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Intra-individual variation of plasma trimethylamine-N-oxide (TMAO), betaine and choline over 1 year

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Abstract

Background: Circulating trimethylamine-*N*-oxide (TMAO) has been implicated in the development of cardiovascular and chronic kidney diseases (CKD). However, while higher TMAO levels have been associated with increased risks of cardiovascular or renal events in first prospective studies, it remained unclear how much plasma TMAO concentrations vary over time.

Methods: We measured fasting plasma levels of TMAO and two of its precursors, betaine and choline by LC-MS, in two samples of 100 participants of the European Investigation into Cancer and Nutrition (EPIC)-Heidelberg study (age range: 47–80 years, 50% female) that were collected 1 year apart, and assessed their intra-individual variation by Spearman's correlation coefficients (ρ).

Results: Correlations of metabolite concentrations over 1 year were at ρ=0.29 (p=0.003) for TMAO, ρ=0.81 (p<0.001) for betaine, and ρ=0.61 (p<0.001) for choline. Plasma levels of TMAO were not significantly associated with food intake, lifestyle factors, or routine biochemistry parameters such as C-reactive protein (CRP), low-density lipoprotein (LDL)-cholesterol, or creatinine.

Conclusions: In contrast to fasting plasma concentrations of betaine and choline, concentrations of TMAO were more strongly affected by intra-individual variation over 1 year in adults from the general population. The modest correlation of TMAO levels over time should be considered when interpreting associations between TMAO levels and disease endpoints.

Keywords: betaine; choline; intra-individual variation; TMAO.

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Introduction

Trimethylamine-*N*-oxide (TMAO), a small organic compound, whose plasma concentrations depend on dietary factors in interaction with the composition of the gut microbiome, is widely discussed as a risk factor and potential biomarker of chronic kidney diseases (CKD) and cardiovascular diseases (CVD) [1–3].

Experimental models indicate that TMAO induces atherosclerosis, possibly via macrophage foam cell formation and effects on cholesterol metabolism [4, 5]. Moreover, first prospective studies from the United States have shown that plasma concentrations of TMAO and two of its precursors, betaine and choline, are predictive of major cardiovascular events in high-risk individuals [5–8], even though these findings were not replicated in two recent studies from Denmark [9] and Austria and Switzerland [10]. Of note, fish is a major dietary source of TMAO and fish consumption has been observed to reduce CVD risk [11]. With regard to CKD, it has been proposed that TMAO may act as a renal toxin, causing tubulointerstitial fibrosis and dysfunction [12]. Alternatively, increased plasma TMAO levels could also be related to a pathological release of TMAO from the renal medulla, where it may act as an osmolyte and also protect protein integrity against denaturants, in kidney disease and to decreased excretion of TMAO due to impaired kidney function [12]. Notwithstanding conflicting evidence on the biological role of TMAO in kidney disease, several studies point to worse outcome in CKD patients with higher plasma levels of TMAO [10, 12–15]. Two recent prospective studies further revealed that plasma concentrations of TMAO in

asymptomatic individuals were associated with increased risks of colorectal and prostate cancer over time [16, 17], while mechanistic links between TMAO and cancer remain spurious and some experimental data actually point to potential anti-carcinogenic effects of TMAO [12].

The synthesis of TMAO comprises two main steps. First, trimethylamine (TMA) is generated from dietary L-carnitine, choline, and, to a lesser extent, betaine by bacteria in the intestine, where it enters the circulation. TMAO is then produced from TMA in the liver by TMA-oxidizing flavin-containing monooxygenases (FMO) [5, 8, 18]. Interestingly, the main sources of TMAO's major precursors L-carnitine and choline are animal foods, such as meat, fish, dairy products, and eggs, and it has been suggested that increased TMAO levels may underlie higher risks of CVD and CKD of individuals with high intakes of animal products and particularly red meat [4, 18, 19]. Besides food intake, the intestinal microbiome strongly affects blood concentrations of TMAO, which is reflected by the fact that dietary L-carnitine and choline challenges in humans lead to increased TMAO levels before, but not after the treatment with broad-spectrum antibiotics [8, 18]. In addition, it has been shown that TMAO levels may be related to specific enterotypes, and first taxa that may affect TMAO levels in the human circulation have been identified [18]. At the same time, data from a genome-wide association study did not point to a strong host genetic influence on circulating TMAO, and it has thus been proposed that dietary factors and the composition of the gut microbiota are the most important determinants of TMAO levels [20].

As dietary habits and the composition of the intestinal microbiota may be prone to changes, despite a certain stability of both factors during adulthood [21, 22], it could be speculated that TMAO levels undergo significant changes over time. In fact, substantial intra-individual variation in circulating TMAO has been observed in a population of overweight individuals with type 2 diabetes [23]. However, the biological variation of TMAO concentrations in the general population is unknown. To address this issue, we examined the intra-individual variation of fasting plasma levels of TMAO as well as betaine and choline over 1 year in 100 adults, who were free of major CVD, kidney diseases and cancer at the time of the blood donations. Further, as high controlled intakes of animal food that may not be entirely representative of individuals' habitual intakes were analyzed in the majority of previous studies on the dietary determinants of TMAO, we evaluated associations between metabolite concentrations and habitual food intake as well as lifestyle factors and routine biochemistry parameters. The overall goal of the present study was to assess whether TMAO and its precursors may be useful as long-term biomarkers in prospective epidemiological studies.

Materials and methods

Study population

The European Investigation into Cancer and Nutrition (EPIC) is a cohort study conducted in 23 study sites across 10 Western European countries [24]. In Heidelberg, 25,540 individuals (53.3% women) aged 35–65 years from the local general population entered the EPIC study between 1994 and 1998 [25]. Between 2010 and 2013, a sub-study on diet, physical activity, and body composition embedded in the EPIC-Heidelberg study was carried out. Details on this sub-study have been given elsewhere [26]. In brief, 798 participants (53.5% women), who had been randomly recruited from EPIC-Heidelberg, took part in a re-examination 14 years after the baseline of the main study (examination 1). The age range of these individuals at examination 1 was 47–80 years. Data on habitual food intake were collected by a validated food frequency questionnaire [27]. Body composition was assessed by whole-body magnetic resonance imaging (MRI) in a subset of 613 individuals [28]. All 798 participants provided questionnaire-information on medication use, smoking, alcohol intake and socio-economic factors. In addition, study physicians carried out interviews on health status and medication use, and took a blood sample. The latter interview and blood draw were repeated in a second re-examination that took place 1 year after the first one, with 765 out of the initial 798 participants attending (examination 2).

For the present study, a sub-sample of 100 participants of the EPIC-Heidelberg sub-study described above was selected. First, only participants who had provided a fasting blood sample at both re-examinations were considered (n=669, 83.8% of the initial sample). Second, considering that clear differences in TMAO levels between vegetarians and non-vegetarians have been described [18], all vegetarians (n=25) were selected. Out of the remaining 644 individuals, those with MRI data were pre-selected (n=483), and 75 out of these 483 individuals were randomly chosen to achieve the target number of 100. The study population only included individuals who had not been diagnosed with cancer or major renal or CVD at the time of the blood draws for the present analyses (see Table 1 for details).

All participants gave written consent prior to participation in the initial study and the sub-study, and ethical approvals were obtained from the Ethics Committee of the Heidelberg University Hospital (Heidelberg, Germany).

Laboratory methods

Blood samples were processed right after the blood draws as plasma, serum and peripheral blood mononuclear cells (PBMC) and stored in gas phase liquid nitrogen at –185 °C. Blood levels of HbA_{1c}, glucose, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, C-reactive protein (CRP), creatinine and leucocytes were measured by routine assays at the central laboratory of Heidelberg University Hospital (Heidelberg, Germany) right after blood donation. The estimated glomerular filtration rate (eGFR) was calculated

Table 1: Characteristics of the study population.

	Men (n=50)	Women (n=50)
Age, years	64.6 (58.6, 71.4)	63.0 (57.3, 70.8)
Education level[a]		
Low	7 (14%)	5 (10%)
Medium	14 (28%)	30 (60%)
High	29 (58%)	15 (30%)
BMI	26.1 (24.7, 27.5)	24.2 (21.6, 27.7)
Waist circumference, cm	97.2 (94.5, 104.3)	89.3 (79.0, 97.0)
Blood pressure		
Systolic	131.5 (124.5, 138.5)	128.5 (116.5, 141.5)
Diastolic	80.0 (72.5, 85.5)	75 (70, 82)
Smoking status		
Never	18 (36%)	28 (56%)
Former	25 (50%)	17 (34%)
Current	7 (14%)	5 (10%)
Prevalent diseases		
Diabetes	2 (4%)	1 (2%)
Cancer	0 (0%)	0 (0%)
Stroke	0 (0%)	0 (0%)
Myocardial infarction	0 (0%)	0 (0%)
eGFR, mL/min/1.73 m^2		
>90	21 (42%)	20 (40%)
60–90	27 (54%)	27 (54%)
<60	2 (4%)	3 (6%)
Red meat intake, g/day	37.5 (14.0, 70.1)	21.9 (5.0, 34.4)
Processed meat intake, g/day	37.6 (20.8, 57.1)	19.5 (9.4, 33.8)
Fish intake, g/day	18.2 (7.8, 29.8)	13.5 (5.2, 27.2)
Cheese intake, g/day	32.5 (23.5, 44.5)	31.3 (24.0, 51.7)
Milk intake, g/day	91.6 (42.3, 179.9)	107.2 (52.7, 185.4)
Egg intake, g/day	13.3 (5.3, 13.8)	5.4 (1.4, 13.7)
Vegetarians	9 (18%)	17 (34%)
Prebiotics use[b]	2 (4%)	5 (10%)
Antibiotics use[c]		
Examination 1	0	1 (2%)
Examination 2	0	1 (2%)
TMAO, µmol/L		
Examination 1	3.6 (2.9, 4.7)	3.7 (2.6, 4.6)
Examination 2	4.5 (2.8, 6.0)	3.4 (2.7, 4.7)
Choline, µmol/L		
Examination 1	36.9 (29.0, 46.3)	27.6 (22.3, 34.6)
Examination 2	37.5 (30.9, 45.3)	29.5 (23.1, 38.0)
Betaine, µmol/L		
Examination 1	11.4 (10.3, 12.7)	10.3 (9.4, 11.5)
Examination 2	12.1 (11.2, 14.1)	10.7 (9.5, 11.1)

All covariates except antibiotics use and metabolite concentrations from examination 1. Continuous variables: median value (25th percentile, 75th percentile). Categorical variables: number (percentage). [a]Low, primary school or no formal school degree; medium, secondary or technical degree; high, university degree. [b]Within the past year before first blood draw. [c]Within 7 days before blood draw.

based on creatinine levels using the BIS1 formula [29] for individuals aged ≥70 years and the CKD-EPI formula [30] for individuals aged <70 years. Total adiponectin was measured in stored plasma samples in the laboratory of the Division of Cancer Epidemiology at the German Cancer Research Center, DKFZ (Heidelberg, Germany) by Sandwich ELISA (A68456; Beckman Coulter, Sinsheim, Germany).

For the measurements of TMAO, betaine, and choline, 20-µL samples of EDTA-plasma were shipped to the Institute of Clinical Chemistry, University Hospital Zurich (Zurich, Switzerland) on dry ice, where they were thawed at room temperature. Metabolite levels were quantified by LC-MS, as previously described [10]. In brief, 360 µL of an internal standard mixture containing TMAO-d9, betaine-d3 and choline-d9 at 1 µmol/L were added. After vortexing and centrifugation at 11,700×*g* for 10 min at 4 °C, the supernatant was analyzed. Separation was achieved on an Accucore HILIC column (50×2.1 mm, 2.6 µm particle size, Thermo Fisher Scientific, Reinach,

Switzerland) under acidic conditions. As mass spectrometer, a Q Exactive hybrid instrument (Thermo Fisher Scientific) was used, that acquired chromatograms in positive heated electrospray ionization fullscan mode at a resolution of 70,000 full width at half maximum (FWHM). Extracted ion chromatograms with mass windows of 10 ppm of TMAO, betaine, choline and the respective internal standards were used for quantification. Calibration ranges were 0.54–71.9 µmol/L for TMAO, 5.07–162 µmol/L for betaine and 1.92–61.5 µmol/L for choline. Between-day imprecision was <10.6% for TMAO, <6.25% for betaine and <6.11% for choline, and between-day accuracy was 92.4% for TMAO, 99.5% for betaine and 97.1% for choline.

Statistical analyses

The biological reproducibility of metabolite concentrations over 1 year was assessed by Spearman's correlation coefficients (ρ) adjusting for age and sex, and by scatter plots. As intra-class coefficients of variation (ICC) were reported in previous reproducibility studies, we further calculated ICCs (ratio of between-person variance divided by the sum of between-person variance and within-person variance) based on log-transformed metabolite concentrations for comparison purposes. Geometric means (95% confidence intervals) of metabolite levels across categories of background factors such as age group, sex, our smoking status were obtained. Correlations matrices were created to visualize correlations of TMAO, betaine, and choline with routine biochemistry parameters.

Results

Intra-individual variation of metabolite concentrations

Characteristics of the study population that comprised 50 women (median age: 63.0 years) and 50 men (median age: 64.6 years) are shown in Table 1. Median fasting plasma concentrations of TMAO, betaine, and choline in women were 3.7, 10.3, and 27.6 µmol/L at the first examination and 3.4, 10.7, and 29.5 µmol/L after 1 year. In men, median levels of TMAO, betaine, and choline were 3.6, 11.4, and 36.9 µmol/L at the first examination and 4.5, 12.1, and 37.5 µmol/L after 1 year. Individual metabolite concentrations from two blood samples taken at the two examinations that were carried out 1 year apart are depicted in Figure 1. Unadjusted Spearman's coefficients for correlations of TMAO, betaine, and choline levels over 1 year (see Table 2) were 0.29 (p=0.003), 0.81 (p<0.001), and 0.61 (p<0.001). After adjustment for age and sex, the estimates were 0.28, 0.76, and 0.52. Unadjusted ICCs (TMAO: 0.22, betaine: 0.76, and choline: 0.63) were slightly lower than unadjusted Spearman's coefficients. The exclusion of high TMAO values (>10 µmol/L) did not lead to clear improvements of the correlation over time, with

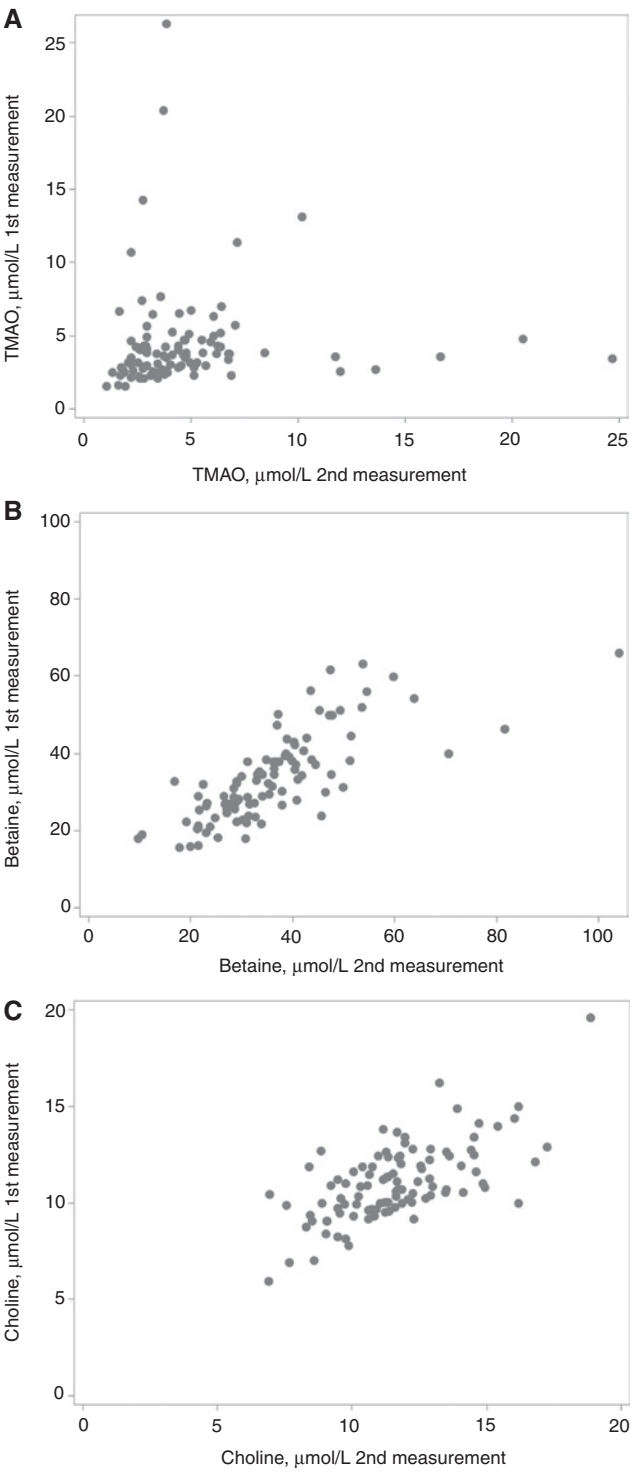

Figure 1: Scatter plots of metabolite levels.
(A) TMAO, (B) betaine, (C) choline.

a Spearman's coefficient of 0.37 and an ICC of 0.39. Correlations remained virtually unchanged when excluding individuals (n=5) with eGFR values <60 mL/min/1.73 m^2 or when adjusting for eGFR values (data not shown). The highest within-person variation (CV_I) was observed for

Table 2: Correlations of metabolite concentrations over 1 year.

	Spearman's ρ		ICC[a]	Coefficient of within-person variation (CV_I), %	Coefficient of between-person variation (CV_G), %
	Unadjusted	Adjusted for age and sex			
Betaine	0.81	0.76	0.76	17.0	30.5
Choline	0.61	0.52	0.63	11.5	15.1
TMAO	0.29	0.28	0.22	46.7	24.7

[a]Intraclass-correlation coefficient calculated as the ratio of between-person variance divided by the sum of between-person variance and within-person variance.

TMAO (46.7%), whereas within-person coefficients of variation were 17.0% and 11.5% for betaine and choline.

Correlates of metabolite concentrations

Cross-sectional associations between metabolite concentrations and epidemiological covariates are presented in Table 3. TMAO levels were significantly higher in users of prebiotic medication compared to non-users. By contrast, plasma choline and betaine were significantly lower in users of prebiotics. No significant differences in TMAO levels across strata of other covariates were observed. Concentrations of choline and betaine were significantly higher in fasting plasma of males, older study participants and individuals with higher intakes of processed meat.

Correlations between TMAO, its precursors and routine clinical biochemistry parameters are depicted in Supplemental Figure 1. None of the routine parameters showed correlations with TMAO, choline, and betaine at Spearman's coefficients of at least 0.3, with the exception of creatinine, which was positively correlated with betaine (ρ=0.27 at examination 1 and ρ=0.40 at examination 2) and choline (ρ=0.39 and ρ=0.50). There were modest correlations between betaine and choline levels at both examinations (ρ=0.55 and ρ=0.47). No strong correlations between concentrations of these two metabolites with concentrations of TMAO were observed.

Discussion

We evaluated the biological reproducibility of plasma levels of TMAO over 1 year in adults aged 64 years on average who had not been diagnosed with cancer or major renal and CVD. Compared to betaine and choline concentrations, which showed intra-individual correlations of ρ=0.81 and ρ=0.61 over 1 year, the correlation of TMAO concentrations was lower (ρ=0.29). The within-person variation of TMAO levels (CV_I=46.7%) was greater than the between-person variation (CV_G=24.7%), again in contrast to betaine (CV_I=17.0%, CV_G=30.5%) and choline (CV_I=11.5%, CV_G=15.1%). No clear associations between TMAO concentrations and epidemiological covariates such as habitual meat intake, obesity, or smoking status were observed. TMAO levels were not related to levels of routine biomarkers of lipid and glucose metabolism, inflammation or renal function.

We are aware of only one previous study, in which intra-individual variation in TMAO levels over time has been evaluated. In the Diabetes Excess Weight Loss (DEWL) study, 243 overweight type 2 diabetic patients (median age: 60 years) provided fasting blood samples four times at 6-month intervals over 2 years [23]. While reliability coefficients for betaine (0.75) and choline (0.67) based on concentrations from all four blood samples pointed to good biological reproducibility over time, plasma TMAO levels were affected by substantial intra-individual variation, with a reliability coefficient of 0.17. The within-person variation in the DEWL trial was higher for TMAO (63.3%) than for betaine (14.9%) and choline (14.4%), which is consistent with the within-person CVs of 46.7%, 17.0%, and 11.5% observed for TMAO, betaine and choline in the present study. Intra-individual variation over time of choline and betaine, but not TMAO has further been assessed in participants of the Nurses' Health Study (NHS) and the Western Norway B Vitamin Intervention Trial (WENBIT) [31]. Spearman's correlations for betaine (ρ=0.64) and choline (ρ=0.33) were slightly worse in NHS compared to our study (ρ=0.81 and ρ=0.61, respectively), even though the duration of 1–2 years between blood draws was longer in the NHS than in EPIC-Heidelberg (1 year) [31]. In the WENBIT study, intra-class correlations based on four samples taken over 38 months were at 0.65 for betaine and 0.45 for choline [31]. Given the longer time periods between blood draws in the NHS and WENBIT, these results and the results of the above mentioned DEWL trial on choline and betaine are consistent with ours.

Table 3: Geometric means (95% CIs) of metabolite concentrations.

	TMAO, µmol/L		Betaine, µmol/L		Choline, µmol/L	
	Ø (95% CI)	p-Value	Ø (95% CI)	p-Value	Ø (95% CI)	p-Value
Sex						
Female	3.9 (3.4, 4.5)		28.1 (25.9, 30.6)		10.3 (9.8, 10.8)	
Male	3.7 (3.2, 4.3)	0.56	36.1 (33.2, 39.3)	<0.001	11.5 (11.0, 12.1)	<0.001
Age						
<60 years	3.7 (3.2, 4.4)		31.21 (28.3, 34.4)		10.84 (10.3, 11.4)	
60–69.9 years	3.5 (2.9, 4.1)		28.88 (26.1, 31.9)		10.18 (9.6, 10.7)	
≥70 years	4.4 (3.7, 5.2)	0.37	36.51 (32.8, 40.6)	0.027	11.80 (11.1, 12.5)	0.048
BMI						
<25	3.9 (3.4, 4.6)		34.0 (31.0, 37.2)		10.8 (10.3, 11.4)	
25–29.9	3.7 (3.2, 4.3)		30.2 (27.6, 33.1)		10.8 (10.3, 11.4)	
≥30	3.8 (2.7, 5.3)	0.71	30.4 (25.0, 37.0)	0.19	11.6 (10.4, 13.0)	0.62
Smoking status						
Never	3.4 (2.9, 3.9)		33.7 (30.9, 36.7)		11.1 (10.5, 11.6)	
Former	4.2 (3.6, 4.9)		31.5 (28.8, 34.5)		10.6 (10.1, 11.2)	
Current	4.3 (3.2, 5.7)	0.10	26.7 (22.6, 31.7)	0.06	11.2 (10.2, 12.3)	0.46
Prebiotics use						
No	3.7 (3.3, 4.1)		32.5 (30.6, 34.5)		11.0 (10.7, 11.4)	
Yes	5.7 (3.9, 8.3)	0.029	24.5 (19.6, 30.7)	0.019	9.2 (8.2, 10.5)	0.008
Vegetarian diet						
No	3.9 (3.5, 4.4)		32.0 (29.8, 34.3)		11.0 (10.5, 11.4)	
Yes	3.6 (3.0, 4.4)	0.57	31.5 (28.0, 35.5)	0.83	10.7 (10.0, 11.4)	0.52
Processed meat intake						
Lowest quartile	3.7 (3.0, 4.5)		31.0 (27.5, 34.9)		10.8 (10.1, 11.6)	
Highest quartile	4.1 (3.3, 5.1)	0.44	35.6 (31.4, 40.3)	0.018	11.3 (10.6, 12.2)	0.033
Red meat intake						
Lowest quartile	3.7 (3.0, 4.6)		32.0 (28.2, 36.2)		10.8 (10.1, 11.6)	
Highest quartile	3.9 (3.2, 4.8)	0.61	31.5 (27.8, 35.6)	0.46	11.2 (10.4, 12.0)	0.06
Fish intake						
Lowest quartile	3.7 (3.0, 4.5)		32.6 (28.8, 36.9)		10.8 (10.1, 11.5)	
Highest quartile	3.9 (3.2, 4.8)	0.90	31.1 (27.5, 35.2)	0.70	10.6 (9.9, 11.4)	0.69
Cheese intake						
Lowest quartile	3.6 (3.0, 4.4)		33.4 (29.7, 37.4)		11.1 (10.3, 11.8)	
Highest quartile	4.0 (3.3, 4.9)	0.98	28.0 (24.9, 31.4)	0.88	10.5 (9.8, 11.2)	0.84
Milk intake						
Lowest quartile	3.8 (3.1, 4.7)		31.1 (27.5, 35.2)		10.8 (10.1, 11.5)	
Highest quartile	3.9 (3.2, 4.8)	0.32	30.7 (27.2, 34.7)	0.62	10.6 (9.9, 11.3)	0.56
Egg intake						
Lowest quartile	3.9 (3.2, 4.8)		32.8 (29.0, 37.0)		11.0 (10.3, 11.8)	
Highest quartile	3.8 (2.7, 5.3)	0.62	33.2 (27.1, 40.6)	0.94	11.0 (9.8, 12.3)	0.73

Geometric means adjusted for age and sex. p-Values from trend tests across categories obtained by linear regression models. All results based on the first examination and blood draw of the EPIC Heidelberg sub-study.

Surprisingly, our study showed no associations between TMAO and the consumption of animal food, which are the main source of TMAO's precursors L-carnitine and choline. With regard to fish, our population had a rather low average habitual intake, which may be the reason for the lack of association with TMAO levels. Habitual intakes of other animal products in our population were still lower than the high intakes of eggs and steak for example that have been demonstrated to cause increases in TMAO levels in previous intervention trials under controlled conditions [18, 32]. This notion is supported by recent results from the Second Bavarian Food Consumption Survey (BVS II) [33] that did not point to strong associations between habitual intake of animal foods and TMAO concentrations overall, even though slightly higher TMAO levels were observed in individuals with higher milk intake. The finding of higher levels of TMAO and lower levels of betaine and choline in users of prebiotics in the present study was unexpected and in contrast to previous studies [34, 35]. However, it must be

noted that only 7% of our participants reported to have used prebiotics, and no further details on duration and frequency of intake were available.

Contrary to previous studies [10, 36], the EPIC-Heidelberg study showed no associations between TMAO and creatinine or the eGFR. Potentially, this finding may be related to the fact that associations between TMAO levels and kidney function were reported from studies in high-risk populations, whereas the individuals in our study were free of major chronic diseases. Actually, only a few of our study participants had eGFR values <60 mL/min/1.73 m^2, at which increases in TMAO concentrations have been observed [10]. Interestingly, however, our study did reveal modest negative correlations between choline levels and eGFR values, which is in line with results from previous studies in individuals undergoing angiography and patients with renal failure [10, 37]. There were no associations between TMAO and its precursors with routine biomarkers of glucose metabolism, lipid metabolism and inflammation in the present study. While correlations of TMAO with HbA_{1c}, LDL, triglycerides and CRP may have been expected in consideration of experimental findings [3, 38], our results are consistent with those of previous epidemiological studies [33]. Again, we cannot rule out that the lack of correlations between TMAO, its precursors and routine biomarkers in the EPIC-Heidelberg study is related to the good overall health status of our participants.

Our study was the first to evaluate the intra-individual variation of TMAO, betaine in choline over 1 year in a general population of adults. While admittedly the dietary determinants of blood concentrations of TMAO and its metabolites could not be analyzed in a controlled manner, we believe that our study was appropriate to evaluate the biological reproducibility of metabolite levels in a real-world setting. Nevertheless, we have to acknowledge that we could not address the role of changes in the intestinal microbiome, which may underlie the observed variation in TMAO levels and thus deserve further study.

Conclusions

In summary, we observed a good biological reproducibility of choline and particularly betaine concentrations over 1 year in fasting blood samples of adults aged 47–80 years without diagnosed cancer or major renal and CVD. By contrast, TMAO concentrations showed clear intra-individual variation over time, driven by a greater within-person than between-person variation, which may speak against the use of TMAO as a risk marker in epidemiological long-term studies. The causes underlying the variation in TMAO levels remain to be clarified in future studies.

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