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ORIGINAL ARTICLE

Trimethylamine-*N*-oxide: A Novel Biomarker for the Identification of Inflammatory Bowel Disease

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Abstract
Background The gastrointestinal (GI) microbiome is recognized for potential clinical relevance in inflammatory bowel disease (IBD). Data suggest that there is a disease-dependent loss of microbial diversity in IBD. Trimethylamine-*N*-oxide (TMAO) is generated by GI anaerobes through the digestion of dietary phosphatidylcholine and carnitine in a microbial-mammalian co-metabolic pathway. IBD-related changes in the gut microbiome may result in disease-specific changes in TMAO plasma concentrations.
Aim To determine whether TMAO plasma levels in IBD are altered compared to controls and whether they correlate with disease presence or activity.
Methods Liquid chromatography–tandem mass spectrometry was used to measure TMAO, choline, and carnitine plasma levels in 479 subjects (373 non-IBD controls, 106 IBD). Subjects were also genotyped for the flavin monooxygenase (FMO)3 variants, E158K and E308G.
Results Plasma TMAO levels were 2.27 μM lower in the IBD population compared to the control population ($p = 0.0001$). Lower TMAO levels were similarly seen in active ulcerative colitis (UC) (1.56 μM) versus inactive disease (3.40 μM) ($p = 0.002$). No difference was seen in active Crohn's disease (CD) versus inactive CD. No intergroup variation existed in plasma TMAO levels based on FMO3 genotype. Choline levels were higher in IBD, while carnitine levels were similar between the two groups, suggesting that lower TMAO levels in IBD were not due to dietary differences.
Conclusions Decreased TMAO levels are seen in IBD compared to a non-IBD population. These data suggest that TMAO may have potential as a biomarker to support IBD diagnosis as well as to assess disease activity in UC.

Keywords Inflammatory bowel disease · Biomarker · Trimethylamine-*N*-oxide · Microbial-mammalian co-metabolism

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Introduction

Inflammatory bowel disease (IBD) is a chronic illness of intestinal inflammation that follows a remitting and relapsing course. It is comprised of two distinct entities: Crohn's disease (CD) and ulcerative colitis (UC). Classically, CD involves the full thickness of the bowel wall and follows a discontinuous path along the gut's full length from mouth to anus. UC is characterized by continuous and superficial, mucosal inflammation confined to the colon and rectum.

The burden of IBD is not insubstantial. IBD affects over 600,000 people in North America, with an estimated 250

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per 100,000 people in UC and 319 per 100,000 people in CD [1]. Some studies suggest that IBD follows a bimodal distribution, with a diagnosis peak seen in the second and third decade of life and a second peak seen in the sixth and seventh decade of life [2]. The pathogenesis of IBD has not been fully elucidated. The most widely held hypothesis suggests that there is an overly aggressive immune response to luminal microbial antigens and other adjuvants that occurs in genetically susceptible individuals facilitated by certain environmental factors [3].

Endoscopic depiction of mucosal inflammation is the gold standard test for diagnosis in combination with histological evidence of chronicity; however, ileocolonoscopy is an invasive test associated with complications which can include bleeding, perforation, and rarely death. Given this, biomarkers of disease have been identified that can aid clinicians in the diagnosis and quantification of disease as well as in predicting the risk of complications and distinguishing between types of IBD. Such biomarkers include, but are not limited to, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fecal leukocyte markers (fecal calprotectin), and antibodies against *Saccharomyces cerevisiae* (ASCA) and perinuclear antineutrophil cytoplasmic proteins (ANCA) [4–7]. Unfortunately, these biomarkers are not without their own respective limitations: CRP and ESR often lack specificity for intestinal inflammation, while fecal calprotectin and serologic antibodies lack accessibility through non-tertiary and quaternary care centers and whose diagnostic accuracy may be confounded by other factors [4, 5]. Therefore, their role in clinical practice has not been consistently defined.

Of note, these biomarkers are derived from the genetic alterations that can occur in IBD or from factors produced due to immune system dysregulation; however, the role of the gut microbiome in the pathogenesis of IBD has also come under scrutiny [8]. Biomarkers derived from possible alterations in the intestinal mircobiota may be an alternative marker of disease presence and severity.

In the healthy individual, the composition of the gastrointestinal (GI) microbiome is unique and consists of hundreds to thousands of species of bacteria; however, these can be categorized into four phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* [9–13]. Factors such as age and diet are known to influence the composition of the gut microbiome [14]. For example, a study by Wu et al. [15] showed that long-term diet was strongly associated with the composition of gut microbiota, while Yatsunenko et al. [16] convincingly demonstrated a similar association with age.

Disease state has also been linked to alterations in the bacterial composition of the gut. Multiple studies demonstrate that diversity in the intestinal microbiota is reduced in IBD compared to non-IBD controls [9, 17]. There is loss of members of multiple genera including *Bacteroides* species, *Clostridia* species, *Eubacterium* species, and *Lactobacillus* species [18, 19]. Microbiota varies with disease activity and distribution [20, 21]. Willing et al. [21] showed that the microbiota in inactive CD was different than that of non-IBD controls, while there was little difference between the microbiota in inactive UC in comparison with non-IBD controls. The impact of this loss of diversity on the pathogenesis and phenotype of IBD is unknown, though it is felt that the gut microbiota may play a role in the former and the latter [8].

Recently, the impact of the gut microbiota on another disease state has come to light. Increased plasma levels of trimethylamine-*N*-oxide (TMAO), a by-product of a gut microbial-mammalian co-metabolic pathway, have been linked with increased risk of cardiovascular disease such as myocardial infarction and stroke in both mouse models and more recently in humans [22–24]. The pathogenesis has not been delineated in detail; however, it is thought to be due to enhanced atherosclerotic plaque formation [25].

TMAO is a microbial-mammalian co-metabolite; its production is dependent on a shared gut microbiome–host metabolic pathway [26]. Specifically, TMAO is the end product of hepatic flavin monooxygenase (FMO)-mediated oxidation of trimethylamine (TMA) in the liver and is excreted mainly in the urine. FMO3 is the most abundant FMO enzyme in humans [27, 28]. Genetic deficiency in FMO3 has been described as the basis for fish odor syndrome, a rare condition that results from inability to convert TMA to TMAO through this enzyme [29]. Homozygous variants of E158K and E308G are associated with reduced enzymatic activity [27]. Little TMA is ingested with food but rather is formed from precursors such as phosphatidylcholine and carnitine found in food products such as eggs, milk, liver, red meat, poultry, fish, and shellfish which then undergo metabolism by the gastrointestinal tract microbiota [22]. Many colonic bacterial species engage in the conversion of phosphatidylcholine and carnitine to TMA, including *Clostridia*, *Proteus*, *Shigella*, and *Aerobacter* [30]. Anaerobes or facultative anaerobes are required [31]. A recent study by Tang et al. demonstrated that TMAO production from phosphatidylcholine is dependent on metabolism by GI tract microbiota [24]. In this study, subjects were fed a meal high in choline, a "choline challenge." TMAO plasma levels were measured over an 8-hour period, with an elevation in the plasma concentration seen within the first hour following the "choline challenge." Subjects were then given a 7-day course of antiobiotics for gut microbiota suppression. A second "choline challenge" was administered following the completion of the antibiotics. TMAO plasma concentrations were suppressed. Following a six-day washout period after completion of the antibiotic course, TMAO

plasma concentrations rebounded after a third "choline challenge." One can theorize that IBD-dependent alterations in the composition of the gut microbiota may lead to IBD-dependent alterations in the production of TMAO, making TMAO a possible biomarker for the identification of IBD.

An ideal biomarker must meet several requirements: It is specific to reduce the risk of false-positive results, is sensitive to allow for the early detection of disease, has a long blood half-life, is quantifiably specific to the disease process being examined, and is robust with a rapid, accurate, and affordable means of detection [32, 33]. TMAO is an easily and rapidly quantifiable metabolite through the use of liquid chromatography–mass spectrometry [34] with plasma concentration results available the same day. Unlike other microbial metabolites, such as butyrate, that are rapidly metabolized and fail to reach readily detectable plasma concentrations [35–37], TMAO remains relatively stable over time (Wilson, unpublished data). Accordingly, we hypothesized that plasma TMAO levels are altered in the setting of IBD and may act as biomarker of disease presence, activity, or phenotype.

Materials and Methods

Subjects

A case-control study was carried out in 106 IBD patients and 373 controls without IBD from southwestern Ontario. IBD subjects and controls were matched on age category (within a 5-year range) and sex with a goal of having three control subjects for each case. All subjects provided written informed consent. Each subject provided one blood sample between August 2009 and July 2014. Subjects from the IBD study arm were required to have a histo-pathological diagnosis of either UC or CD. Subjects with IBD were excluded from the study if there was no documentation pertaining to the activity of their IBD at the time the blood sample was drawn or if there was a history of probiotic or antibiotic use 4 weeks prior to blood sampling. Individuals without a history of IBD were selected as controls based on age and sex. Controls were excluded if there was a history of diarrheal symptoms, GI tract illness of any kind, or if there was a history of probiotic or antibiotic use 4 weeks prior to blood sampling. All cases and controls were required to be over the age of 18. The study protocol was approved by the Western University Health Sciences Research Ethics Board.

Data collected included age, type of IBD, disease phenotype, disease activity, medication use, and history of surgical resections. This information was collected from patient records. Disease activity was assessed using the Simple Clinical Colitis Activity Index for UC and the Harvey–Bradshaw Index for CD [38, 39].

Sample Size

Published data by Wang et al. [34] indicated a mean plasma TMAO level of 3.45 μM in a cohort of healthy volunteers. To detect a difference of at least 1.59 μM in the mean random plasma TMAO levels between cases and controls, a two-sided significance level of 5 % was used and power was set to 80 %. Based on these assumptions, a sample size of 100 subjects in each group was necessary.

TMAO, Choline, and Carnitine Measurement

One-milliliter aliquots of plasma were separated from whole blood collected in EDTA tubes and stored at -80 °C until mass spectrometer analysis. Plasma concentrations of TMAO, choline, and carnitine were measured by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS). Plasma samples (50 μl) were precipitated by adding 150 μl of acetonitrile (ACN) containing internal standards (TMAO-d9, carnitine-d9, and choline-d9). Standards made in water were similarly diluted with internal standards in ACN (all standards were obtained from Toronto Research Chemicals, Toronto, Ontario, Canada). Samples were centrifuged at 14,000 rpm for 30 min at 4 °C. One hundred and fifty microliters of supernatant was used for analysis. Mobile phases included 95:5 and 5:95 water: ACN, both containing 0.05 % formic acid and 5 mM ammonium formate.

Chromatographic separation was performed using a HILIC Plus silica column (Agilent Technologies), with an injection volume of 20 μl and a run-time of 6 min. The mass spectrometer (Thermo TSQ Vantage) with heated electrospray ionization source was set in positive mode for the detection of TMAO ($76 \rightarrow 58$, $76 \rightarrow 59$). TMAO, carnitine, and choline analyte areas were obtained using Xcalibur 11.0, and TMAO, carnitine, and choline plasma concentrations were quantified using LCQuan 8.0 software. TMAO plasma concentrations were accepted if the quantified levels for the qualitative mass transition were within 20 % of that quantified with the quantitative mass transition.

Genotyping

Genomic DNA was extracted from peripheral lymphocytes using a standard DNA extraction protocol (QIAmp DNA Mini Kit, Qiagen, Valencia, California). *FMO3* genotypes of subjects with available DNA were determined ($n = 440$). Allelic discrimination using TaqMan Drug Metabolism Genotyping assays with the 7500 RT-PCR

System (Applied Biosystems, Carlsbad, CA) was used to determine the presence of the common variants of *FMO3,* E158K and E308G.

Statistical Analysis

All data were analyzed using R [40] and package "nlme" for mixed-effects models [41]. To control the dependence among subjects matched by the age and sex, we performed a series of linear mixed-effects models to investigate the association between plasma TMAO levels and the fixed effects of disease presence (IBD or no IBD) and disease type (CD, UC, or no IBD) with sex and age category variables as random effects, allowing TMAO levels to vary over sex and age category groups; TMAO levels were compared between cases and controls for each sex *and* age category group.

Multivariable linear regression models were used for examining the effects of disease type (CD or UC), disease activity (yes or no), history of intestinal resection (yes or no), and disease location (colonic or extra-colonic) on plasma TMAO levels among IBD patients, adjusting for age and sex variables.

Plasma TMAO levels separated by FMO3 E158K and E308G genotype were compared using a one-way ANOVA test within the IBD population and then within the non-IBD control population.

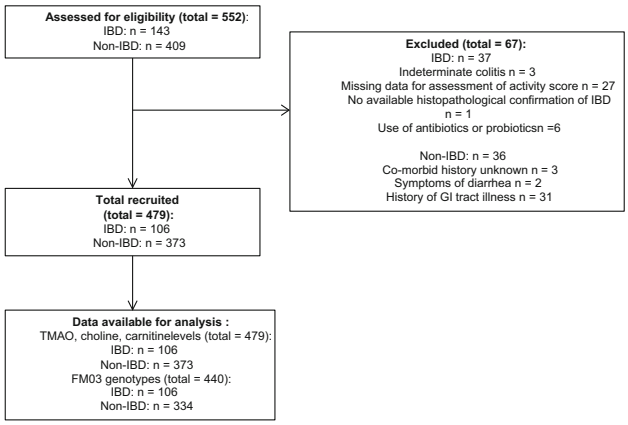

Fig. 1 Subject selection pathway. *TMAO* trimethylamine-*N*-oxide, *IBD* inflammatory bowel disease, *FMO3* flavin monooxygenase 3

Results

Figure 1 highlights patient selection based on the STROBE statement [42]. Five hundred and fifty-two subjects were screened for eligibility, and 479 subjects were enrolled in this study with 106 in the IBD arm and 373 in the control arm. The baseline characteristics of the IBD cohort are highlighted in Table 1. More IBD subjects had CD

Table 1 Demographic and baseline characteristics of the IBD and non-IBD populations

Characteristic	IBD subjects[a,b,c] (n = 106, %)	Non-IBD subjects[a,b,c] (n = 373, %)
Age (years)	44.6 (18–88)	55.3 (19–74)
Female sex	57 (53.8)	141 (37.8)
UC diagnosis	33 (31.1)	–
Pan-colitis	20 (18.9)	–
Left-sided colitis	13 (12.3)	–
Proctitis	0 (0)	–
CD diagnosis	73 (68.9)	–
Ileal disease	36 (32.1)	–
Ileo-colonic disease	25 (23.4)	–
Colonic disease	12 (10.7)	–
Active disease[d]	52 (49.1)	–
CD	33 (29.4)	–
UC	19 (17.9)	–
Previous resection	19 (17.9)	–
Medications		–
Use of prednisone	39 (34.8)	–

UC ulcerative colitis, *CD* Crohn's disease

[a] All participants were Caucasian

[b] Race is self reported

[c] Differences in the mean age and sex between the two populations are controlled for in the final analysis

[d] Disease activity is based on the Simple Clinical Colitis Index for individuals with UC and the Harvey–Bradshaw Index for individuals with CD

(68.9 %) than UC. An almost equal proportion of the IBD patients had active disease as had inactive disease. One third of the IBD patients were on prednisone. Differences in mean age and sex between the IBD subjects and the control population are noted and are controlled for in the final analysis.

TMAO plasma levels were measured in IBD patients and controls stratified by age, sex, and presence of disease (Fig. 2). There were no significant sex differences in TMAO plasma levels in either the IBD or the control populations (IBD, $p = 0.90$; control, $p = 0.89$). There was a trend toward increasing levels with increasing age; however, this failed to achieve statistical significance in either group (IBD, $p = 0.29$; control, $p = 0.15$) (Fig. 2).

The mean plasma TMAO levels, unadjusted for sex, age, or dietary factors (choline and carnitine levels), of the IBD population and the control population were 3.07 and 5.97 μM, respectively ($p < 0.0001$) (Fig. 3a). On average, plasma TMAO levels were 2.27 μM (standard error = 0.59) lower in the IBD population compared to the control population when adjusted for age, sex, and dietary factors ($p = 0.0001$). A similar finding was observed when comparing active IBD to inactive IBD ($p < 0.05$) (Fig. 3b).

The mean plasma choline level of the IBD population was significantly higher than the mean plasma choline level of the control population (13.67 vs 10.83 μM, $p \leq 0.0001$), while plasma carnitine levels were similar between the two groups (17.96 vs 16.92 μM, $p = 0.21$) (Fig. 4). The FMO3 variants E158K (GA) and E308G (AA) were most commonly seen in both populations (Table 2). There was no significant difference between the plasma TMAO levels separated by FMO3 genotype within either the IBD population or the control population (Fig. 5a, b).

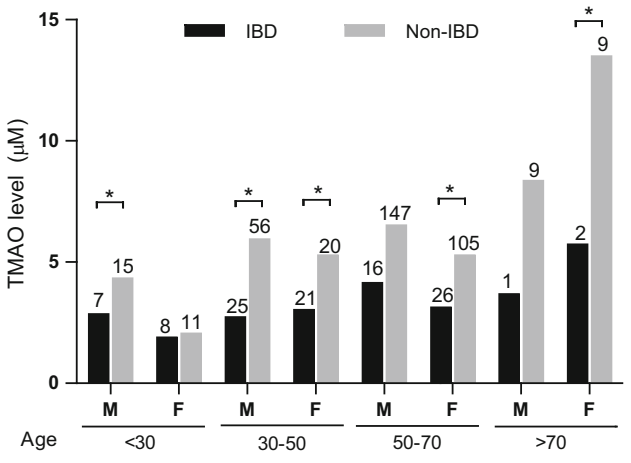

Fig. 2 Mean plasma TMAO levels in IBD patients and non-IBD controls. *Bars* (IBD, *black*; non-IBD, *gray*) depict the mean plasma TMAO level based on sex, separated into four distinct age categories (in years). *Numbers above each bar* represent the number of subjects. *M* male, *F* female; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

Both the unadjusted (for sex, age, and dietary factors) TMAO levels of the UC population and of the CD population were lower (2.34 μM; 3.40 μM) than that of the control population (5.97 μM). When controlling for age category, sex, and dietary factors, plasma TMAO levels

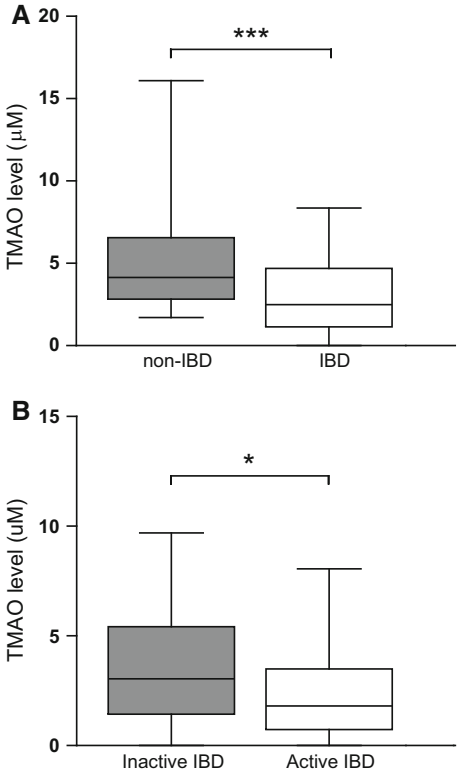

Fig. 3 Mean TMAO levels of the IBD and non-IBD populations (**a**) and of the active and non-active IBD populations (**b**), adjusted for age and sex, are represented by *box* and *whisker plot*. Median values (*thick horizontal line*), 25th and 75th percentile values (*box outline*), 5–95 % confidence interval (*whiskers*); $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

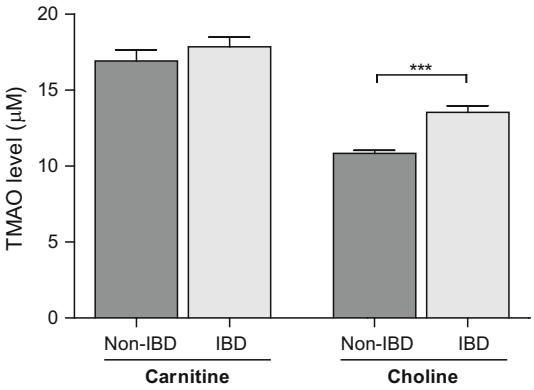

Fig. 4 Plasma choline and carnitine levels of the non-IBD (*dark gray bars*) and IBD (*light gray bars*) populations. *Error bars* indicate the 95 % confidence interval; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

were lower by 3.05 μM in the UC population compared to the control population ($p = 0.0017$) and by 1.94 μM in the CD population compared to the control population (0.0042).

Furthermore, plasma TMAO levels were found to be significantly lower in individuals with active UC (1.56 μM) compared to those with inactive UC (3.40 μM), with a difference of −1.82 μM ($p = 0.002$) when controlling for age category, sex, and dietary factors (Fig. 6a). There was no difference between plasma choline or carnitine levels in active versus inactive UC (Fig. 6b, c).

No statistically significant difference in TMAO levels was found between inactive and active CD ($p = 0.78$), disease type ($p = 0.28$), or the presence or absence of a surgical resection ($p = 0.69$). While no significant difference was found based on disease location ($p = 0.83$), there was a trend toward lower TMAO levels in those with colonic disease. There were no adverse events.

Discussion

The intricate relationship between the gut microbiome and IBD is of growing interest [43, 44]. It is increasingly accepted that the pathogenesis of IBD is likely driven by altered and deleterious interactions between the host's immune system and its own commensal GI microbiota [45, 46].

In the healthy individual, the microbiota of the gut are involved in a number of physiologic processes including immune system modeling, digestion of macro- and micronutrients, and suppression of the growth of foreign microorganisms [47]. There is often an interaction with host processes to produce "a network of microbial–host co-metabolism to process nutrients and drugs" [48]. It is well established that there is a loss of microbial diversity in the GI tracts of those with IBD [9, 49–51]. The impact of this on IBD activity, phenotype, and severity has not been well elucidated.

Table 2 Plasma trimethylamine-*N*-oxide (TMAO), choline, carnitine levels, and prevalence of flavin monooxygenase 3 (FMO3) genotypes separated by FMO3 genotype

Population	Item	FMO3 E158K			FMO3 E308G		
		GG	GA	AA	AA	AG	GG
IBD	Prevalence (%)	29 (27.4)	52 (49.1)	25 (23.5)	66 (62.2)	33 (31.1)	7 (6.6)
	Mean plasma TMAO level (μM)	3.53	3.17	2.73	3.39	2.89	2.56
	Mean plasma choline level (μM)	12.38	14.31	13.38	13.41	13.71	14.27
	Mean plasma carnitine level (μM)	18.08	17.82	17.64	18.77	16.58	15.35
Non-IBD	Prevalence (%)	100 (30.0)	186 (55.9)	48 (19.4)	227 (68.0)	99 (29.6)	8 (2.4)
	Mean plasma TMAO level (μM)	6.89	5.62	6.44	6.22	5.73	8.01
	Mean plasma choline level (μM)	11.36	10.89	10.98	10.96	11.04	12.16
	Mean plasma carnitine level (μM)	18.02	17.15	17.30	17.51	17.22	13.53

TMAO trimethylamine-*N*-oxide, *IBD* inflammatory bowel disease

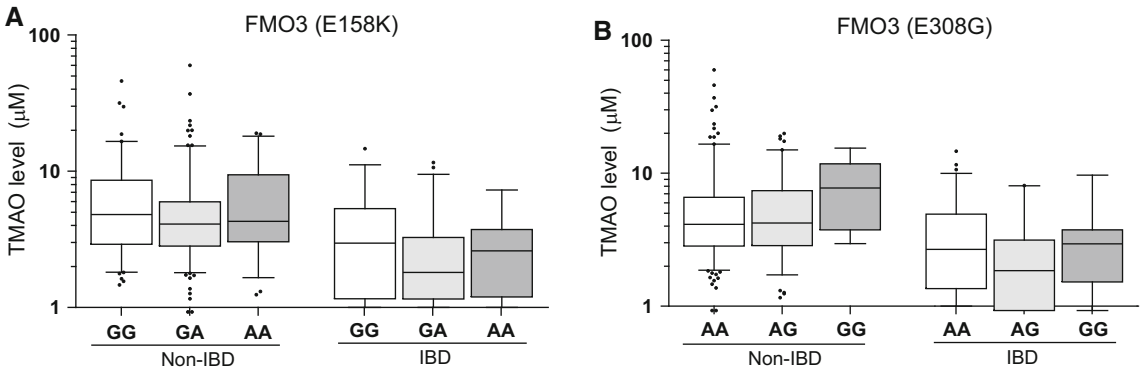

Fig. 5 Plasma TMAO levels of the non-IBD and IBD populations are separated by FMO3 genotype [E158K (**a**), E308G (**b**)]. *GG* represents homozygous wild type, *GA* represents heterozygous variant, and *AA* represents homozygous variant for E158K. *AA* represents homozygous wild type, *AG* represents heterozygous variant, and *GG* represents homozygous variant for E308K. Median values (*thick horizontal line*), 25th and 75th percentile values (*box outline*), minimum and maximum values (*whiskers*), and outlier values (*circle*); $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

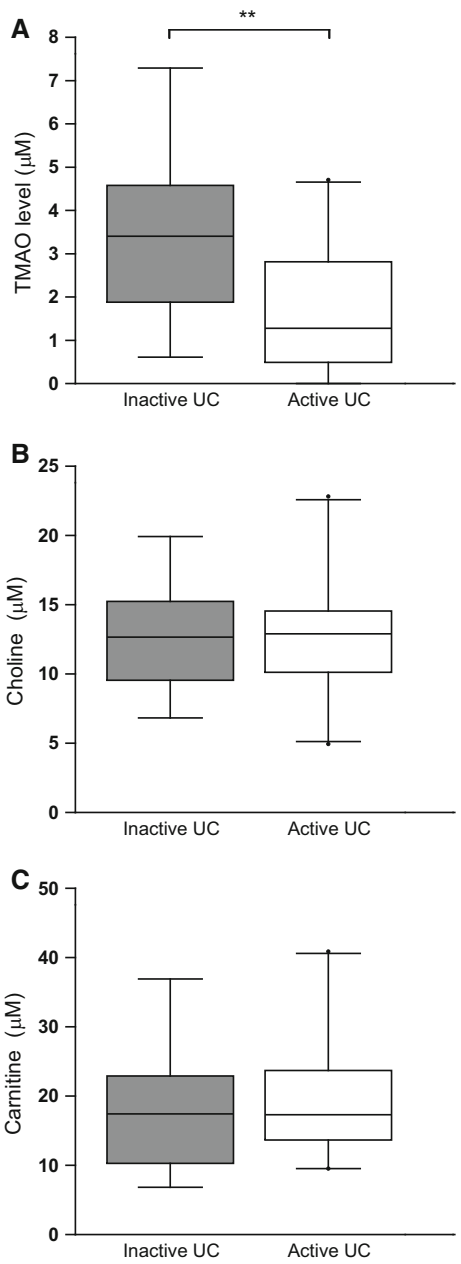

Fig. 6 Mean TMAO (**a**), choline (**b**), and carnitine (**c**) levels of the active UC and inactive UC populations are represented in a *box-plot*. Median values (*horizontal line*), 25th and 75th percentile values (*box outline*), and minimum and maximum values (*whiskers*). *TMAO* trimethylamine-*N*-oxide, *UC* ulcerative colitis; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

This study is the first of its kind to link plasma TMAO, a by-product of a gut microbial-mammalian co-metabolic pathway, to IBD. Many studies have examined how the gut microbiome is altered in disease, but very few have recognized that alterations in the gut microbiome may be harnessed as a biomarker of disease presence and activity [43, 52]. Based on our findings that TMAO is altered in the setting of IBD compared to a non-IBD population, independent of hepatic FMO3 activity and diet, we speculate that TMAO may serve as an example of how alterations in the gut microbiome in those with IBD could possibly be used to identify and characterize disease. In our study, plasma TMAO levels were significantly lower in patients in IBD compared with a cohort of sex and age category-matched non-IBD controls, suggesting that plasma TMAO may be clinically relevant for the noninvasive identification of those with IBD. Figure 7 summarizes the relationship of TMAO to IBD. Of note, the plasma TMAO levels of our control population were in keeping with plasma TMAO levels reported in the literature using a similar measurement technique in healthy volunteers [34].

Similarly, in the UC population, plasma TMAO levels were significantly lower in the group with active disease, suggesting that TMAO may be clinically useful in monitoring UC patients. A similar trend was seen in active IBD versus inactive IBD, though statistical significance was barely achieved. This may have been driven by the larger proportion of subjects with CD included in our study. No difference was seen between those with active versus inactive CD. There was a trend toward lower plasma TMAO levels in individuals with colonic disease. Most subjects in the CD cohort had isolated ileal disease. Given that the largest amount of gut bacteria is in the colon, one could hypothesize that the microbiota of the colon are driving the production of TMAO. It may be that the colonic microbiota in active ileal CD are similar to that of non-active CD, resulting in similar TMAO levels.

It should also be noted that plasma TMAO levels may be affected by several factors including diet, antibiotic use, and age. A strength of this study was that we measured plasma choline and carnitine levels concurrently with plasma TMAO levels. Recent studies show that objective measures of dietary intake are more accurate than relying on patient-reported questionnaires [53]. An individual's eating habits over the short term as well as their dietary patterns over time have been shown to alter the concentration of certain species in the gut [15, 54]. Furthermore, TMAO is a by-product of the metabolism of carnitine- and choline-containing food products [55, 56]. Individuals with certain dietary preferences or restrictions persistent over time may have overly inflated or reduced TMAO levels based on the composition of the gut flora population and the provision of carnitine- and choline-containing food products. With the measurement of plasma carnitine and choline levels in this study, we were able to address this possible confounder. Plasma choline levels were significantly higher in the IBD population compared to the control population. Reasons for this may be dependent on varied diets. Given this, one would expect TMAO levels would be proportionately higher in the IBD population; however, this was not the case. Plasma carnitine levels

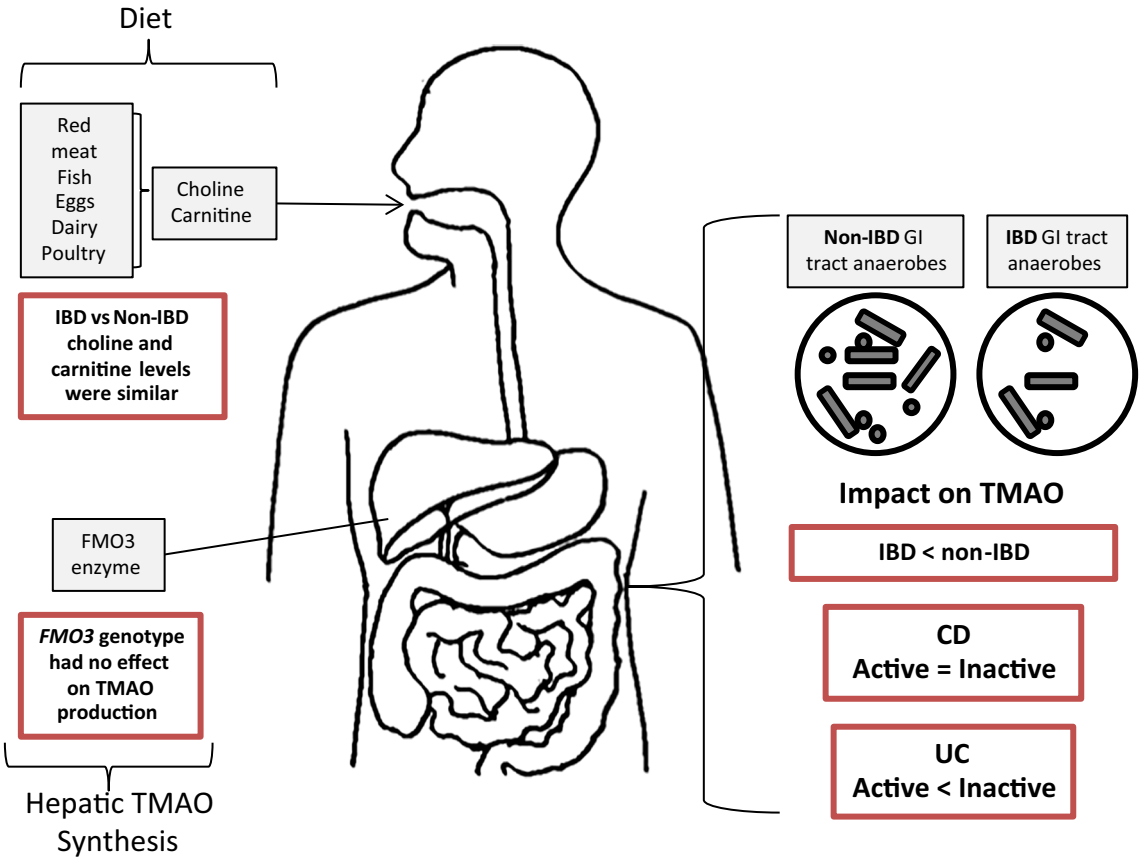

Fig. 7 A schematic showing the pathway by which dietary products are converted to TMAO and the relationship of TMAO to IBD compared to that of a non-IBD population

were similar between the two groups. These findings strengthen the argument that there is a clinically significant difference between plasma TMAO in IBD versus non-IBD populations independent of dietary intake, therefore likely reflective of gut microbiome differences.

It should be noted that certain gut microbiota from the *Proteobacteria* phylum, such as *E. coli*, possess a flavin-containing monoxygenase (bFMO) known as trimethylamine monooxygenase (tmm) that catalyzes the conversion of TMA to TMAO [57]. Given that we see lower TMAO plasma levels in IBD patients, this suggests that there may also be a change in bFMO activity in addition to a change in bacterial diversity.

Moreover, this study used a population of asymptomatic, non-IBD controls as a comparator group. It did not examine the difference between individuals with IBD-related diarrhea versus those with non-IBD-related diarrhea nor did it assess if intra-individual TMAO levels remain constant over time or fluctuate with disease activity. Noninvasive markers accurately assessing response to therapy as well differentiating IBD-related clinical symptoms from other conditions such as irritable bowel syndrome (IBS) concomitantly affecting those with IBD would be useful in clinical practice [58, 59]. This would need to be examined and replicated in future studies. Moreover, future studies comparing TMAO plasma concentrations to endoscopically determined disease activity would further strength the use of TMAO as a marker of disease activity.

A perceived limitation of this study may be that the composition of the gut microbiota was not directly assessed in either cohort; however, it has been well established, as described above, that the gut microbiome is altered in IBD compared to a healthy population. Furthermore, TMAO production has been convincingly shown to be dependent on gut microbiome metabolism [24]. Our study builds on these concepts; however, it would be useful to conduct a follow-up mechanistic trial, whereby TMAO levels in IBD patients and controls are compared to the composition of their gut microbiomes.

It is unknown whether TMAO could play more than the role of a biomarker in IBD. In cardiovascular disease, Tang et al. [24] showed that elevated plasma TMAO levels are an independent predictor of disease. Furthermore, it has been postulated that TMAO plays a role in the pathophysiology of atherosclerosis by enhancing the uptake of cholesterol in macrophages by inducing receptors involved

in the uptake of lipoproteins as well as in reducing reverse cholesterol transport [22, 23]. It is unclear in IBD, if TMAO plays a similar, but mechanistically different, pathophysiological role.

It should be noted that increased plasma TMAO levels have been linked to the over-expression of hepatic FMO3 in mice [55]. The same study showed that FMO3 expression is induced by dietary bile acids via farnesoid X receptor (FXR) in mice. Homozygous carriers of the most common *FMO3* variants, E158K and E308G, are known to have decreased activity of the FMO3 enzyme, resulting in reduced conversion of TMA to TMAO [27, 60]. Despite this, no significant difference in plasma TMAO levels was seen within either population based on FMO3 E158K or E308G genotype (Fig. 5a, b).

Bile acid metabolism and FXR expression have been linked to IBD. A number of studies using in vivo and in vitro models have shown that bile acids contribute to the regulation of the intestinal epithelium and reduce its permeability via intermediary proteins such as occludin in humans and GP-BAR1 in mice [61, 62]. Furthermore, FXR, a bile acid-activated nuclear receptor, regulates the transcription of genes protective against inflammation and FXR agonists reduce the mRNA expression of pro-inflammatory proteins in mouse models of colitis [63, 64]. FXR activation has also been shown to be reduced in human subjects with IBD [65]. Such data suggest that, in addition to the loss of bacterial diversity, reduced FXR activation is another pathway by which TMAO levels could be reduced in the setting of IBD. Interestingly, further study of the relationship between TMAO, FMO3 expression, and IBD may provide new insights or support evolving hypotheses relating to the pathogenesis of the disease.

In conclusion, these data highlight a significant association between TMAO and IBD presence and activity. We established that plasma TMAO levels are significantly lower in individuals with IBD and levels are further reduced in active UC when compared to inactive UC. With further study, plasma TMAO levels may become a clinically relevant, noninvasive means of identifying individuals with IBD as well monitoring disease activity in those with UC.

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Conflict of interest None.

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