles based on the distribution of the controls. Conditional logistic regression models were used to estimate ORs and 95% confidence intervals (CI) of colorectal cancer risk among quartiles of choline metabolites, using the lowest quartiles as reference groups. Because risk-set sampling was used for selecting matched controls, the conditional ORs yielded estimates of the incidence rate ratio in a full cohort study. We further explored the associations between the ratios of choline metabolites (i.e., betaine:choline, DMG:choline, and DMG:betaine) and colorectal cancer risk, because the ratios of these metabolites (vs. individual metabolite alone) are suggested to be stronger predictors of metabolic disturbances (27). The models were first adjusted only for age (continuous) and then further adjusted for baseline confounding factors selected *a priori*: BMI, pack-years of smoking, physical activity, use of postmenopausal hormone therapy, history of colonoscopy, RBC folate, plasma vitamin B12, PLP, and homocysteine. All of these factors were added in the model as continuous variables except for postmenopausal hormone therapy use (categorical: never, past, or current). Tests of linear trend across increasing quartiles of choline metabolites were

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conducted by the Wald test, using the median value for each quartile as a single continuous variable.

To explore whether the associations between choline metabolites and colorectal cancer risk were modified by B vitamins involved in one-carbon metabolism, we conducted analyses stratified into high/low plasma concentrations of folate, PLP, and vitamin B12 based on median values among controls. We also examined the influence of FA fortification by stratifying into the following FA fortification periods based on the timing of baseline blood draw: prefortification (1994–1995), perifortification (1996–1997; when initial fortification began, but was not yet mandated), and postfortification (1998; ref. 28). The Wald test was used to evaluate the effect modification including a two-way interaction term between the ordinal trend variables (choline metabolites) and effect modifiers (B vitamins or FA fortification period). Because the matching was broken, unconditional multiple logistic regression models were used in these stratified analyses, further adjusting for days to colorectal cancer diagnosis and ethnicity. Significance was defined as $P < 0.05$, and all statistical tests were two-sided. Analyses were conducted by SAS version 9.3 (SAS Institute Inc.).

Results

Characteristics of the study population

Baseline characteristics of the colorectal cancer cases and controls are shown in Table 1. Compared with the controls, the cases had a higher BMI, a greater number of cigarettes smoked among current smokers, fewer weekly minutes of moderate or strenuous physical activity, and had a different distribution pattern of postmenopausal hormone therapy use. The colorectal cancer group also had a lower percentage of previous colonoscopy, but a higher percentage of having history of a colon polyp removed.

Plasma choline, betaine, and DMG concentrations did not differ between cases and controls (Table 1). However, the cases (vs. controls) had higher ($P = 0.005$) median plasma concentrations of TMAO (4.0 vs. 3.8 μmol/L) and tended to have a lower ($P = 0.07$) mean plasma betaine:choline ratio (2.9 vs. 3.0). In addition, the cases had lower median plasma folate, PLP, and vitamin B12 as well as higher median plasma homocysteine.

Among the cases, tumors were classified by tumor site (proximal, distal, or rectal) and stage (local/regional or metastatic). More than half (59%; $n = 489$) of the tumors were proximal followed by distal (21%; $n = 177$) and rectal (19%; $n = 155$). Two percent ($n = 14$) of the tumors were not classified by tumor site because they were unknown or had overlapping lesions. In addition, when stratified by tumor stage, the majority of the cases (85%; $n = 712$) had localized or regional tumors, whereas 12% of the cases ($n = 104$) had distant metastases. Two percent ($n = 18$) of the tumors were not stratified by tumor stage because their stages were unknown or not determined.

Correlations among plasma concentrations of choline metabolites

Spearman correlation coefficients (r) were computed to examine associations among plasma choline metabolites. There were statistically significant, but modest, positive associations of plasma choline with plasma betaine ($r = 0.22$; $P < 0.001$), DMG ($r = 0.21$; $P < 0.001$) and TMAO ($r = 0.18$; $P < 0.001$). Plasma betaine was also positively correlated with plasma DMG ($r = 0.39$; $P < 0.001$).

Associations between plasma choline metabolites and colorectal cancer risk

In multivariable-adjusted analyses, women in the highest (vs. lowest) choline quartile were at an estimated 2.4 times greater risk of rectal cancer (P trend = 0.08; Table 2). Conversely, women in the highest (vs. lowest) betaine quartile were at 32% lower colorectal cancer risk overall [OR (95% CI)$_{\text{highest vs. lowest quartile}} = 0.68$ (0.47–0.99); P trend = 0.01], 36% lower risk of local/regional tumors [0.64 (0.42–0.99); P trend = 0.009], and 31% lower risk of proximal tumors [0.69 (0.43–1.10); P trend = 0.05; Table 3]. No association between DMG quartiles and colorectal cancer risk was observed (Supplementary Table S1).

Notably, after controlling for covariates, women in the highest (vs. lowest) quartile of the plasma betaine:choline ratio were at an estimated 44% lower colorectal cancer risk overall [0.56 (0.39–0.82); P trend = 0.004] as well as 34% lower risk of proximal tumors [0.66 (0.41–1.06); P trend = 0.049], 73% lower risk of rectal tumors [0.27 (0.10–0.78); P trend = 0.02], and 50% lower risk of local/regional tumors [0.50 (0.33–0.76); P trend = 0.001; Table 4]. The plasma DMG:choline ratio tended to be inversely associated with colorectal cancer risk overall [0.69 (0.48–0.98); P trend = 0.06; Supplementary Table S2]. The inverse association was statistically significant for local/regional tumors [0.62 (0.42–0.91); P trend = 0.04] and borderline significant for proximal tumors [0.57 (0.36–0.93); P trend = 0.07]. Last, the DMG:betaine ratio tended to be positively associated with rectal cancer risk [2.56 (0.98–6.64); P trend = 0.09; Supplementary Table S3].

Plasma TMAO, an oxidative derivative of choline produced by intestinal bacteria, was positively associated with colorectal cancer risk in age-adjusted analyses [1.78 (1.32–2.40); P trend = 0.005; Table 5]. Women in the highest (vs. lowest) TMAO quartile were at approximately 1.9 times greater risk of proximal tumors (P trend = 0.04), 2.3 times greater risk of rectal tumors (P trend = 0.02), and 1.8 times greater risk of local/regional tumors (P trend = 0.008). After controlling for covariates, the positive association remained strong and statistically significant for rectal cancer with approximately 3.4 times greater risk among women in the highest (vs. lowest) TMAO quartile (P trend = 0.02). A borderline significant positive association was also observed for local/regional tumors with approximately 1.8 times greater risk in the highest (vs. lowest) TMAO quartile (P trend = 0.08). Notably, although the linear trend across TMAO quartiles was not statistically significant, higher risk was observed from the second (vs. lowest) quartile of TMAO for colorectal cancer overall [1.90 (1.36–2.64)] and for proximal tumors [2.37 (1.52–3.70)]. Similarly, women in the second (vs. lowest) quartile of TMAO were at an estimated 1.9 times higher risk for local/regional tumors and 3.6 times higher risk for metastatic tumors, but this was not consistently observed in the other quartiles.

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Table 1. Characteristics of colorectal cancer cases and controls[a]

	Cases		Controls		
Characteristics	***n***	**Value**	***n***	**Value**	***P* value**
Age (years)[b]	835	66 ± 7	835	67 ± 7	0.52
BMI (kg/m^2)[b]	824	28.2 ± 6.1	827	27.1 ± 5.9	0.004
Race/ethnicity[c]	835	100	835	100	1.0
White	711	85	711	85	
Other[d]	124	15	124	15	
Family income ($)[c]	801	100	793	100	0.30
<34,999	374	47	351	44	
35,000–74,999	294	37	282	36	
≥75,000	111	14	137	17	
Do not know	22	3	23	3	
Education (high school or less)[c]	160	19	186	22	0.11
Residence location (US region)[c]	835	100	835	100	0.57
Northeast	210	25	189	23	
South	188	23	203	24	
Midwest	196	23	191	23	
West	241	29	252	30	
Pack-years smoking[b]	802	13 ± 22	799	9 ± 17	<0.001
Moderate or strenuous activity (min/wk)[b]	824	98 ± 136	827	111 ± 145	0.05
Use of postmenopausal hormone therapy[c]	834	100	835	100	<0.001
Never	415	50	346	41	
Past	138	17	135	16	
Current	281	34	354	42	
Family history of colorectal cancer (yes)[c]	167	22	143	19	0.17
History of colonoscopy or sigmoidoscopy (yes)[c]	431	53	500	61	<0.001
History of colon polyp removal (yes)[c]	102	24	90	18	0.03
Plasma choline (μmol/L)[b]	835	9.5 ± 2.3	835	9.4 ± 2.2	0.25
Plasma betaine (μmol/L)[b]	835	26.6 ± 10.8	835	27.1 ± 10.7	0.31
Plasma DMG (μmol/L)[e]	835	2.3 (1.9–2.9)	834	2.3 (1.9–2.9)	0.89
Plasma TMAO (μmol/L)[e]	835	4.0 (2.9–6.0)	835	3.8 (2.6–5.7)	0.005
Plasma betaine:choline ratio[b]	835	2.9 ± 1.2	835	3.0 ± 1.3	0.07
Plasma DMG:choline ratio[b]	835	0.27 ± 0.12	834	0.28 ± 0.11	0.52
Plasma DMG:betaine ratio[b]	835	0.10 ± 0.05	834	0.10 ± 0.05	0.51
Plasma folate (ng/mL)[e]	835	15.6 (8.9–25.3)	835	17.2 (9.9–27.1)	0.02
RBC folate (ng/mL)[e]	832	564 (410–742)	835	591 (431–751)	0.16
Plasma PLP (nmol/L)[e]	821	60 (39–101)	817	67 (44–113)	0.002
Plasma vitamin B12 (pg/mL)[e]	833	477 (336–661)	835	505 (376–691)	0.02
Plasma homocysteine (μmol/L)[e]	835	8.1 (6.8–9.9)	835	7.7 (6.7–9.4)	0.002

[a]Differences between cases and controls were analyzed by *t* tests (normally distributed continuous variables); Wilcoxon tests (non-normally distributed continuous variables); and χ^2 tests (categorical variables).
[b]Values are mean ± SD for normally distributed continuous variables.
[c]Values are percentage for categorical variables.
[d]Black or African American, Hispanic, Asian or Pacific Islander, American Indian or Alaskan native, or missing.
[e]Values are median (interquartile range) for non-normally distributed continuous variables.

Associations of choline metabolites with colorectal cancer risk according to plasma B-vitamin concentrations

To further explore whether B vitamins (folate, PLP, and vitamin B12) modified the associations between choline metabolites and colorectal cancer risk, we stratified into high/low plasma concentrations of B vitamins and assessed the interaction. After controlling for covariates, vitamin B12 status modified the association between plasma TMAO and colorectal cancer risk (P interaction = 0.003; Table 6). Specifically, higher colorectal cancer risk was observed with higher TMAO quartiles among women with low plasma vitamin B12 (i.e., ≤505 pg/mL; P trend = 0.001), but not among those with high B12 levels. Other than this finding, no effect modifications

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Table 2. ORs (95% CIs) of colorectal cancer by quartile of plasma choline[a]

	Quartiles of choline (μmol/L)				
	1 (≤7.9)	2 (>7.9–9.2)	3 (>9.2–10.6)	4 (>10.6)	*P* trend[b]
n	412	403	408	447	
All participants					
Age-adjusted	1	1.06 (0.80–1.40)	0.96 (0.71–1.29)	1.30 (0.97–1.74)	0.09
Multivariable[c]	1	1.01 (0.74–1.39)	0.95 (0.68–1.31)	1.22 (0.88–1.70)	0.26
By tumor site					
Proximal					
Age-adjusted	1	1.16 (0.80–1.70)	1.11 (0.75–1.62)	1.33 (0.91–1.95)	0.17
Multivariable[c]	1	1.06 (0.68–1.66)	1.07 (0.69–1.65)	1.21 (0.78–1.87)	0.39
Distal					
Age-adjusted	1	1.02 (0.58–1.82)	0.69 (0.34–1.39)	1.12 (0.61–2.05)	0.73
Multivariable[c]	1	0.92 (0.48–1.77)	0.68 (0.31–1.49)	1.07 (0.51–2.23)	0.91
Rectal					
Age-adjusted	1	1.08 (0.56–2.08)	1.00 (0.51–1.95)	1.79 (0.88–3.64)	0.13
Multivariable[c]	1	1.38 (0.59–3.22)	1.37 (0.56–3.34)	2.44 (0.93–6.40)	0.08
By stage					
Local/regional					
Age-adjusted	1	1.11 (0.82–1.51)	1.07 (0.78–1.48)	1.33 (0.97–1.81)	0.08
Multivariable[c]	1	1.01 (0.71–1.44)	1.01 (0.70–1.45)	1.23 (0.86–1.76)	0.24
Metastatic					
Age-adjusted	1	0.84 (0.37–1.90)	0.41 (0.16–1.04)	1.12 (0.46–2.73)	0.82
Multivariable[c]	1	1.66 (0.56–4.92)	0.55 (0.18–1.73)	2.32 (0.69–7.83)	0.30

[a]ORs (95% CIs) of colorectal cancer were determined by conditional logistic regression.

[b]Medians for each quartile used in trend test: quartile 1 = 7.0 μmol/L; quartile 2 = 8.6 μmol/L; quartile 3 = 9.8 μmol/L; and quartile 4 = 11.8 μmol/L.

[c]Multivariable analyses were adjusted for age, baseline BMI, pack-years of smoking, moderate or strenuous physical activity (min/wk), use of postmenopausal hormone therapy, history of colonoscopy, RBC folate, plasma PLP, plasma vitamin B12, and plasma homocysteine.

by B vitamins were observed on the associations of plasma choline metabolites and their ratios with colorectal cancer risk (data not shown).

Associations of choline metabolites with colorectal cancer risk according to FA fortification period

We next explored the possible effect modification by FA fortification. The association of plasma choline, DMG, TMAO, and the ratios of choline metabolites with colorectal cancer risk did not differ by fortification periods (data not shown). However, after controlling for covariates, plasma betaine tended to interact with FA fortification period in association with colorectal cancer risk (P interaction = 0.08; Table 7). Specifically, lower colorectal cancer risk was observed with higher plasma betaine during the pre-(P trend = 0.02) and peri-(P trend = 0.02) fortification periods, but not during the postfortification period.

Discussion

To the best of our knowledge, this is the first study to assess associations between plasma biomarkers of choline metabolism and colorectal cancer risk among postmenopausal women in the United States. The following main findings emerged: (i) plasma choline (modest positive) and betaine (inverse) were divergently associated with colorectal cancer risk; (ii) the plasma betaine:choline ratio was more strongly associated with colorectal cancer risk than was either metabolite alone; and (iii) higher plasma TMAO concentrations were associated with higher risk of colorectal cancer especially among women with low plasma vitamin B12.

The divergent associations of plasma choline and betaine with colorectal cancer risk are unexpected given that betaine is derived from choline and increases in response to a higher choline intake (24). Thus, the divergent associations may arise from the disease process itself, which could alter choline metabolism before diagnosis (29, 30). For example, postmenopausal women harboring undiagnosed, precancerous lesions may have a higher demand for choline due to its greater use for membrane biosynthesis by abnormally dividing cells (31, 32). This in turn may upregulate *de novo* choline production through the hepatic phosphatidylethanolamine *N*-methyltransferase (PEMT) pathway. Enhanced hepatic PEMT activity would be expected to elevate choline, a product of the PEMT reaction, while depleting betaine, a source of methyl groups for

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Table 3. ORs (95% CIs) of colorectal cancer by quartile of plasma betaine[a]

	Quartiles of betaine (μmol/L)				
	1 (≤18.8)	2 (>18.8–26.6)	3 (>26.6–34.0)	4 (>34.0)	*P* trend[b]
n	413	464	417	376	
All participants					
Age-adjusted	1	1.32 (1.01–1.73)	1.02 (0.77–1.36)	0.93 (0.70–1.24)	0.29
Multivariable[c]	1	1.03 (0.75–1.43)	0.74 (0.52–1.06)	0.68 (0.47–0.99)	0.01
By tumor site					
Proximal					
Age-adjusted	1	1.33 (0.95–1.87)	1.07 (0.74–1.54)	0.80 (0.55–1.17)	0.16
Multivariable[c]	1	1.26 (0.84–1.89)	0.87 (0.55–1.38)	0.69 (0.43–1.10)	0.05
Distal					
Age-adjusted	1	1.51 (0.81–2.81)	1.25 (0.67–2.36)	1.12 (0.58–2.16)	0.95
Multivariable[c]	1	0.89 (0.37–2.11)	0.82 (0.33–2.02)	0.63 (0.23–1.73)	0.32
Rectal					
Age-adjusted	1	1.44 (0.74–2.80)	0.65 (0.33–1.27)	1.13 (0.60–2.14)	0.71
Multivariable[c]	1	1.02 (0.43–2.42)	0.35 (0.13–0.96)	0.61 (0.22–1.70)	0.16
By stage					
Local/regional					
Age-adjusted	1	1.31 (0.98–1.74)	0.91 (0.67–1.23)	0.93 (0.67–1.28)	0.23
Multivariable[c]	1	1.01 (0.71–1.44)	0.64 (0.43–0.96)	0.64 (0.42–0.99)	0.009
Metastatic					
Age-adjusted	1	1.34 (0.57–3.15)	2.13 (0.87–5.25)	0.91 (0.45–1.85)	0.55
Multivariable[c]	1	0.97 (0.33–2.82)	1.91 (0.61–5.95)	0.85 (0.31–2.37)	0.70

[a]ORs (95% CIs) of colorectal cancer were determined by conditional logistic regression.

[b]Medians for each quartile used in trend test: quartile 1 = 14.4 μmol/L; quartile 2 = 22.8 μmol/L; quartile 3 = 29.9 μmol/L; and quartile 4 = 39.1 μmol/L.

[c]Multivariable analyses were adjusted for age, baseline BMI, pack-years of smoking, moderate or strenuous physical activity (min/wk), use of postmenopausal hormone therapy, history of colonoscopy, RBC folate, plasma PLP, plasma vitamin B12, and plasma homocysteine.

the PEMT reaction. This metabolic scenario is observed during pregnancy (33), which like cancer is a state of rapidly dividing cells and exhibits several of the same molecular characteristics (34). However, unlike pregnancy where providing substrate for the PEMT reaction may beneficially influence fetal growth and development, betaine supplementation for the purposes of colorectal cancer reduction among postmenopausal women appears unwise because the prevalence of colonic neoplasia increases with age (35) and extra betaine may accelerate tumor progression.

The divergent associations of plasma choline and betaine with colorectal cancer risk observed in our study cohort differ from findings of a recent case–control study nested within the EPIC cohort, where, in the subgroup analyses of women, plasma choline (but not plasma betaine) was inversely associated with colorectal cancer risk (13). One major difference between the study cohorts that could explain these discordant findings is folate status. Specifically, median plasma folate concentrations were approximately 3.5 times higher in the WHI (vs. EPIC) cohort. Other contributing factors may include age of participants, follow-up period, blood sample collection (fasting vs. nonfasting), use of different cutpoints for categories of choline metabolites, and the status of other nutrients involved in one-carbon metabolism.

In the present study, the plasma betaine:choline ratio was more strongly associated with colorectal cancer risk than either metabolite alone. After adjusting for potential confounders, women in the highest (vs. lowest) betaine:choline quartile were at 44% lower colorectal cancer risk overall, 34% lower proximal tumors, 50% lower local/regional tumors, and 73% lower rectal tumors. The association between the betaine:choline ratio and colorectal cancer risk did not appear to differ according to B-vitamin status or FA fortification period. In contrast, FA exposure appeared to modify the association between plasma betaine and colorectal cancer risk with an inverse association observed in the pre- and perifortification periods, but not in the postfortification period. As such, the association between plasma betaine and colorectal cancer risk appears to be dependent on folate availability and may be more evident when folate availability is low (i.e., before FA fortification). Overall, these data support the utility of the plasma betaine:choline ratio as a potential biomarker for excess risk of colorectal cancer in postmenopausal women.

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Table 4. ORs (95% CIs) of colorectal cancer by quartile of plasma betaine:choline ratio[a]

	Quartiles of betaine:choline ratio				
	1 (≤2.0)	2 (>2.0–2.8)	3 (>2.8–3.8)	4 (>3.8)	*P* trend[b]
n	416	446	436	372	
All participants					
Age-adjusted	1	1.12 (0.85–1.48)	1.08 (0.83–1.41)	0.79 (0.59–1.05)	0.08
Multivariable[c]	1	0.83 (0.60–1.15)	0.87 (0.62–1.22)	0.56 (0.39–0.82)	0.004
By tumor site					
Proximal					
Age-adjusted	1	1.26 (0.88–1.79)	1.09 (0.77–1.55)	0.74 (0.51–1.09)	0.08
Multivariable[c]	1	1.12 (0.73–1.70)	0.98 (0.63–1.53)	0.66 (0.41–1.06)	0.049
Distal					
Age-adjusted	1	0.90 (0.48–1.69)	1.07 (0.61–1.87)	0.83 (0.43–1.60)	0.76
Multivariable[c]	1	0.53 (0.24–1.18)	0.86 (0.40–1.84)	0.45 (0.19–1.10)	0.24
Rectal					
Age-adjusted	1	1.06 (0.55–2.06)	0.94 (0.51–1.73)	0.75 (0.39–1.41)	0.32
Multivariable[c]	1	0.56 (0.22–1.43)	0.45 (0.18–1.13)	0.27 (0.10–0.78)	0.02
By stage					
Local/regional					
Age-adjusted	1	1.17 (0.86–1.58)	1.04 (0.78–1.38)	0.74 (0.54–1.02)	0.04
Multivariable[c]	1	0.88 (0.61–1.27)	0.81 (0.56–1.18)	0.50 (0.33–0.76)	0.001
Metastatic					
Age-adjusted	1	0.81 (0.38–1.75)	1.05 (0.49–2.24)	0.95 (0.43–2.10)	0.95
Multivariable[c]	1	0.55 (0.20–1.54)	0.80 (0.27–2.32)	0.79 (0.25–2.50)	0.98

[a]ORs (95% CIs) of colorectal cancer were determined by conditional logistic regression.

[b]Medians for each quartile used in trend test: quartile 1 = 1.6; quartile 2 = 2.4; quartile 3 = 3.2; and quartile 4 = 4.4.

[c]Multivariable analyses were adjusted for age, baseline BMI, pack-years of smoking, moderate or strenuous physical activity (min/wk), use of postmenopausal hormone therapy, history of colonoscopy, RBC folate, plasma PLP, plasma vitamin B12, and plasma homocysteine.

In humans, choline can undergo catabolism by anaerobic intestinal bacteria to produce TMA, which is further converted to TMAO by the hepatic enzyme FMO (14, 15). Similarly, L-carnitine also serves as a precursor of TMAO through a gut microbiota–dependent metabolism (i.e., choline/carnitine → gut microbiota → TMA/TMAO; refs. 36, 37). This metabolic pathway mediated by intestinal microbiota has been linked to several diseases (37–41), suggesting the potential role of gut-microbial metabolism and their metabolic products in carcinogenesis among humans. The present study, for the first time to our knowledge, examined an association between circulating concentrations of TMAO and colorectal cancer risk. We found that women in the highest (vs. lowest) TMAO quartile had an approximately 3.4 times greater risk of rectal cancer. Although no statistically significant linear trend was observed, increased risk was also detected from the second quartile of TMAO with 1.9 times greater risk for colorectal cancer overall and for local/regional tumors, approximately 2.4 times greater risk for proximal tumors, and approximately 3.6 times greater risk for metastatic tumors. These findings collectively suggest that plasma TMAO may serve as a potential predictor of increased colorectal cancer risk.

Alterations in the intestinal microbiota may predispose to the development and progression of colorectal cancer through affecting multiple processes, including colonic epithelial cell proliferation, immune system, and chronic inflammation (16, 18). For example, compared with healthy individuals, increased number and diversity as well as the decreased stability of a colonic bacterial group, *Clostridium*, have been characterized in patients with colorectal cancer (16, 42). Indeed, *Clostridium* is also suggested to play a role in the conversion of choline (41, 43) and carnitine (37, 44) to TMA, thereby contributing to TMAO production. Thus, it is possible that the positive association between plasma TMAO and colorectal cancer risk may arise from abnormal changes in particular colonic bacteria, which could occur early in disease development before diagnosis. Given that TMAO is a gut bacteria–derived metabolite, it may also represent evidence for an etiologic correlation between intestinal microbiota and colorectal cancer and could potentially serve as a novel biomarker of colorectal cancer risk.

Notably, the association between plasma TMAO and colorectal cancer risk appeared to be modified by vitamin B12 status. Specifically, the risk of colorectal cancer increased across increasing TMAO quartiles in the low B12 group, but

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Table 5. ORs (95% CIs) of colorectal cancer by quartile of plasma TMAO[a]

	Quartiles of TMAO (μmol/L)				
	1 (≤2.6)	2 (>2.6–3.7)	3 (>3.7–5.6)	4 (>5.6)	*P* trend[b]
n	358	435	426	451	
All participants					
Age-adjusted	1	1.67 (1.25–2.23)	1.55 (1.16–2.07)	1.78 (1.32–2.40)	0.005
Multivariable[c]	1	1.90 (1.36–2.64)	1.47 (1.06–2.05)	1.65 (1.17–2.34)	0.13
By tumor site					
Proximal					
Age-adjusted	1	2.06 (1.40–3.03)	2.06 (1.39–3.04)	1.93 (1.31–2.83)	0.04
Multivariable[c]	1	2.37 (1.52–3.70)	1.92 (1.23–3.00)	1.69 (1.09–2.63)	0.42
Distal					
Age-adjusted	1	1.50 (0.77–2.92)	1.20 (0.63–2.27)	1.54 (0.78–3.06)	0.41
Multivariable[c]	1	1.96 (0.86–4.48)	1.19 (0.56–2.53)	1.69 (0.73–3.90)	0.59
Rectal					
Age-adjusted	1	1.03 (0.53–1.98)	0.99 (0.52–1.89)	2.26 (1.06–4.79)	0.02
Multivariable[c]	1	1.42 (0.62–3.28)	1.20 (0.53–2.72)	3.38 (1.25–9.16)	0.02
By stage					
Local/regional					
Age-adjusted	1	1.59 (1.16–2.19)	1.56 (1.13–2.14)	1.78 (1.28–2.46)	0.008
Multivariable[c]	1	1.90 (1.31–2.74)	1.46 (1.00–2.11)	1.78 (1.21–2.60)	0.08
Metastatic					
Age-adjusted	1	2.81 (1.23–6.41)	1.61 (0.78–3.32)	2.26 (0.96–5.31)	0.17
Multivariable[c]	1	3.63 (1.29–10.23)	2.27 (0.86–5.96)	2.09 (0.63–6.97)	0.47

[a]ORs (95% CIs) of colorectal cancer were determined by conditional logistic regression.

[b]Medians for each quartile used in trend test: quartile 1 = 2.0 μmol/L; quartile 2 = 3.1 μmol/L; quartile 3 = 4.5 μmol/L; and quartile 4 = 8.1 μmol/L.

[c]Multivariable analyses were adjusted for age, baseline BMI, pack-years of smoking, moderate or strenuous physical activity (min/wk), use of postmenopausal-hormone-therapy, history of colonoscopy, RBC folate, plasma PLP, plasma vitamin B12, and plasma homocysteine.

not in the high B12 group. These data suggest that postmenopausal women with higher TMAO and lower vitamin B12 may be more susceptible to developing colorectal cancer. Certain groups of intestinal bacteria can synthesize (45, 46) and consume (47, 48) vitamin B12, which may affect the vitamin B12 requirement/status of the host. Indeed, overgrowth of intestinal bacteria that take up vitamin B12 has been implicated in B12 malabsorption (47–50). In human intestine, overgrowth of a specific bacterial group can also block colonization of other bacterial groups (16), yielding an imbalance between their metabolic production and consumption. Therefore, elevated colorectal cancer risk among women with high TMAO and low vitamin B12 may in part be associated with the disturbances in colonic bacterial populations. Additional studies are required to confirm these findings, and potential biologic mechanisms need further elucidation.

Key strengths of the present study include: (i) the prospective design; (ii) the large sample size, which allowed for stratified analyses by tumor site/stage as well as by B vitamins and FA fortification periods; and (iii) assessment of choline metabolite ratios (especially betaine:choline ratio), which provided more robust colorectal cancer risk estimates. Several limitations should also be noted: (i) although we attempted to control confounding, there is a potential for residual confounding by factors that were either not collected in the WHI-OS or not measured with sufficient precision; (ii) although the concentrations of plasma choline and its metabolites are stable through time in healthy women (24), single measures of these metabolites may not fully reflect long-term associations with colorectal cancer risk; and (iii) although baseline hysterectomy status was used as a matching factor based on the evidence that female sex hormones (e.g., estrogen) are associated with colorectal cancer risk (51–53), it may not comprehensively account for estrogen status. However, this would not be expected to have an influence on the results, as the analyses were adjusted for the use of postmenopausal hormone therapy (which would more comprehensively account for estrogen status).

In conclusion, the results of this study indicate that alterations in choline metabolism, which may arise early in disease development, associate with higher risk of colorectal cancer in postmenopausal women. Our data also indicate that the plasma betaine:choline ratio may be a potential indicator of colorectal cancer risk, which, if confirmed, could have clinical implications for colorectal cancer screening. This study also

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Table 6. ORs (95% CIs) of colorectal cancer associated with quartiles of plasma TMAO by vitamin B12 status[a]

	Quartiles of TMAO (μmol/L)[b]				
	1 (≤2.6)	2 (>2.6–3.7)	3 (>3.7–5.6)	4 (>5.6)	*P* interaction[c]
Vitamin B12 status					
Age-adjusted					0.0007
Multivariable[d]					0.003
Low B12 (≤505 pg/mL)					
Number of cases	77	107	122	153	
Age-adjusted	1	1.74 (1.17–2.58)	2.01 (1.35–2.98)	2.49 (1.68–3.67)	
Multivariable[d]	1	2.00 (1.30–3.06)	2.06 (1.34–3.17)	2.44 (1.59–3.75)	
High B12 (>505 pg/mL)					
Number of cases	71	122	95	86	
Age-adjusted	1	1.45 (0.97–2.18)	1.11 (0.73–1.69)	1.00 (0.66–1.53)	
Multivariable[d]	1	1.49 (0.96–2.32)	0.98 (0.63–1.55)	0.92 (0.58–1.47)	

[a]ORs (95% CIs) of colorectal cancer were determined by unconditional logistic regression due to case–control matching being broken in these subset analyses. Models were additionally adjusted for ethnicity and time to diagnosis.
[b]Medians for each quartile: quartile 1 = 2.0 μmol/L; quartile 2 = 3.1 μmol/L; quartile 3 = 4.5 μmol/L; and quartile 4 = 8.1 μmol/L.
[c]*P* value for test of interaction between TMAO (as an ordinal variable) and plasma B vitamin status.
[d]Multivariable analyses were adjusted for days to colorectal cancer diagnosis, ethnicity, age, baseline BMI, pack-years of smoking, moderate or strenuous physical activity (min/wk), use of postmenopausal hormone therapy, history of colonoscopy, RBC folate, plasma PLP, and plasma homocysteine.

Table 7. ORs (95% CIs) of colorectal cancer associated with quartiles of plasma betaine by FA fortification periods[a]

	Quartiles of betaine (μmol/L)[b]				
	1 (≤18.8)	2 (>18.8–26.6)	3 (>26.6–34.0)	4 (>34.0)	*P* interaction[c]
Fortification period					
Age-adjusted					0.04
Multivariable[d]					0.08
Prefortification					
Number of cases	50	65	49	38	
Age-adjusted	1	1.45 (0.84–2.51)	0.85 (0.49–1.48)	0.73 (0.41–1.29)	
Multivariable[d]	1	1.06 (0.55–2.01)	0.65 (0.32–1.31)	0.46 (0.22–0.98)	
Perifortification					
Number of cases	107	147	116	89	
Age-adjusted	1	1.43 (0.99–2.07)	0.98 (0.67–1.42)	0.78 (0.53–1.15)	
Multivariable[d]	1	1.10 (0.72–1.67)	0.74 (0.47–1.15)	0.64 (0.39–1.04)	
Postfortification					
Number of cases	44	48	38	44	
Age-adjusted	1	1.09 (0.62–1.92)	1.39 (0.74–2.60)	1.58 (0.85–2.91)	
Multivariable[d]	1	0.88 (0.46–1.69)	0.87 (0.41–1.86)	0.97 (0.45–2.06)	

[a]ORs (95% CIs) of colorectal cancer were determined by unconditional logistic regression due to case–control matching being broken in these subset analyses. Models were additionally adjusted for ethnicity and time to diagnosis.
[b]Medians for each quartile: quartile 1 = 14.4 μmol/L; quartile 2 = 22.8 μmol/L; quartile 3 = 29.9 μmol/L; and quartile 4 = 39.1 μmol/L.
[c]*P* value for test of interaction between betaine (as an ordinal variable) and FA fortification periods.
[d]Multivariable analyses were adjusted for days to colorectal cancer diagnosis, ethnicity, age, baseline BMI, pack-years of smoking, moderate or strenuous physical activity (min/wk), use of postmenopausal hormone therapy, history of colonoscopy, RBC folate, plasma PLP, plasma vitamin B12, and plasma homocysteine.

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provides new evidence that plasma TMAO, an oxidative derivative of choline produced by intestinal bacteria, may serve as a potential biomarker for increased risk of colorectal cancer especially among those with low plasma vitamin B12 concentrations. Although further investigations are needed to delineate the underlying mechanisms, these novel findings may advance understanding of an etiologic correlation between intestinal bacteria and colorectal cancer pathogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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