

Plasma Concentrations of Trimethylamine-N-oxide Are Directly Associated with Dairy Food Consumption and Low-Grade Inflammation in a German Adult Population^{1,2}

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Abstract

Background: Trimethylamine-N-oxide (TMAO) is a metabolite of carnitine, choline, and phosphatidylcholine, which is inversely associated with survival of cardiovascular disease (CVD) patients.

Objective: We examined the associations of diet with plasma concentrations of TMAO, choline, and betaine and the associations of TMAO with plasma concentrations of various cytokines.

Methods: Plasma TMAO, choline, and betaine concentrations were measured using LC-high resolution mass spectrometry in 271 participants, ≥ 18 y old, of the Second Bavarian Food Consumption Survey, conducted in 2002 and 2003. Food consumption was assessed using at least two 24-h dietary recalls. Cytokines were measured in plasma with enzyme-linked immunosorbent assays. Geometric mean concentrations of TMAO, choline, and betaine by categories of meat, dairy food, egg, and fish consumption were computed, adjusted for sex, age, and BMI. Multivariable-adjusted geometric mean concentrations of cytokines [tumor necrosis factor- α (TNF- α), soluble TNF receptors (sTNF-R) p55, sTNF-R p75, interleukin-6 (IL-6), and C-reactive protein (CRP)] were computed by quartiles of TMAO concentration using general linear models.

Results: Meat, egg, or fish consumption was not associated with TMAO, choline, or betaine concentrations (all P -trend ≥ 0.05). With increases in milk and other dairy food consumption, the plasma TMAO concentration increased [geometric mean bottom quartile of milk consumption: 2.08 μM (95% CI: 1.69, 2.57 μM); compared with top quartile: 3.13 μM (95% CI: 2.56, 3.84 μM); P -trend = 0.008]. Participants in the top TMAO quartile had higher plasma concentrations of TNF- α , sTNF-R p55, and sTNF-R p75 than participants in the bottom quartile (percentage difference ranging between 14.4% and 17.3%; all P -trend < 0.05), but there were no differences in plasma concentrations of CRP and IL-6 (all P -trend ≥ 0.05).

Conclusions: Results of this study conducted among healthy adults from the general population do not indicate a strong effect of diet on plasma concentrations of TMAO, choline, or betaine, with the exception of a positive association between dairy food consumption and plasma TMAO concentrations. Also, plasma TMAO concentrations were positively associated with inflammation. Whether habitual diet is strongly linked to the plasma TMAO concentration, a potential marker of CVD risk, needs to be determined in further studies.

Introduction

High red meat consumption is associated with increased risk of cardiovascular disease (CVD)⁶ incidence and mortality, as well

as some types of cancer and overall mortality (1–4). A number of hypotheses have been put forward that might explain these associations, such as high intake of heme iron, increased intake of nitrite and increased formation of N-nitroso compounds, and formation of heterocyclic aromatic amines and polycyclic aromatic hydrocarbons when preparing meat at high temperatures, as well as higher intake of cholesterol and saturated fats. A

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⁶ Abbreviations: BVS II, Second Bavarian Food Consumption Survey (Bayrische Verzehrsstudie II); CRP, C-reactive protein; CVD, cardiovascular disease; FMO3, flavin monooxygenase 3; s-TNF-R p55 and s-TNF-R p75, soluble TNF receptors; TMAO, trimethylamine-N-oxide.

rather new hypothesis has been developed by US scientists, who observed that CVD patients with higher concentrations of trimethylamine-N-oxide (TMAO) have a higher risk for major adverse cardiovascular events such as death, myocardial infarction, or stroke than patients with low TMAO concentrations (5). Trimethylamine, the precursor of TMAO, is synthesized by intestinal bacteria predominantly from carnitine, phosphatidylcholine (lecithin), choline, and betaine. After absorption, in a second step, trimethylamine is oxidized to TMAO in the liver mainly by flavin monooxygenase 3 (FMO3) (6–8). The TMAO precursors carnitine, lecithin (phosphatidylcholine), and choline are abundant in red meat, liver, fish, milk, cheese, and eggs, whereas sources of betaine include wheat bran, wheat germ, and spinach (9). Thus, it might be possible that TMAO metabolism is an important link between diet and chronic diseases, in particular CVD. To the best of our knowledge, no study has yet examined whether circulating TMAO concentrations are associated with consumption of foods of animal origin, such as red meat, eggs, or dairy products. It was the aim of our study to address the question whether these foods are associated with circulating concentrations of TMAO, its precursor choline, and betaine, a choline metabolite, in a cross-sectional study. From a mechanistic view, we aimed to investigate a possible link between TMAO concentrations and low-grade inflammation markers.

Methods

Study participants. The second Bavarian Food Consumption Survey (BVSII) was conducted between September 2002 and June 2003 as a representative cross-sectional study. Potential participants were German-speaking individuals from the Bavarian population who lived in private homes (i.e., non-institutionalized) and were between 13 and 80 y old. A total of 1050 participants (participation rate 70%) were recruited using a 3-stage random route sampling procedure (10). In a personal interview, demographics, lifestyle factors, and medical history were assessed. The study was approved by the ethical committee of “Bayrische Landesärztekammer.” Written informed consent was obtained from all study participants.

Data assessment. The 24-h dietary recalls were assessed with the standardized, interactive PC-guided program EPIC-Soft (11, 12). A total of 847 participants, ≥ 18 y old (only these were invited for anthropometry assessment and a blood sample), completed at least two (most of them three) 24-h recalls. Trained study personnel assessed the 24-h recalls in a telephone interview. The data from the recalls per person were weighted for weekday and weekend day to calculate the average daily food intake. The different foods reported during the 24-h dietary recalls were grouped into 17 food groups (12). Nutrient intakes were calculated using food content data from the German food composition database “Bundeslebensmittelschlüssel” (version II.3; BgVV, Berlin, Germany).

Participants with at least 1 completed 24-h recall, who were ≥ 18 y of age, were invited to their nearest public health office for blood sampling and standardized anthropometric measurements. Of those invited, 568 men and women participated in blood sampling (under nonfasting conditions). As described previously (13), blood was stabilized with sodium ethylenediamine tetra-acetic acid (1 g/L) and immediately centrifuged; the plasma was frozen at -80°C . Cytokines were measured with commercial enzyme-linked immunosorbent assays (TNF- α , sTNF-R p55, sTNF-R p75, and IL-6 [Biosource, Brussels, Belgium]). For all assays, the intra- and interassay coefficients of variation were below 7% and 9%, respectively.

TMAO was measured in 274 out of 568 participants for which the highest plasma volume was obtained and, thus, more aliquots are available. Although this is not a random selection process, we do not expect a differential bias by using this procedure. TMAO, choline, and

betaine were measured by LC-high resolution mass spectrometry. The method was fully validated according to generally accepted guidelines. Intra- and interassay coefficients of variation were below 2% and 11%, respectively.

Statistical analysis. Baseline characteristics are presented by sex showing categorical variables as absolute numbers and percentages and continuous variables as median and interquartile range. To examine whether plasma concentrations of TMAO, choline, and betaine were associated with food consumption, we computed means by categories of red meat, processed meat, cheese, milk, and egg consumption. Three participants were excluded due to missing dietary information, resulting in a sample size of 271 participants. The categorization of food consumption depended on consumption habits, such that processed meat and milk and dairy product consumption was categorized into quartiles and red meat and egg consumption were divided into 3 groups (nonconsumers and consumers divided by the median). Because many participants did not consume fish or white meat, their consumption was divided into consumers and nonconsumers. We also categorized participants into vegetarians and nonvegetarians based on self-report during the interview because not all participants who did not report meat consumption on two 24-h recalls might actually indeed be vegetarians.

Smoking was categorized into never, former, and current smokers. Social class was divided into 5 groups based on educational level, social position, and net household income (14). Based on the responses to the question about whether the participants had previously been told by their doctor if they had a specific disease, participants were categorized into yes/no of having a history of hypertension, heart attack, stroke, gastric or duodenal ulcer, chronic bowel inflammation (morbus crohn, colitis ulcerosa), colorectal adenomas, diabetes mellitus, hypercholesterolemia or hypertriglyceremia, gout or hyperuricemia, osteoporosis, or a malign tumor. BMI was computed as kilograms per square meter from measured body height and weight and categorized into quartiles.

Because TMAO, choline, and betaine concentrations were not normally distributed, we computed the geometric mean and 95% CIs instead of arithmetic mean and standard deviation using general linear models adjusting for sex, age, and BMI. Tests for trend were conducted using integer scores for categories of food intake (analysis of TMAO, choline, betaine concentrations by food groups) and TMAO concentrations (analysis of cytokines and blood lipids by TMAO quartiles), respectively. A value of $P < 0.05$ was considered statistically significant.

In subanalyses, we excluded under-reporters according to their ratio of total energy intake to estimated basal metabolic rate (ratio < 0.80) (15) and participants with self-reported chronic diseases.

Results

Of our study participants, 104 (38.4%) were male and 167 (61.6%) were female. Baseline characteristics of the study participants are shown by sex in Table 1. Of diseases self-reported by the participants, the most common were hypertension, hypercholesterolemia or hypertriglyceridemia, hyperuricemia (among men), gastric or duodenal ulcer, and diabetes mellitus. All other diseases, including heart attack, stroke, and cancer, were infrequent because the study participants were young.

TMAO plasma concentrations did not differ by sex (Table 2), but plasma betaine and choline concentrations were higher among men than among women. With increasing age, the concentrations of all 3 markers increased statistically significantly, but we did not observe any statistically significant trend by BMI for plasma TMAO and betaine concentrations, whereas plasma choline concentrations were positively associated with BMI. Because age, sex, and BMI were associated with TMAO, choline, and/or betaine concentrations, we adjusted all the following analyses for these 3 factors. Further taking into

TABLE 1 Dietary and lifestyle characteristics of adult participants in the Bavarian Food Consumption Survey II (2002 and 2003) by sex¹

	Male	Female
<i>n</i> (%)	104 (38.4)	167 (61.6)
Age, y	50 (37, 63)	44 (36, 59)
Food consumption, g/d		
Milk and dairy products	125 [63.2, 277]	157 [70.5, 229]
Milk	37.9 [0, 151]	59.1 [10.4, 141]
Cheese	25.4 [6.7, 45.0]	21.4 [10.7, 34.4]
Total meat	157 [99.5, 207]	80.9 [44.1, 132]
Red meat	31.7 [0, 77.7]	13.8 [0, 46.4]
Processed meat	73.4 [39.1, 121]	34.3 [7.4, 61.2]
White meat	0 [0, 20.0]	0 [0, 17.1]
Fish and shellfish	0 [0, 27.2]	0 [0, 20.3]
Eggs and egg products	0 [0, 17.1]	0 [0, 16.1]
Total energy intake, kcal/d	2246 [1988, 2688]	1662 [1377, 1976]
BMI, kg/m ²	26.1 [24.0, 29.4]	25.2 [22.4, 28.9]
Smoking status, <i>n</i> (%)		
Never	45 (44.3)	103 (61.9)
Former	26 (24.5)	30 (17.9)
Current	33 (31.1)	34 (20.2)
Social class, <i>n</i> (%)		
Lower	5 (6.6)	25 (14.8)
Lower middle	24 (22.6)	39 (23.2)
Middle	40 (37.7)	57 (33.9)
Upper middle	20 (18.8)	34 (20.8)
Upper	15 (14.2)	12 (7.1)
Prevalent diseases, <i>n</i> (%)		
Hypertension	16 (15.1)	29 (17.3)
Heart attack (myocardial infarction)	4 (3.9)	1 (0.6)
Stroke	2 (1.9)	1 (0.6)
Gastric/duodenal ulcer	10 (9.4)	9 (5.2)
Chronic bowel disease	2 (1.9)	2 (1.2)
Colorectal adenomas	2 (1.9)	2 (1.2)
Diabetes mellitus	8 (7.7)	11 (6.6)
Hypercholesterolemia/ hypertriglyceridemia	24 (23.1)	28 (16.7)
Gout/hyperuricemia	15 (14.4)	7 (4.2)
Osteoporosis	1 (0.9)	10 (6.0)
Malign tumor	3 (2.8)	1 (0.6)

¹ Values are *n* (%) or medians [IQRs].

account smoking habits and energy intake did not alter the observed associations (data not shown).

Neither TMAO nor betaine nor choline plasma concentrations differed by intake of red, processed, or white meat (Table 3). Furthermore, there were no differences by consumption of eggs and fish. Also, we did not observe a difference between vegetarians and nonvegetarians in plasma TMAO [geometric mean vegetarians: 3.35 μM (95% CI: 1.88, 5.97 μM); compared with nonvegetarians: 2.51 μM (95% CI: 2.27, 2.78 μM)], betaine [35.4 μM (95% CI: 28.6, 43.8 μM) compared with 40.0 μM (95% CI: 38.5, 41.5 μM)], or choline [10.6 μM (95% CI: 9.28, 12.0 μM) compared with 10.6 μM (95% CI: 10.4, 10.8 μM)] concentrations (all *P* ≥ 0.05). However, the number of vegetarians was small (*n* = 8). Participants with higher total milk and dairy consumption had higher plasma TMAO concentrations than participants with low consumption, but we did not observe any difference in betaine or choline plasma concentrations by milk and dairy consumption. In particular, milk, but

TABLE 2 Geometric mean plasma concentrations of TMAO, betaine, and choline by sex, age, and BMI in German adults, Bavarian Food Consumption Survey II, 2002 and 2003¹

	<i>n</i>	TMAO, μM	Betaine, μM	Choline, μM
Sex				
Men	104	2.55 (2.17, 2.99)	45.0 (42.4, 47.7)	10.9 (10.5, 11.3)
Women	167	2.52 (2.22, 2.86)	35.3 (33.7, 37.0)	10.3 (10.0, 10.6)
<i>P</i> -trend		0.94	<0.0001	0.008
Age, y				
<40	102	1.99 (1.68, 2.36)	38.4 (36.1, 40.9)	10.0 (9.65, 10.4)
40 to <60	94	2.58 (2.18, 3.07)	39.1 (36.8, 41.7)	10.3 (9.87, 10.6)
≥60	75	3.44 (2.83, 4.17)	42.8 (39.9, 45.9)	11.9 (11.4, 12.4)
<i>P</i> -trend		<0.0001	0.03	<0.0001
BMI, kg/m ²				
<22.8	68	2.46 (1.97, 3.06)	40.0 (36.9, 43.3)	10.2 (9.69, 10.7)
22.8 to <25.6	66	2.64 (2.17, 3.22)	40.1 (37.3, 43.1)	10.3 (9.83, 10.7)
25.6 to <29.0	68	2.50 (2.05, 3.06)	39.5 (36.7, 42.5)	10.5 (10.0, 10.9)
≥29.0	69	2.53 (2.06, 3.10)	39.9 (37.0, 43.0)	11.5 (10.1, 12.0)
<i>P</i> -trend		0.98	0.88	0.0003
Smoking				
Never	148	2.56 (2.23, 2.95)	39.9 (37.9, 42.0)	10.5 (10.2, 10.9)
Former	56	2.87 (2.30, 3.57)	37.9 (35.0, 41.1)	11.0 (10.5, 11.5)
Current	67	2.24 (1.83, 2.75)	41.4 (38.5, 44.7)	10.4 (10.0, 10.9)
<i>P</i> -trend		0.43	0.59	0.99

¹ Values are geometric mean concentrations (95% CIs) from general linear models adjusted for age, sex, and BMI (where appropriate). TMAO, trimethylamine-N-oxide.

not cheese consumption, was related to higher TMAO plasma concentrations.

One proposed mechanism by which TMAO might affect CVD risk is via inflammation. Hence, we examined whether high plasma TMAO concentrations were associated with circulating concentrations of inflammatory markers. Participants in the top quartile of plasma TMAO concentrations had higher plasma concentrations of TNF-α, sTNF-R p55, and sTNF-R p75, but there were no differences in plasma concentrations of CRP and IL-6 (Table 4).

We also examined the associations between plasma TMAO and lipid concentrations. However, neither total cholesterol nor HDL cholesterol nor TG plasma concentrations were statistically significantly associated with plasma TMAO concentrations (Table 4).

Participants with prevalent diseases (as described in Methods) did not have statistically significantly higher plasma concentrations of TMAO, betaine, and choline than participants without prevalent diseases (results not shown). When we excluded participants with self-reported diseases from the analyses, none of the observed results changed appreciably (results not shown). Similarly, results did not change when we excluded 22 under-reporters of energy intake from the analysis (data not shown).

Discussion

In our cross-sectional analysis of a German population, we did not observe any associations between the consumption of red, processed, or white meat; fish; or eggs and the plasma concentrations of TMAO or its precursors choline and betaine. Only high consumption of milk was related to increased plasma concentrations of TMAO, but not choline and betaine.

TABLE 3 Geometric mean plasma concentrations of TMAO, betaine, and choline by consumption of red meat, milk and dairy products, eggs, fish, and vegetarian status in German adults, Bavarian Food Consumption Survey II, 2002 and 2003¹

	<i>n</i>	TMAO, μM	Betaine, μM	Choline, μM
Red meat				
0	111	2.50 (2.13, 2.93)	39.4 (37.1, 41.7)	10.6 (10.2, 11.0)
0.1 to <55.0	92	2.62 (2.20, 3.12)	39.4 (36.9, 42.0)	10.8 (10.4, 11.2)
≥ 55.0	68	2.37 (1.95, 2.89)	41.4 (38.5, 44.5)	10.5 (10.0, 10.9)
<i>P</i> -trend ²		0.77	0.33	0.75
Processed meat				
Q1 (<15.0)	68	2.69 (2.19, 3.30)	38.0 (35.2, 41.0)	10.6 (10.1, 11.1)
Q2 (15.0 to <45.4)	68	2.88 (2.35, 3.53)	41.6 (38.6, 44.8)	10.7 (10.3, 11.2)
Q3 (45.4 to <82.5)	67	2.25 (1.85, 2.74)	42.2 (39.2, 45.3)	10.6 (10.1, 11.0)
Q4 (≥ 82.5)	68	2.32 (1.90, 2.82)	38.2 (35.6, 41.1)	10.6 (10.2, 11.1)
<i>P</i> -trend ²		0.15	0.80	0.99
White meat				
No	187	2.53 (2.15, 2.98)	39.9 (38.1, 41.7)	10.6 (10.3, 10.9)
Yes	84	2.34 (1.85, 2.95)	40.0 (37.5, 42.8)	10.7 (10.3, 11.2)
<i>P</i> -trend ²		0.56	0.92	0.48
Milk and dairy products				
Q1 (<68.2)	68	2.08 (1.69, 2.57)	40.8 (37.8, 44.1)	10.4 (10.0, 10.9)
Q2 (68.2 to <139.3)	68	2.34 (1.92, 2.85)	40.0 (37.2, 43.1)	10.5 (10.0, 10.9)
Q3 (139.3 to <253.3)	68	2.51 (2.05, 3.06)	39.4 (36.6, 42.4)	10.7 (10.2, 11.2)
Q4 (≥ 253.3)	67	3.13 (2.56, 3.84)	39.4 (36.5, 42.5)	11.0 (10.5, 11.5)
<i>P</i> -trend ²		0.008	0.41	0.06
Milk				
Q1 (<5.7)	66	2.01 (1.65, 2.44)	42.8 (38.9, 47.1)	9.58 (9.08, 10.1)
Q2 (5.7 to <53.8)	69	2.29 (1.88, 2.78)	34.5 (31.2, 38.2)	9.81 (9.26, 10.4)
Q3 (53.8 to <143.4)	68	2.69 (2.21, 3.27)	37.4 (34.1, 40.9)	9.99 (9.50, 10.5)
Q4 (≥ 143.4)	68	3.17 (2.61, 3.86)	38.2 (35.2, 41.5)	10.6 (10.1, 11.1)
<i>P</i> -trend ²		0.0007	0.18	0.05
Cheese				
Q1 (<7.9)	68	2.33 (1.90, 2.85)	37.9 (34.3, 41.9)	9.73 (9.21, 10.3)
Q2 (7.9 to <21.4)	62	2.77 (2.26, 3.39)	39.2 (35.8, 43.0)	9.91 (9.42, 10.4)
Q3 (21.4 to <37.9)	74	2.26 (1.86, 2.75)	36.8 (33.6, 40.4)	10.3 (9.73, 10.8)
Q4 (≥ 37.9)	67	2.70 (2.22, 3.28)	38.8 (35.4, 42.5)	10.2 (9.72, 10.8)
<i>P</i> -trend ²		0.61	0.66	0.29
Eggs				
0	161	2.56 (2.25, 2.92)	39.9 (38.0, 41.9)	10.7 (10.4, 11.0)
0.1 to <17.1	38	2.23 (1.71, 2.92)	38.2 (34.6, 42.1)	9.79 (9.23, 10.4)
≥ 17.1	72	2.51 (2.07, 3.04)	40.8 (38.0, 43.8)	10.9 (10.5, 11.4)
<i>P</i> -trend ²		0.75	0.73	0.68
Fish				
No	180	2.53 (2.24, 2.87)	40.2 (38.4, 42.1)	10.7 (10.4, 11.0)
Yes	91	2.45 (2.07, 2.91)	39.3 (36.9, 41.9)	10.5 (10.1, 10.9)
<i>P</i> -trend ²		0.40	0.56	0.45

¹ Values are geometric mean concentrations (95% CIs) from general linear models adjusted for age, sex, and BMI. Q, quartile; TMAO, trimethylamine-N-oxide.

² Tests for trend were conducted using integer scores for categories of food intake in the linear regression model.

Several studies examined the associations of TMAO, choline, and betaine concentrations with heart and/or kidney disease (5, 8, 16–22) and cancer (23, 24). Some of these studies argued that TMAO might link diet with the risk of CVD because high intakes of red and processed meat have been found to be associated with CVD incidence and mortality. However, to the best of our knowledge, no study has yet examined the association between diet and plasma TMAO concentrations in healthy humans. Our results do not support strong associations

between consumption of foods of animal origin and plasma concentrations of TMAO, choline, or betaine, with the exception of milk. In our analysis, we accounted for age, sex, and BMI; doing so slightly changed the geometric means per category, but not the overall interpretation of results compared with the unadjusted results. Considering, based on previous studies, that participants with prevalent disease might have increased levels of TMAO and its precursors, we excluded those participants from the analysis. This, however, did not appreciably change our results.

Only a few studies examined TMAO, choline, and betaine concentrations in a healthy general population. In our study, geometric mean TMAO plasma concentrations were 2.55 μM in men and 2.52 μM in women. These concentrations are lower than reported by studies conducted among patients with medians ranging between 3.5 μM (5) and 12.1 μM (19). Even in the healthy control sample of the Women's Health Initiative (24), the mean concentration was 3.8 μM .

Our method used to quantify TMAO in plasma is comparable with the methods used by others (20, 25). All methods used LC mass spectrometry. The performance characteristics are similar to the ones reported by Wang et al. (25).

We observed higher plasma concentrations of betaine and choline, but not of TMAO among men than among women. Circulating choline and betaine concentrations correlate with dietary intake (9, 26, 27); thus, higher food intake of men might explain this finding, although adjustment for energy intake did not alter this finding. However, other studies reported that moderate changes in dietary choline intake are not reflected by blood choline concentrations (28). In a Norwegian cohort study of healthy men and women, only egg consumption, but no other foods of animal origin, were associated with plasma choline concentrations, and plasma betaine concentrations were predicted by high-fiber bread and high-fat dairy product consumption (29). In a human randomized intervention study, the consumption of two or more eggs resulted in the increased formation of TMAO (30). However, there was considerable variation between individuals in the response to egg consumption with respect to TMAO formation. Another intervention trial in 15 free-living lacto-ovo-vegetarian women of reproductive age did not find any associations between egg consumption and plasma TMAO concentrations (31).

In the study population, choline, but neither TMAO nor betaine, plasma concentrations increased with increasing BMI. This contrasts the results of a Scandinavian study among patients with chronic heart failure (19), in which BMI was associated only with betaine, but not with TMAO or choline concentrations. In the same study, a positive association of age with TMAO and choline, but not betaine, concentrations was observed (19), whereas in our data set, age was significantly associated with either metabolite. Neither Trøseid et al. (19) nor we observed associations of smoking status with any of the 3 metabolites.

Fish consumption, in particular consumption of fatty fish, may be inversely associated with CVDs (32, 33). On the other hand, TMAO is an organic osmolyte in many marine fish (34), and in a study among 44 men, fish consumption correlated statistically significantly with urinary TMAO concentrations (35). Likewise, an intervention study in 6 healthy volunteers revealed a significant increase in TMAO plasma concentrations in response to fish intake (36). If TMAO is indeed associated with CVD risk, it might be that metabolism from carnitine, choline, and phosphatidylcholine is more relevant to total TMAO plasma concentration than TMAO from fish intake.

TABLE 4 Geometric mean plasma concentrations of cytokines and lipids by quartiles of plasma TMAO concentration in German adults, Bavarian Food Consumption Survey II, 2002 and 2003¹

	Quartiles of TMAO concentration, μM				<i>P</i> -trend ²
	Q1 (<1.44)	Q2 (1.44 to <2.7)	Q3 (2.7 to <4.25)	Q4 (\geq 4.25)	
<i>n</i>	67	68	68	68	
Cytokines					
CRP, mg/L	1.87 (1.47, 2.36)	1.68 (1.34, 2.11)	1.82 (1.45, 2.29)	1.72 (1.36, 2.16)	0.75
IL-6, pg/mL	1.44 (1.20, 1.74)	1.51 (1.26, 1.81)	1.59 (1.32, 1.90)	1.48 (1.23, 1.78)	0.79
TNF- α , pg/mL	11.0 (10.0, 12.0)	11.4 (10.4, 12.4)	11.3 (10.4, 12.4)	12.7 (11.6, 14.0)	0.04
sTNF-R p55, $\mu\text{g/L}$	1.65 (1.58, 1.73)	1.69 (1.62, 1.77)	1.88 (1.79, 1.96)	1.96 (1.87, 2.05)	<0.0001
sTNF-R p75, $\mu\text{g/L}$	4.12 (3.89, 4.38)	4.29 (4.05, 4.55)	4.54 (4.28, 4.81)	4.90 (4.62, 5.20)	<0.0001
Blood lipids					
Total cholesterol, mg/dL	214 (205, 223)	202 (194, 211)	206 (198, 215)	202 (193, 211)	0.13
HDL cholesterol, mg/dL	44.5 (42.7, 46.4)	45.4 (43.6, 47.3)	46.0 (44.2, 47.9)	47.1 (45.3, 49.1)	0.05
TGs, mmol/L	1.32 (1.13, 1.54)	1.34 (1.14, 1.58)	1.57 (1.36, 1.82)	1.36 (1.16, 1.59)	0.48

¹ Values are geometric mean concentrations (95% CIs) from general linear models adjusted for age, sex, and BMI. CRP, C-reactive protein; Q, quartile; sTNF-R p55 and sTNF-R p75, soluble TNF receptors; TMAO, trimethylamine-N-oxide.

² Tests for trend were conducted using integer scores for categories of food intake in the linear regression model.

This might be true particularly in populations such as our study population, where fish consumption is generally low.

Some studies examined whether higher TMAO concentrations were related to inflammatory markers, mostly CRP (17, 19, 20, 30, 37), but of these studies, all besides 1 (30) were conducted among participants with heart or kidney disease. Of these, only 1 study reported an inverse association of TMAO with CRP concentrations (20). In our analysis, TMAO concentrations were not related to CRP or IL-6 concentrations, but with TNF- α , and two soluble TNF receptors (sTNF-R p55 and sTNF-R p75). In 46 healthy nonsmoking US women, soluble TNF receptors were indicators of early vascular changes associated with CVD (38). Longitudinal studies revealed significant associations of TNF-R p75 with CVD events, cardiovascular and total and mortality (39); progression of chronic kidney disease; and heart failure, which all have also been associated with TMAO concentrations (40–43). From the current evidence it is yet unclear if and how TMAO and the pathway leading to TMAO formation are causally linked to inflammation in general (44) and specifically the TNF system. Just recently, it has been shown that FMO3 is a positive regulator of the liver X receptor (45), which in vitro improves TNF- α -induced endothelial dysfunction (46). In the liver, TMAO decreases the bile acid pool and lowers the expression of key bile acid synthesis and transport proteins (6), and decreased bile acid production is linked to systemic inflammation (47).

We did not observe any statistically significant differences in concentrations of total cholesterol and triglycerides by quartiles of TMAO concentration, confirming previous studies (5, 7). This contrasts results in mice, showing that TMAO concentrations were significantly correlated with plasma lipid concentrations and reduced reverse cholesterol transport (7, 44). The positive association between HDL cholesterol and TMAO plasma concentrations was of borderline significance, which is opposite of what would have been expected from results in mice (7, 44).

The following limitations of our study need to be taken into account. First, creatinine was not measured in this survey. Therefore, we were not able to estimate glomerular filtration rate (eGFR), which is inversely associated with TMAO concentrations (17, 19). To a certain extent, we captured the influence of prevalent diseases on the observed associations by excluding

those participants who had reported an existing disease, for example, diabetes mellitus and heart disease, but not kidney diseases. Second, diet was assessed generally using three 24-h recalls. Although 24-h recalls are good to determine dietary habits on the population level, they are less able to capture the long-term dietary habits of an individual. However, we took into consideration weighting factors to account for differences in consumption depending on the day of the week, and at least two 24-h recalls were filled in by the study participants. Still, consumption of some foods such as fish and eggs might be underreported. Therefore, our study had a limited capacity to assess the particular effect of fish consumption, an important source of TMAO, on circulating TMAO. This is further limited by the fact that the sample size of the study was rather small ($n = 271$) and that half of the study participants did not consume any fish. Third, data of multiple dietary recalls are often interpreted as indicators of usual food intake, which is correct at the group level. We did not assess food intake on the day or the day before blood collection. Consequently, we cannot aim at short-term biomarkers of intake of rarely consumed food, but meat and milk products are frequently consumed foods. An exception are food components that are barely absorbed or rapidly metabolized or excreted, and choline may refer to this group. However, epidemiologic studies such as ours are not interested in identifying biomarkers of intake that can be detected only a few hours after ingestion. Such biomarkers can be used only in the metabolic ward or clinical setting. Fourth, our study sample included only 8 vegetarians, and we cannot exclude that we might have seen a difference between vegetarians and nonvegetarians with a larger sample size. Finally, we have no information on recent antibiotic use of the participants, which influences gut microbiota (5).

In conclusion, our study among participants from the general population does not provide any strong evidence for associations between consumption of foods of animal origin, perhaps with the exception of milk, and TMAO plasma concentrations. What we did see, however, was a link between TMAO plasma concentrations and some cytokines. Future studies need to first address whether circulating TMAO is an indicator of the gut microbiota without any strong diet-TMAO relation and, second, the importance of gut microbiota and FMO3 as effect modifiers of potential associations between diet, TMAO, and chronic diseases.

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