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Serum Trimethylamine N-oxide, Carnitine, Choline and Betaine in Relation to Colorectal Cancer Risk in the Alpha Tocopherol and Beta Carotene Study

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Abstract

Background—TMAO, a choline-derived metabolite produced by gut microbiota, and its biomarker precursors have not been adequately evaluated in relation to colorectal cancer risk.

Methods—We investigated the relationship between serum concentrations of TMAO and its biomarker precursors (choline, carnitine and betaine) and incident colorectal cancer risk in a nested case-control study of male smokers in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study. We measured biomarker concentrations in baseline fasting serum samples from 644 incident colorectal cancer cases and 644 controls using LC-MS/MS. Logistic regression models estimated the odds ratio (OR) and 95% confidence interval (CI) for colorectal cancer by quartile (Q) of serum TMAO, choline, carnitine and betaine concentrations.

Results—Men with higher serum choline at ATBC baseline had approximately 3-fold greater risk of developing colorectal cancer over the ensuing (median ± IQR) 14 ±10 years (in fully adjusted models, Q4 vs. Q1 OR, 3.22; 95% CI, 2.24–4.61; P trend<0.0001). The prognostic value of serum choline for prediction of incident colorectal cancer development was similarly robust for proximal, distal and rectal colon cancers (all P<0.0001). The association between serum TMAO, carnitine, or betaine and colorectal cancer risk was not statistically significant (P=0.25, P=0.71 and P=0.61, respectively).

Conclusions—Higher serum choline concentration (but not TMAO, carnitine, or betaine) was associated with increased risk of colorectal cancer.

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Disclosure

All other authors have no relationships to disclose.

Impact—Serum choline levels showed strong prognostic value for prediction of incident colorectal cancer risks across all anatomical subsites, suggesting a role of altered choline metabolism in colorectal cancer pathogenesis.

Keywords

Trimethylamine N-oxide (TMAO); carnitine; choline; betaine; colorectal cancer

INTRODUCTION

Recent studies provide convincing evidence that individuals with higher serum trimethylamine N-Oxide (TMAO) have greater risk of several detrimental outcomes, including atherosclerosis, cardiovascular disease (CVD) and adverse thrombotic events (1–4). TMAO is a metabolite formed by host hepatic metabolism of intestinal bacteria-derived trimethylamine (TMA), which is in turn derived from several nutrients that can be obtained through the diet—choline, carnitine, or (to a lesser extent) betaine (2, 5–7). Despite the potential relevance of TMAO to the gut, there is limited evidence evaluating the association between TMAO and its biomarker precursors in relation to the risk of colorectal cancer, the third leading cause of cancer-related deaths in the United States (8).

Only one study, to the best of our knowledge, has investigated the association between baseline circulating TMAO concentrations and incident risk of colorectal cancer (9). In 835 matched case-control female pairs from the Women's Health Initiative (WHI) Observational Study, higher plasma TMAO concentration was associated with a 3-fold greater risk of rectal cancer (9). Moreover, in that study, plasma choline concentration was positively associated with rectal cancer risk, whereas plasma betaine concentration was inversely associated with colorectal cancer (9). The association between TMAO and colorectal cancer risk among men has not yet been examined. Further epidemiologic evidence is needed to gain a better understanding of the relationship between serum TMAO, its precursor biomarkers and incident colorectal cancer risks, particularly among men.

Several mechanistic links between TMAO, its biomarker precursors and colorectal cancer risk are plausible. One potential link between TMAO and colorectal cancer risk is its involvement in inflammatory pathway upregulation (10). Choline and betaine may be involved in carcinogenesis through their role as methyl donors in one-carbon metabolism (11, 12). There are also several lines of evidence linking diet in general and choline specifically, to both colorectal cancer and TMAO. Red and processed meats are a shared risk factor for both cardiovascular disease (CVD) (13, 14) and colorectal cancer (8, 15–20) and these foods are also dietary sources of TMAO precursors (carnitine and choline); in humans, higher meat intake is associated with higher circulating and urinary TMAO levels (2, 21).

Collectively, these studies suggest the need for further examination of TMAO and related metabolites in relation to colorectal cancer risk. Herein we investigated the association between serum levels of TMAO and its nutrient precursors choline, carnitine and betaine and prospective colorectal cancer risk in a nested case-control study of men.

MATERIALS AND METHODS

Study population

We conducted a nested case-control study within the Alpha Tocopherol and Beta Carotene Cancer Prevention (ATBC) Study, described in detail elsewhere (22). Briefly, the ATBC Study was a large randomized, double-blind, placebo-controlled, primary prevention trial of vitamin E (50 mg/day DL-α-tocopheryl acetate) and beta-carotene (20 mg/day β-carotene) among 29,133 male Finnish smokers aged 50–69 at baseline; the primary endpoint in the ATBC Study was lung cancer occurrence. ATBC excluded men with a prior cancer or serious illness and men who reported current use of high levels of vitamin E, A, or beta-carotene. Study supplementation occurred from enrollment (1985–1988) until death or the end of the trial (April 30, 1993) and follow-up is continuing through the Finnish Cancer Registry and the Register of Causes of Death (23). Our study includes follow-up through December 31, 2011. The ATBC Study obtained written informed consent from all participants and was approved by institutional review boards at the US National Cancer Institute and the Finnish National Public Health Institute.

Selection of cases and controls—We included all identified colorectal cancer cases (n=644; International Classification of Diseases 9, codes 153–154) and an equivalent number of controls. Incident colorectal cancer cases in ATBC were identified by the Finnish Cancer Registry (23), which provides nearly complete ascertainment of cases. Outcomes of interest included total and site-specific colorectal cancers (proximal colon ICD-9 153.1, 153.4–153.6; distal colon ICD-9 153.2, 153.3, 153.7; rectum ICD-9 154.0–154.1).

ATBC participants were eligible for this nested case-control study if they had an available baseline serum specimen of adequate volume with ≤1 prior freeze-thaw cycle and no prior rare cancer (whose specimens were reserved for other studies). 20,846 participants met these criteria, including 644 cases and 20,199 potential controls. Incidence density matching was used to select one control alive and free of colorectal cancer from each case's risk set without replacement. Within the risk sets of cases, controls were matched 1:1 on age at randomization (±5 years) and within the pool of eligible controls we selected the specimen that minimized the difference in thaw count and serum draw date between the chosen control and the case. Two colorectal cancer cases were diagnosed with cancer at both the proximal colon and rectum and thus are counted once in the overall analyses for colorectal cancer but also contribute to each of the site-specific cases.

Laboratory analysis

ATBC collected overnight fasting serum samples at the pre-randomization baseline study visit. Samples were stored at −70°C and the median time from blood collection until colorectal cancer diagnosis was 14 years (range 1 month to 26 years). Biospecimens were shipped overnight on dry ice to the Cleveland Clinic laboratory that measured serum TMAO, choline, carnitine and betaine concentrations. Metabolites were analyzed by stable isotope dilution liquid chromatography-tandem mass spectrometry (LC/MS/MS) using established methods (1, 2) on a Shimadzu LCMS-8050 CL Triple Quadrupole Liquid Chromatograph Mass Spectrometer with Nexera LC-30AD CL UHPLC interface. Investigators performing

analyses were blinded to sample identity (other than barcode label) and to case-control status. Specimens were divided into 27 batches and case-control pairs were included in the same batch. Blinded quality control specimens were randomly inserted into each batch; these samples comprised approximately 10% of all specimens assayed and assay values from these specimens were used to calculate coefficients of variation for the three assays. The average inter-batch coefficients of variation for the blind duplicate control specimens across the entire analyses were between 3–5% as follows: carnitine 3%, choline 4%, TMAO 5% and betaine 5%.

Covariate Assessment

At baseline, ATBC administered a questionnaire that collected data on demographics, medical history, physical activity and smoking and height and weight were measured. Total energy intake was estimated by a 276-item food frequency questionnaire (FFQ) that participants completed at baseline; usual intake of specific foods in grams per day (g/day) over the past 12 months was calculated by linkage to a food-composition database of the National Public Health Institute in Finland.

Statistical analysis

We compared baseline characteristics of colorectal cancer cases and controls using t-tests. We used Spearman correlations to describe the association between the serum biomarkers (TMAO, choline, carnitine, betaine). We used unconditional logistic regression models to estimate the odds ratio (OR) and 95% confidence interval (CI) for colorectal cancer for each quartile of the serum biomarkers (based on the distribution of controls). P-values for trend (denoted as P-trend) were calculated by testing if the regression coefficient for a continuous exposure, which was defined as the median value within each quartile, differed from zero. All models were adjusted for batch (categorical) and age (continuous). The fully adjusted model included age, batch, years smoked, cigarettes per day, education, body mass index (BMI), physical activity and total energy intake. We also evaluated models adjusted for alcohol consumption and aspirin use and considered models excluding cases that occurred within the first two years and, separately, the first five years of follow-up. We explored potential interactions between the biomarker concentration quartile and years smoked, number of cigarettes per day, quartile of alcohol intake and BMI (<median, ≥median). In supplemental analyses we investigated whether there were differences in serum biomarker concentrations by cancer stage at diagnosis or by the ATBC Study randomization arm. Statistical significance was defined as $P<0.05$ and tests of significance were two-sided. Analyses were conducted in SAS 9.3 (SAS Institute, Cary, NC).

RESULTS

At baseline, the mean age of study participants was 57 years and most (>80%) men were married (Table 1). On average, these smokers initiated smoking at age 19, smoked about one pack of cigarettes per day and had regularly smoked for 36 years. Most baseline characteristics, including demographics, smoking and dietary intake, were comparable between incident colorectal cancer cases and controls. There was a small difference between cases and controls in body weight (mean 80.4 versus 78.9 kg among cases and controls

(P=0.03), respectively) but the difference in BMI was not statistically significant (P=0.06). Nominal but non-statistically significant differences between cases and controls were observed for aspirin use (P=0.09) and alcohol consumption (P=0.05); there was no difference in reported intake of folate (P=0.94). Comparing dietary intake of choline- and carnitine-containing foods between cases and controls (Supplementary Table S1), there were no significant differences for red meat (P=0.79), processed meat (P=0.30), fish (P=0.07), or eggs (P= 0.13).

Serum TMAO, choline, carnitine and betaine concentrations were moderately inter-correlated (Supplementary Table S2). Among controls, the Spearman correlation coefficients (P-value) for these biomarkers were as follows: TMAO vs. carnitine 0.22 (P<0.0001), TMAO vs. choline 0.23 (P<0.001), carnitine vs. choline 0.36 (P<0.0001) and betaine vs. choline 0.40 (P<0.0001). The magnitude and significance of these associations were similar among cases.

In this study, no statistically significant association was observed between serum levels of TMAO and risk of total or site-specific colorectal cancer (Table 2). In the fully adjusted model the estimated risk of colorectal cancer in the highest quartile of serum TMAO was not significantly different (P=0.25) compared to the lowest quartile (OR 1.20; 95% CI 0.86–1.68). In investigations by anatomical subsite, we observed similarly elevated (but non-significant) point estimates among those in the highest quartile of serum TMAO for cancer of the proximal colon; there was no association between TMAO and rectal cancer. OR estimates from models that were further adjusted for alcohol intake and aspirin use were largely unchanged (Supplementary Table S3).

In addition to serum TMAO concentrations, we also examined serum metabolites that are precursors of TMAO— namely, choline, carnitine and betaine. Serum choline was strongly and statistically significantly associated with incident colorectal cancer risk (Table 3); participants with higher serum choline had greater risk of developing colorectal cancer over the ensuing follow-up period (P-trend<0.0001). Compared to those in the lowest quartile of choline, the ORs (95% CIs) for colorectal cancer development in the fully-adjusted model among increasing choline quartiles 2–4 were 1.05 (0.73–1.51), 1.26 (0.86–1.84), and 3.38 (2.37–4.80). The direction and significance of the association between serum choline levels and colorectal cancer development risk was consistent for cancers of the proximal colon, distal colon and rectum furthermore, the risk associated with choline persisted after eliminating cases that occurred early during follow-up (first two years, and separately, first five years) (Supplementary Table S4). Further adjustment for alcohol intake and aspirin use did not appreciably alter estimates Supplementary Table S3). In fully adjusted models estimating colorectal cancer risk, there were no significant interactions between serum choline and years smoked (p=0.47), number of cigarettes smoked per day (p=0.74), quartile of alcohol intake (p=0.97), or BMI (p=0.54).

There was no significant association between serum carnitine and risk of total or site-specific colorectal cancer (Table 4). Comparing the highest quartile to the lowest quartile of carnitine, the OR (95% CI) for colorectal cancer was 1.03 (0.73–1.44) in the fully-adjusted model (P-trend=0.71). The P-trend for total colorectal cancer and cancers of the proximal

colon, distal colon and rectum were not statistically significant. Similarly, no statistically significant association was observed between serum betaine and colorectal cancer risk (Table 4) in fully adjusted models; comparing the highest quartile to the lowest quartile of betaine the OR (95% CI) for colorectal cancer was 1.12 (0.81–1.55).

Several additional analyses were undertaken in efforts to fully describe the aforementioned biomarker-cancer associations. We investigated whether the biomarker concentrations differed by ATBC intervention arm, as our case-control study population is derived from a large randomized trial and thus some (74%) participants were randomized to one of the three active intervention arms (alpha-tocopherol supplements, beta-carotene supplements, or both) with the remaining 26% in the placebo arm. However, as expected there was no evidence that these serum biomarker concentrations differed by intervention arm; in fully-adjusted logistic regression models, for example, ATBC intervention arm was not predicted by serum choline (p=0.93) or, separately, by serum TMAO (p=0.10). We found no evidence of variation in serum biomarker concentrations according to stage of cancer diagnosis; in fully-adjusted logistic regression models neither serum choline (p=0.65) nor TMAO (p=0.40) predicted stage of cancer at diagnosis (p=0.65). There was no interaction between BMI and quartile of serum TMAO (Type III p=0.09) or choline (Type III p=0.54). In order to facilitate comparisons to other studies, we also present the associations between each biomarker and risk of colon cancer, defined as cancer diagnoses of either the proximal or distal colon, in Supplementary Table S5; risk estimates for colon cancer are similar to overall colorectal cancer findings in that serum choline was positively associated with risk.

DISCUSSION

We identified a strong association between serum choline (the presumed major dietary source of TMA (24), from which TMAO is derived) and the risk of colorectal cancer, whereby men in the highest quartile of serum choline demonstrated a significantly increased 3-fold risk of developing colorectal cancer compared to men in the lowest quartile. This association was consistent across all three examined anatomical subsites of colorectal cancer including cancers of the proximal colon, distal colon and rectum. In this first prospective study of TMAO and colorectal cancer risk among men, we did not observe a significant association. There was also no association noted between serum levels of either carnitine or betaine, alternative dietary precursors of TMAO, and colorectal cancer development.

To our knowledge, only one prospective study has previously investigated the association between serum TMAO and colorectal cancer risk (9). In contrast to our null TMAO-colorectal cancer findings for total and site-specific colorectal cancer, the WHI observed that women with higher plasma TMAO had an increased risk of rectal cancer and, among women with low plasma B12, greater risk of overall colorectal cancer. While statistically significant, the WHI point estimate for rectal cancer risk in the highest quartile of TMAO had a very wide confidence interval; additionally, despite the positive finding for rectal cancer, TMAO was not significantly associated with risk of overall colorectal cancer or cancers of the proximal or distal colon in the WHI (9). Whether sex explains the different TMAO findings in ATBC and the WHI with respect to colorectal cancer risk is unknown. However, it should be noted that prior epidemiologic studies examining predictors of TMAO did not observe an

influence of sex on TMAO concentrations, though these studies have predominantly included post-menopausal aged women (6, 25). Several other differences between the ATBC and WHI populations, including differences in the underlying distribution of biomarker concentrations, may have contributed to the divergent findings. Serum choline concentrations (μmol/L), for example, were more variable and slightly higher, on average, among controls in this study (mean 10.4 SD 9.9) compared to controls in the WHI (mean 9.4 SD 2.2). Serum TMAO concentrations, in contrast, were comparable between controls in ATBC and WHI, with median (25^{th}–75^{th} percentile) concentrations of 3.6(2.5–5.2) and 3.8 (2.6–5.7), respectively (9); it is thus unlikely that the lack of association between TMAO and colorectal cancer in ATBC, in contrast to the positive association reported by WHI, is due to differences in the distribution of serum TMAO concentrations. Although ATBC and WHI utilized different specimen types (serum and plasma, respectively), this is unlikely to explain the divergent TMAO findings given that studies comparing side-by-side plasma versus serum levels of TMAO recovered from subjects at the same time show no differences in TMAO levels from the two matrices (26). Further epidemiologic studies are needed in order to fully evaluate the association between serum TMAO and colorectal cancer in both sexes.

In addition to TMAO and choline, we investigated serum carnitine and betaine in relation to colorectal cancer risk. As expected, we found a modest direct correlation between serum concentrations of TMAO and carnitine; however, there was no association between serum carnitine concentration and colorectal cancer risk. We did not detect an association between serum betaine and colorectal cancer risk; this is in contrast to two previously reported inverse associations between betaine, colorectal cancer (9), colorectal adenoma (27). While betaine was reported to have an inverse correlation with colorectal cancer risk in the WHI study (9), we observed no association between betaine concentration and incident colorectal cancer development in this study of men.

A link between choline and colorectal cancer risk has been previously reported (9, 28). The gut microbiota converts dietary choline, typically in the form of phosphatidylcholine, to TMA (6), which is the precursor for TMAO. We observed a strong increased risk of colorectal cancer with higher serum choline. This observation is consistent with the modest positive association detected by the nested case-control study in the WHI (9), although our risk estimates are substantially higher [OR (95% CI); 3.38 (2.37–4.80) compared to 1.22 (0.88–1.70) for colorectal cancer and 4.09 (2.35–7.12) compared to 2.44 (0.93–6.40) for rectal cancer]. In contrast, a nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) detected an inverse association between serum choline and colorectal cancer risk among women and a null association among men (29). Null associations were also reported for serum choline and colorectal adenoma in a cross-sectional Norwegian study (27). The mixed findings for serum choline and colorectal cancer risk reported by observational epidemiologic studies may be due to a variety of factors including differences in study populations. The ATBC study population was confined exclusively to male smokers. Whether tobacco use impacts the relationship between choline and colorectal cancer risk is unknown. However, sex may contribute to differences in the association between choline and colorectal cancer risk; estrogens are known to increase the activity of the Phosphatidylethanolamine N-methyltransferase (PEMT) pathway by which phosphatidylcholine is synthesized (30). Additionally, there may be differences in the

distribution of serum choline concentration between different study populations. As previously mentioned, controls in this study had slightly higher mean serum choline concentration compared to controls in WHI (9); this may partly explain the higher ORs observed in this study compared to WHI. Furthermore, there is evidence that the magnitude of the upper end of range of serum choline concentrations (95^{th} percentiles) was higher in colorectal cancer cases in this study (37.4 μmol/L) compared to EPIC cases (14.2 μmol/L) (29).

The potential relation of choline to cancer is complex (30). Currently, there is a limited understanding of choline's role in cancer etiology, although a prior study demonstrated that choline kinase is overexpressed in human colorectal cancer cells (28); this enzyme initiates the first and rate-limiting step of converting choline to phosphatidylcholine. Choline kinase is a potential new target for cancer treatments, as associations have been reported for choline kinase-α expression/activity and both malignancy and increased cellular proliferation (31). Increased total choline-containing compounds, referred to as the 'colonic phenotype,' is a recently identified metabolic hallmark of malignant transformations (32). Differential uptake of choline, which can be measured by positron emission tomography (33), has been noted in several cancers. There is other evidence that activated choline metabolism may result from the malignancy itself, rather than as a result of enhanced proliferation (34). In our study, the elevated risk of colorectal cancer with higher serum choline persisted even after excluding cases that occurred early during follow-up (the first two years and separately, the first five years); thus, it is unlikely, but not impossible, that our risk estimates reflect the promotion of growth of precancerous lesions by choline. A similar case exists for folate, a nutrient with a central role in one-carbon metabolism, where deficiency promotes carcinogenesis while folate supplementation is thought to promote tumor growth and progression (35). Studies in mice have shown that diets deficient in methyl donors (choline, folic acid, methionine, and vitamin B12) and supplemented with homocysteine can change the intestinal epithelium and result in prolonged protection against colorectal tumor development (36).

Strengths of this study include the prospective design, large sample size, the ability to stratify by tumor site and state-of-the-art assay methods. The consistency in the direction of the significant choline risk estimates in this study and the WHI lends support to the validity of our choline findings and a connection between choline metabolism and colorectal cancer development. Limitations of this study included use of a single blood specimen to measure biomarkers, which may not reflect long-term concentrations. Additionally, since this sample is comprised of male Finnish smokers the results herein may not be generalizable to other populations; the epidemiologic data to date raise the need for additional studies that evaluate both sexes. Lastly, choline status can be modulated by several factors including folate nutritional status (37, 38) and the composition of the intestinal gut microbiome (39); however, neither factor was measured in this study. Folate nutritional status may differ between the ATBC and WHI study populations given that Finland does not require mandatory folic acid fortification of staple foods, in contrast to the US (40); however, WHI analyses did not detect differences in the association between plasma metabolites and colorectal cancer risk by fortification period (9) and thus folate fortification (or lack thereof) is unlikely to have a substatntial impact on our findings. Though no significant association between TMAO and colorectal cancer risk were observed in the present study, whether or

not alternative choline and gut microbial processes or pathways contribute to colorectal cancer development remain to be examined.

In this study of male Finnish smokers, we did not detect an association between serum TMAO and colorectal cancer risk. Men with high serum choline had a statistically significant three-fold increase in colorectal cancer risk compared to men with low serum choline. Future studies should investigate serum choline and colorectal cancer risk in more diverse study populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline characteristics of colorectal cancer cases and controls in a nested case-control study within the ATBC Study

	Colorectal cancer cases (N=644)		Controls (N=644)		P-value
	N	**Value**	**N**	**Value**	
Age at baseline	644	57 (5)	644	57 (5)	0.39
Married	644	530 (82)	644	539 (84)	0.46
Education	644		644		0.19
<HS		186 (29)		216 (34)	
Some college or technical school		421 (65)		396 (61)	
College graduate		37 (6)		32 (5)	
Height (cm)	643	174.0 (6.0)	644	173.7 (6.1)	0.39
Weight (kg)	643	80.4 (12.6)	644	78.9 (11.4)	**0.03**
Body mass index[a] (kg/m2)	643	26.5 (3.8)	644	26.1 (3.4)	0.06
Heavy physical activity					
Leisure time	644	39 (6)	643	45 (7)	0.49
Occupational	644	51 (8)	644	64 (10)	0.21
Physical activity ≥3 times/week[a]	644	118 (18)	644	120 (19)	0.89
Family history of colorectal cancer	436	25 (6)	485	22 (5)	0.41
Cigarettes per day	644	19.4 (8.4)	644	20 (9)	0.93
Years Smoked	644	35.9 (8.3)	644	35.9 (7.9)	0.95
Pack-years	644	35.2 (17.5)	644	35.4 (18.0)	0.90
Age at smoking initiation)	644	19.6 (4.9)	644	19 (4)	0.11
Aspirin use (% yes)	489	70 (14)	536	98 (18)	0.09
Supplement Use	643	126 (20)	643	125 (19)	0.94
Calcium	637	64 (10)	638	70 (11)	0.59
Vitamin D	637	39 (6)	638	52 (8)	0.16
Dietary Intake (per day)					
Energy (kcal)	614	2691.5 (708.6)	616	2668.3 (708.2)	0.57
Folate including supplements (μg)	614	339.8 (98.7)	616	340.2 (102.2)	0.94
Alcohol (g)	614	18.8 (20.9)	616	16.5 (20.3)	0.05

	Colorectal cancer cases (N=644)		Controls (N=644)		P-value
	N	Value	N	Value	
Serum biomarkers (μmol/L)					
Trimethylamine N-oxide (TMAO)	644	4.8 (3.9)	644	4.7 (3.9)	0.51
Median (25th–75th percentile)		3.73 (2.61–5.46)		3.6 (2.5–5.2)	
Choline	644	15.2 (15.2)	644	10.4 (9.9)	**<0.01** *
Median (25th–75th percentile)		10.24 (7.90–15.68)		8.7 (7.0–10.5)	
Carnitine	644	34.2 (7.4)	644	33.9 (7.2)	0.40
Median (25th–75th percentile)		33.79 (29.91–38.23)		33.5 (29.8–38.3)	
Betaine	644	33.08 (13.72)	644	32.4 (11.2)	0.33
Median (25th–75th percentile)		30.72 (25.05–38.54)		30.6 (24.8–37.6)	

ATBC Study, The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. 2 participants had diagnoses of both colon and rectal cancers on the same date and only contribute once to these data. Values are number (percent) or mean (standard deviation) unless otherwise noted. P-values represent a t-test for difference in unadjusted means.

[a] Leisure-time physical activity

* P<0.0001

Table 2

ORs (95% CIs) of colorectal cancer ranked by quartile of serum TMAO

	OR (95% CI) by quartile of serum TMAO (μmol/L)					
	N	**1 (≤2.5)**	**2 (>2.5–3.6)**	**3 (>3.6 to 5.4)**	**4 (>5.4)**	**P trend**
Colorectal cancer						
No. cases/ No. controls	644/644	154/167	159/166	163/156	168/155	
Age and batch adjusted	644/644	1.00 (Ref.)	1.04 (0.76–1.42)	1.14 (0.83–1.57)	1.20 (0.86–1.68)	0.26
Fully adjusted[a]	642/643	1.00 (Ref.)	1.04 (0.76–1.42)	1.15 (0.83–1.58)	1.20 (0.86–1.68)	0.25
Cancer of the Proximal Colon						
No. cases/ No. controls	169/169	43/53	38/47	51/37	37/32	
Age and batch adjusted	169/169	1.00 (Ref.)	0.99 (0.54–1.84)	1.83 (0.99–3.41)	1.60 (0.80–3.22)	0.12
Fully adjusted[a]	169/169	1.00 (Ref.)	1.00 (0.54–1.86)	1.84 (0.99–3.43)	1.60 (0.80–3.22)	0.12
Cancer of the Distal Colon						
No. cases/ No. controls	153/153	30/35	50/36	34/45	39/37	
Age and batch adjusted	153/153	1.00 (Ref.)	1.63 (0.82–3.24)	0.85 (0.41–1.78)	1.22 (0.58–2.56)	0.95
Fully adjusted[a]	152/153	1.00 (Ref.)	1.73 (0.86–3.46)	0.81 (0.38–1.70)	1.21 (0.57–2.56)	0.89
Rectal Cancer						
No. cases/ No. controls	282/282	76/68	62/71	63/67	81/76	
Age and batch adjusted	282/282	1.00 (Ref.)	0.77 (0.48–1.25)	0.83 (0.50–1.36)	0.97 (0.59–1.60)	0.87
Fully adjusted [a]	281/281	1.00 (Ref.)	0.76 (0.47–1.24)	0.83 (0.50–1.36)	0.94 (0.57–1.57)	0.92

Logistic regression models adjusted for batch (categorical, 27 batches) and age (continuous). Quartiles of serum TMAO were based on the distribution among controls. Medians for each quartile (based on controls) were used in trend test; medians for TMAO quartiles 1 through 4 were 2.0, 3.1, 4.4 and 7.7, respectively. 2 participants had cancer of the proximal colon and rectum and are counted in both disease-stratified analyses.

[a]Model additionally adjusted for the number of years smoked regularly (continuous), number of cigarettes smoked per day (continuous), education (college graduate, some college or technical school, versus ≤ high school), body mass index (continuous, kg/m^2), heavy physical activity in leisure (yes/no), heavy occupational physical activity (yes/no), physical activity frequency (≥3 times per week, versus <3), total energy intake (continuous, kcal/day)

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Table 3

ORs (95% CIs) of colorectal cancer ranked by quartile of serum choline

		OR (95%CI) by quartile of serum choline (μmol/L)				
	N	**1 (≤7.0)**	**2 (>7.0–8.7)**	**3 (>8.7 to 10.5)**	**4 (>10.5)**	**P trend**
Colorectal cancer						
No. cases/ No. controls	644/644	113/161	108/161	116/160	307/162	
Age and batch adjusted	644/644	1.00 (Ref.)	1.04 (0.73–1.500	1.25 (0.86–1.83)	3.37 (2.37–4.79)	**<0.01** *
Fully adjusted[a]	642/643	1.00 (Ref.)	1.05 (0.73–1.51)	1.26 (0.86–1.84)	3.38 (2.37–4.80)	**<0.01** *
Cancer of the Proximal Colon						
No. cases/ No. controls	169/169	33/44	28/39	31/46	77/40	
Age and batch adjusted	169/169	1.00 (Ref.)	1.02 (0.50–2.06)	1.10 (0.52–2.31)	3.35 (1.66–6.76)	**<0.01** *
Fully adjusted[a]	169/169	1.00 (Ref.)	1.02 (0.50–02.06)	1.08 (0.51–2.29)	3.37 (1.67–6.81)	**<0.01** *
Cancer of the Distal Colon						
No. cases/ No. controls	153/153	24/39	22/40	30/31	77/43	
Age and batch adjusted	153/153	1.00 (Ref.)	1.01 (0.46–2.22)	2.36 (1.02–5.51)	4.24 (1.98–9.05)	**<0.01** *
Fully adjusted[a]	152/153	1.00 (Ref.)	0.99 (0.45–2.18)	2.27 (0.97–5.31)	4.07 (1.89–8.75)	**<0.01** *
Rectal Cancer						
No. cases/ No. controls	282/282	76/68	62/71	63/67	81/76	
Age and batch adjusted	282/282	1.00 (Ref.)	1.19 (0.68–2.07)	1.09 (0.61–1.95)	4.06 (2.34–7.04)	**<0.01** *
Fully adjusted[a]	281/281	1.00 (Ref.)	1.22 (0.70–2.14)	1.09 (0.61–1.96)	4.09 (2.35–7.12)	**<0.01** *

Logistic regression models adjusted for batch (categorical, 27 batches) and age (continuous). Quartiles of serum choline were based on the distribution among controls. Medians for each quartile (based on controls) were used in trend test; medians for choline quartiles 1 through 4 were 6.0, 7.8, 9.4 and 12.9, respectively.

*P<0.0001

**P<0.001

[a] Model additionally adjusted for the number of years smoked regularly (continuous), number of cigarettes smoked per day (continuous), education (college graduate, some college or technical school, versus ≤ high school), body mass index (continuous, kg/m^2), heavy physical activity in leisure (yes/no), heavy occupational physical activity (yes/no), physical activity frequency (≥3 times per week, versus <3), total energy intake (continuous, kcal/day)

Table 4

ORs (95% CIs) of colorectal cancer ranked by quartile of serum carnitine and betaine

OR (95% CI) by quartile of serum biomarker (μmol/L)						
Carnitine	N	1 (≤29.8)	2 (>29.8 to 33.5)	3 (>33.5 to 38.3)	4 (>38.3)	P trend
Colorectal cancer						
No. cases/ No. controls	644/644	157/161	151/161	178/161	158/161	
Age and batch adjusted	644/644	1.00 (Ref.)	0.97 (0.70–1.35)	1.15 (0.83–1.59)	1.02 (0.73–1.43)	0.74
Fully adjusted[a]	642/643	1.00 (Ref.)	0.97 (0.70–1.35)	1.15 (0.83–1.59)	1.03 (0.73–1.44)	0.71
Cancer of the Proximal Colon						
No. cases/ No. controls	169/169	38/50	42/46	50/34	39/39	
Age and batch adjusted	169/169	1.00 (Ref.)	1.34 (0.70–2.59)	2.22 (1.13–4.36)	1.44 (0.72–2.87)	0.16
Fully adjusted[a]	169/169	1.00 (Ref.)	1.33 (0.69–2.58)	2.21 (1.12–4.34)	1.42 (0.71–2.84)	0.17
Cancer of the Distal Colon						
No. cases/ No. controls	153/153	42/36	25/42	46/41	40/34	
Age and batch adjusted	153/153	1.00 (Ref.)	0.49 (0.24–1.00)	0.96 (0.49–1.89)	1.05 (0.50–2.20)	0.60
Fully adjusted[a]	152/153	1.00 (Ref.)	0.49 (0.24–1.02)	0.97 (0.49–1.90)	1.06 (0.51–2.22)	0.60
Cancer of the Rectum						
No. cases/ No. controls	282/282	69/66	73/62	70/77	70/77	
Age and batch adjusted	282/282	1.00 (Ref.)	1.12 (0.67–1.85)	0.85 (0.51–1.41)	0.84 (0.51–1.40)	0.37
Fully adjusted[a]	281/281	1.00 (Ref.)	1.15 (0.69–1.91)	0.84 (0.51–1.41)	0.85 (0.51–1.41)	0.37
Betaine	**N**	**1 (≤24.75)**	**2 (>24.75 to 30.62)**	**3 (>30.62 to 37.56)**	**4 (>37.56)**	**P trend**
Colorectal cancer						
No. cases/ No. controls	644/644	152/161	169/161	154/161	169/161	
Age and batch adjusted	644/644	1.00 (Ref.)	1.12 (0.82–1.54)	1.02 (0.74–1.41)	1.13 (0.82–1.56)	0.58

		OR (95% CI) by quartile of serum biomarker (μmol/L)				
Fully adjusted[a]	642/643	1.00 (Ref.)	1.12 (0.82–1.53)	1.02 (0.74–1.40)	1.12 (0.81–1.55)	0.61
Cancer of the Proximal Colon						
No. cases/ No. controls	169/169	40/49	41/31	43/45	45/44	
Age and batch adjusted	169/169	1.00 (Ref.)	1.68 (0.87–3.23)	1.20 (0.64–2.25)	1.33 (0.69–2.53)	0.62
Fully adjusted[a]	169/169	1.00 (Ref.)	1.71 (0.88–3.30)	1.24 (0.66–2.33)	1.34 (0.70–2.57)	0.60
Cancer of the Distal Colon						
No. cases/ No. controls	153/153	38/37	41/40	34/40	40/36	
Age and batch adjusted	153/153	1.00 (Ref.)	1.00 (0.52–1.92)	0.81 (0.41–1.58)	1.11 (0.57–2.18)	0.83
Fully adjusted[a]	152/153	1.00 (Ref.)	1.00 (0.52–1.93)	0.79 (0.40–1.57)	1.17 (0.59–2.31)	0.73
Rectal Cancer						
No. cases/ No. controls	282/282	62/60	81/78	68/67	71/71	
Age and batch adjusted	282/282	1.00 (Ref.)	1.12 (0.69–1.82)	1.09 (0.66–1.81)	1.08 (0.65–1.80)	0.86
Fully adjusted[a]	281/281	1.00 (Ref.)	1.10 (0.68–1.78)	1.05 (0.63–1.75)	1.02 (0.61–1.71)	0.98

Logistic regression models adjusted for batch (categorical, 27 batches) and age (continuous). Quartiles of serum carnitine were based on the distribution among controls. Medians for each quartile (based on controls) were used in trend test; medians for carnitine quartiles 1 through 4 were 26.4, 31.9, 35.5 and 41.1, respectively; medians for betaine quartiles 1 through 4 were 24.75, 30.62 and 37.56, respectively.

*P<0.0001

**P<0.001

[a]Model additionally adjusted for the number of years smoked regularly (continuous), number of cigarettes smoked per day (continuous), education (college graduate, some college or technical school, versus ≤ high school), body mass index (continuous, kg/m^2), heavy physical activity in leisure (yes/no), heavy occupational physical activity (yes/no), physical activity frequency (≥3 times per week, versus <3), total energy intake (continuous, kcal/day)