# Plasma concentrations of trimethylamine-N-oxide are directly associated with dairy food consumption and low-grade inflammation in a German adult population

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# 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 sTNF-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 "Bundeslebensmittelschluessel" (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 sex[1]

| | Male | Female |
|---|---|---|
| *n* (%) | 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, *n* (%) | | |
| Never | 45 (44.3) | 103 (61.9) |
| Former | 26 (24.5) | 30 (17.9) |
| Current | 33 (31.1) | 34 (20.2) |
| Social class, *n* (%) | | |
| 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, *n* (%) | | |
| 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/ hypertryglyceridemia | 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 $P \geq 0.05$). 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 not cheese consumption, was related to higher TMAO plasma concentrations.

**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 2003[1]

| | *n* | 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) |
| *P*-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) |
| *P*-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) |
| *P*-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) |
| *P*-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.

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 2003[1]

| | *n* | 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) |
| *P*-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) |
| *P*-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) |
| *P*-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) |
| *P*-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) |
| *P*-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) |
| *P*-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) |
| *P*-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) |
| *P*-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 2003[1]

| | Quartiles of TMAO concentration, μM | | | | |
|---|---|---|---|---|---|
| | Q1 (<1.44) | Q2 (1.44 to <2.7) | Q3 (2.7 to <4.25) | Q4 (≥4.25) | *P*-trend[2] |
| *n* | 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, μg/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, μg/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 microbia and FMO3 as effect modifiers of potential associations between diet, TMAO, and chronic diseases.

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## References

1. Micha R, Michas G, Mozaffarian D. Unprocessed red and processed meats and risk of coronary artery disease and type 2 diabetes–an updated review of the evidence. Curr Atheroscler Rep 2012;14:515–24.
2. Chen GC, Lv DB, Pang Z, Liu QF. Red and processed meat consumption and risk of stroke: a meta-analysis of prospective cohort studies. Eur J Clin Nutr 2013;67:91–5.
3. Abete I, Romaguera D, Vieira AR, Lopez de Munain A, Norat T. Association between total, processed, red and white meat consumption and all-cause, CVD and IHD mortality: a meta-analysis of cohort studies. Br J Nutr 2014;112:762–75.
4. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Washington (DC): AICR; 2007.
5. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 2013;368:1575–84.
6. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 2013;19: 576–85.
7. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57–63.
8. Wang Z, Tang WH, Buffa JA, Fu X, Britt EB, Koeth RA, Levison BS, Fan Y, Wu Y, Hazen SL. Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-N-oxide. Eur Heart J 2014;35:904–10.
9. Hamlin JC, Pauly M, Melnyk S, Pavliv O, Starrett W, Crook TA, James SJ. Dietary intake and plasma levels of choline and betaine in children with autism spectrum disorders. Autism Res Treat 2013;2013:578429.
10. Himmerich S, Gedrich K, Karg G. Bayerische Verzehrsstudie (BVS) II - Abschlussbericht [Second Bavarian Food Consumption Survey- Final report.]: Bayerisches Staatsministerium fuer Umwelt, Gesundheit und Verbraucherschutz; 2003 (in German).
11. Slimani N, Deharveng G, Charrondiere RU, van Kappel AL, Ocke MC, Welch A, Lagiou A, van Liere M, Agudo A, Pala V, et al. Structure of the standardized computerized 24-h diet recall interview used as reference method in the 22 centers participating in the EPIC project. European Prospective Investigation into Cancer and Nutrition. Comput Methods Programs Biomed 1999;58:251–66.
12. Voss S, Charrondiere UR, Slimani N, Kroke A, Riboli E, Wahrendorf J, Boeing H. [EPIC-SOFT: a European computer program for 24-hour dietary protocols.] Z Ernahrungswiss 1998;37:227–33.
13. Himmerich H, Fulda S, Linseisen J, Seiler H, Wolfram G, Himmerich S, Gedrich K, Pollmächter T. TNF-alpha, soluble TNF receptor and interleukin-6 plasma levels in the general population. Eur Cytokine Netw 2006;17:196–201.
14. Winkler J, Stolzenberg H. [Social class index in the Federal Health Survey.] Gesundheitswesen 1999;61:S178–83.
15. WHO. Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation. Geneva (Switzerland): WHO;1985.
16. Tang WH, Wang Z, Kennedy DJ, Wu Y, Buffa JA, Agatisa-Boyle B, Li XS, Levison BS, Hazen SL. Gut microbiota-dependent trimethylamine N-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. Circ Res 2015;116:448–55.
17. Tang WH, Wang Z, Shrestha K, Borowski AG, Wu Y, Troughton RW, Klein AL, Hazen SL. Intestinal microbiota-dependent phosphatidylcholine metabolites, diastolic dysfunction, and adverse clinical outcomes in chronic systolic heart failure. J Card Fail 2015;21:91–6.
18. Tang WH, Wang Z, Fan Y, Levison B, Hazen JE, Donahue LM, Wu Y, Hazen SL. Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: refining the gut hypothesis. J Am Coll Cardiol 2014;64:1908–14.
19. Trøseid M, Ueland T, Hov JR, Svardal A, Gregersen I, Dahl CP, Aakhus S, Gude E, Bjorndal B, Halvorsen B, et al. Microbiota-dependent metabolite trimethylamine-N-oxide is associated with disease severity and survival of patients with chronic heart failure. J Intern Med 2015;277:717–26.
20. Kaysen GA, Johansen KL, Chertow GM, Dalrymple LS, Kornak J, Grimes B, Dwyer T, Chassy AW, Fiehn O. Associations of trimethylamine N-oxide with nutritional and inflammatory biomarkers and cardiovascular outcomes in patients new to dialysis. J Ren Nutr 2015;25:351–6.
21. Lever M, George PM, Slow S, Bellamy D, Young JM, Ho M, McEntyre CJ, Elmslie JL, Atkinson W, Molyneux SL, et al. Betaine and trimethylamine-N-oxide as predictors of cardiovascular outcomes show different patterns in diabetes mellitus: an observational study. PLoS One 2014;9:e114969.
22. Mueller DM, Allenspach M, Othman A, Saely CH, Muendlein A, Vonbank A, Drexel H, von Eckardstein A. Plasma levels of trimethylamine-N-oxide are confounded by impaired kidney function and poor metabolic control. Atherosclerosis 2015;243:638–44.
23. Mondul AM, Moore SC, Weinstein SJ, Karoly ED, Sampson JN, Albanes D. Metabolomic analysis of prostate cancer risk in a prospective cohort: the alpha-tocopherol, beta-carotene cancer prevention study. Int J Cancer 2015;137:2124–32.
24. Bae S, Ulrich CM, Neuhouser ML, Malysheva O, Bailey LB, Xiao L, Brown EC, Cushing-Haugen KL, Zheng Y, Cheng TY, et al. Plasma choline metabolites and colorectal cancer risk in the Women's Health Initiative Observational Study. Cancer Res 2014;74:7442–52.
25. Wang Z, Levison BS, Hazen JE, Donahue L, Li XM, Hazen SL. Measurement of trimethylamine-N-oxide by stable isotope dilution liquid chromatography tandem mass spectrometry. Anal Biochem 2014;455:35–40.
26. Keaveney EM, Price RK, Hamill LL, Wallace JM, McNulty H, Ward M, Strain JJ, Ueland PM, Molloy AM, Piironen V, et al. Postprandial plasma betaine and other methyl donor-related responses after consumption of minimally processed wheat bran or wheat aleurone, or wheat aleurone incorporated into bread. Br J Nutr 2015;113:445–53.
27. Fischer LM, da Costa KA, Galanko J, Sha W, Stephenson B, Vick J, Zeisel SH. Choline intake and genetic polymorphisms influence choline metabolite concentrations in human breast milk and plasma. Am J Clin Nutr 2010;92:336–46.
28. Abratte CM, Wang W, Li R, Axume J, Moriarty DJ, Caudill MA. Choline status is not a reliable indicator of moderate changes in dietary choline consumption in premenopausal women. J Nutr Biochem 2009;20:62–9.
29. Konstantinova SV, Tell GS, Vollset SE, Ulvik A, Drevon CA, Ueland PM. Dietary patterns, food groups, and nutrients as predictors of plasma choline and betaine in middle-aged and elderly men and women. Am J Clin Nutr 2008;88:1663–9.
30. Miller CA, Corbin KD, da Costa KA, Zhang S, Zhao X, Galanko JA, Blevins T, Bennett BJ, O'Connor A, Zeisel SH. Effect of egg ingestion on trimethylamine-N-oxide production in humans: a randomized, controlled, dose-response study. Am J Clin Nutr 2014;100:778–86.
31. West AA, Shih Y, Wang W, Oda K, Jaceldo-Siegl K, Sabate J, Haddad E, Rajaram S, Caudill MA, Burns-Whitmore B. Egg n-3 fatty acid composition modulates biomarkers of choline metabolism in free-living lacto-ovo-vegetarian women of reproductive age. J Acad Nutr Diet 2014;114:1594–600.
32. Zheng J, Huang T, Yu Y, Hu X, Yang B, Li D. Fish consumption and CHD mortality: an updated meta-analysis of seventeen cohort studies. Public Health Nutr 2012;15:725–37.
33. Larsson SC, Orsini N. Fish consumption and the risk of stroke: a dose-response meta-analysis. Stroke 2011;42:3621–3.
34. Eisert R, Oftedal OT, Lever M, Ramdohr S, Breier BH, Barrell GK. Detection of food intake in a marine mammal using marine osmolytes and their analogues as dietary biomarkers. Mar Ecol Prog Ser 2005;300:213–228.
35. Svensson BG, Akesson B, Nilsson A, Paulsson K. Urinary excretion of methylamines in men with varying intake of fish from the Baltic Sea. J Toxicol Environ Health 1994;41:411–20.

36. Zhang AQ, Mitchell SC, Smith RL. Dietary precursors of trimethylamine in man: a pilot study. Food Chem Toxicol 1999;37:515–20.
37. Srinivasa S, Fitch KV, Lo J, Kadar H, Knight R, Wong K, Abbara S, Gauguier D, Capeau J, Boccara F, et al. Plaque burden in HIV-infected patients is associated with serum intestinal microbiota-generated trimethylamine. AIDS 2015;29:443–52.
38. Cortez-Cooper M, Meaders E, Stallings J, Haddow S, Kraj B, Sloan G, McCully KK, Cannon JG. Soluble TNF and IL-6 receptors: indicators of vascular health in women without cardiovascular disease. Vasc Med 2013;18:282–9.
39. Schnabel RB, Yin X, Larson MG, Yamamoto JF, Fontes JD, Kathiresan S, Rong J, Levy D, Keaney JF Jr., Wang TJ, et al. Multiple inflammatory biomarkers in relation to cardiovascular events and mortality in the community. Arterioscler Thromb Vasc Biol 2013;33:1728–33.
40. Carlsson AC, Larsson TE, Helmersson-Karlqvist J, Larsson A, Lind L, Arnlov J. Soluble TNF receptors and kidney dysfunction in the elderly. J Am Soc Nephrol 2014;25:1313–20.
41. Marti CN, Khan H, Mann DL, Georgiopoulou VV, Bibbins-Domingo K, Harris T, Koster A, Newman A, Kritchevsky SB, Kalogeropoulos AP, et al. Soluble tumor necrosis factor receptors and heart failure risk in older adults: Health, Aging, and Body Composition (Health ABC) Study. Circ Heart Fail 2014;7:5–11.
42. Guzmán-Fulgencio M, Medrano J, Rallon N, Echeverria-Urabayen A, Miguel Benito J, Restrepo C, Garcia-Alvarez M, Vispo E, San Roman J, Sanchez-Piedra C, et al. Soluble markers of inflammation are associated with Framingham scores in HIV-infected patients on suppressive antiretroviral therapy. J Infect 2011;63:382–90.
43. Carlsson AC, Juhlin CC, Larsson TE, Larsson A, Ingelsson E, Sundstrom J, Lind L, Arnlov J. Soluble tumor necrosis factor receptor 1 (sTNFR1) is associated with increased total mortality due to cancer and cardiovascular causes—findings from two community based cohorts of elderly. Atherosclerosis 2014;237:236–42.
44. Shih DM, Wang Z, Lee R, Meng Y, Che N, Charugundla S, Qi H, Wu J, Pan C, Brown JM, et al. Flavin containing monooxygenase 3 exerts broad effects on glucose and lipid metabolism and atherosclerosis. J Lipid Res 2015;56:22–37.
45. Warrier M, Shih DM, Burrows AC, Ferguson D, Gromovsky AD, Brown AL, Marshall S, McDaniel A, Schugar RC, Wang Z, et al. The TMAO-generating enzyme flavin monooxygenase 3 is a central regulator of cholesterol balance. Cell Reports 2015;10:326–8.
46. Spillmann F, Van Linthout S, Miteva K, Lorenz M, Stangl V, Schultheiss HP, Tschope C. LXR agonism improves TNF-alpha-induced endothelial dysfunction in the absence of its cholesterol-modulating effects. Atherosclerosis 2014;232:1–9.
47. Sipka S, Bruckner G. The immunomodulatory role of bile acids. Int Arch Allergy Immunol 2014;165:1–8.