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Nahua Xu
Central South University
Ying Li
Central South University
Yihui Li
Central South University
Pingting Yang
Central South University
Xuejing Dai
Central South University
Jiangang Wang
600410@csu.edu.cn

Central South University

Research Article

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Low plasma trimethylamine N-oxide is associated with vitamin D deficiency: toward a U-shaped TMAO–health hypothesis

Nahua Xu[a,b,c], Ying Li[a,b,c], Yihui Li[a,b,c], Pingting Yang[a,b,c], Xuejing Dai[d], Jiangang Wang[a,b,c]*

Affiliations:

a. Health Management Medicine Center, Third Xiangya Hospital, Central South University, Changsha, Hunan, China.

b. Hunan Engineering Technology Research Center for Sub-health Diagnosis and Intervention, Changsha, Hunan, China.

c. Hunan Clinical Medical Research Center for Chronic Disease Health Management, Changsha, Hunan, China.

d. Department of Pharmacology, Xiangya School of Pharmaceutical Sciences, Central South University, Changsha, Hunan, China.

Corresponding author at: Health Management Medicine Center, Third Xiangya Hospital, Central South University, Changsha, Hunan, China.

E-mail address: 600410@csu.edu.cn (Jiangang Wang)

Highlights

- Low plasma TMAO is associated with vitamin D deficiency in Chinese adults.
- Per 1-SD higher logTMAO, 25(OH)D was 2.18 ng/mL higher.
- Vitamin D deficiency prevalence decreased from 60.3% to 33.9% with increasing TMAO.
- The TMAO–vitamin D association weakened with increasing BMI.

Abstract

Background & aims: Trimethylamine N-oxide (TMAO), a gut microbiota-derived metabolite, has been studied predominantly in relation to adverse outcomes at high concentrations, whereas the health relevance of low circulating TMAO remains largely unexplored. Because fatty fish provides both preformed TMAO and vitamin D, we postulated that plasma TMAO is positively associated with circulating 25-hydroxyvitamin D [25(OH)D].

Methods: We cross-sectionally analyzed 621 adults undergoing health examinations (median age 52.0 years; 52.7% men), using multivariable linear regression of serum 25(OH)D on plasma TMAO (per 1-SD logTMAO; quartiles), restricted cubic splines for dose–response, and likelihood ratio tests for BMI effect modification.

Results: Median TMAO was 170.3 ng/mL (IQR 116.1–247.1); mean 25(OH)D was 22.1 ± 11.7 ng/mL. Adjusted for age, sex, BMI, and season, each 1-SD higher logTMAO was associated with 2.18 ng/mL higher 25(OH)D (95% CI 1.29–3.07), and Q4 vs Q1 differed by 4.56 ng/mL (2.05–7.07; both $P < 0.001$); across TMAO percentile bands, mean 25(OH)D rose from 20.1 to 29.8 ng/mL and deficiency prevalence fell from 60.3% to 33.9%. The association was nonlinear (P for nonlinearity = 0.018) and modified by BMI (P for interaction = 0.018): positive in normal-weight and overweight participants but attenuated, with a reversed point estimate, in obesity. It remained positive after excluding participants with obesity, high-leverage observations, and renal impairment.

Conclusions: In Chinese adults, lower plasma TMAO was associated with lower

circulating 25(OH)D, with BMI-dependent effect modification. Together with the established harms of high TMAO concentrations, these findings motivate a U-shaped TMAO–health hypothesis that awaits prospective confirmation.

Keywords: trimethylamine N-oxide; vitamin D; 25-hydroxyvitamin D; body mass index; gut microbiota.

1. Introduction

Vitamin D deficiency is highly prevalent worldwide[1,2] and is associated with musculoskeletal disorders[3,4], cardiometabolic disease and all-cause mortality[5,6], and immune dysfunction[7,8], although large supplementation trials have yielded mixed cardiovascular results[9,10]. In Chinese adults, more than half have insufficient 25-hydroxyvitamin D[25(OH)D] levels[11,12]; identifying modifiable and metabolism-related determinants of vitamin D status is therefore of substantial public health importance[13,14].

Trimethylamine N-oxide (TMAO) is a small amine oxide derived from dietary choline, carnitine, and betaine. Its production involves sequential metabolism by the gut microbiota and the liver[15,16]. Early work linked elevated circulating TMAO to higher cardiovascular risk[17], and subsequent meta-analyses have summarized its associations with a wide range of health outcomes[18]. Since then, a growing body of evidence has specifically implicated high TMAO levels in heart failure[19–21], chronic kidney disease (CKD) progression[22–24], type 2 diabetes and its renal complications[25–27], and all-cause mortality[28–30]. Additional studies have reported associations with hypertension, stroke, and abdominal aortic aneurysm[31–

33]. This evidence has focused research almost exclusively on the upper end of the TMAO distribution.

In contrast, the health relevance of low circulating TMAO has received little attention. TMAO is abundant in fish and seafood[34,35], which are also among the few natural dietary sources of vitamin D[4,36]. In free-living adults, circulating TMAO tracks with habitual diet and gut microbial ecology[37]; low TMAO concentrations may therefore reflect a dietary pattern or gut microbial phenotype that simultaneously limits vitamin D intake or compromises vitamin D status. Direct evidence linking vitamin D with TMAO is limited but suggestive: vitamin D–based supplementation lowered plasma TMAO in a small randomized trial, more so with added B vitamins[38]; cholecalciferol supplementation reduced circulating TMAO in obese, vitamin D-deficient adults[39]; and vitamin D repletion lowered plasma TMAO in mice by reshaping the gut microbiota[40]. To our knowledge, no study in a general health-examination setting has examined the TMAO–vitamin D association. The only previous observational study, conducted in 104 adults recruited at an Italian university endocrinology unit (outpatients, hospital volunteers, and employees; 54% with obesity), reported an inverse association ($r = -0.59$) that was no longer significant after adjustment for the fatty liver index, a surrogate of nonalcoholic fatty liver disease[41]; however, that analysis was not adjusted for BMI, and the association within non-obese individuals was not separately reported.

We therefore performed a cross-sectional analysis among participants undergoing routine health examinations, examining the association between plasma TMAO and

25(OH)D. As a secondary analysis, we further assessed whether this association was modified by BMI, given that the inverse association in that study appeared to strengthen across increasing BMI classes[41].

2. Materials and methods

2.1. Study population

All participants were recruited from the Department of Health Management at the Third Xiangya Hospital of Central South University, a tertiary medical center in Changsha, China. The source population consisted of 662 consecutive examinees (aged ≥18 years) who underwent both plasma TMAO and serum 25(OH)D measurement between January and July 2026, all of whom completed baseline physical examinations that included measurements of blood pressure, hepatic and renal function, and serum lipid levels. After exclusion of participants with a history of malignant neoplasm (n = 12), 650 remained; further exclusion of severe hepatic impairment (ALT/AST>120 U/L, n = 2) left 648; and exclusion of those missing covariate information required for the prespecified models (age, sex, BMI, or examination season; n = 27) yielded a final analytic sample of 621 adults (Supplementary Fig. S1). Because these participants lacked the covariates used in the adjusted analyses by definition, a formal comparison of included versus excluded participants was not undertaken. This study was approved by the Independent Ethics Committee of the Third Xiangya Hospital of Central South University (Approval No. 25955). The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants.

2.2. Laboratory and clinical measurements

Demographic characteristics and self-reported medical and family history were collected using standardized questionnaires; dietary intake, supplement use, and sun exposure were not systematically recorded. Venous blood samples were obtained after an overnight fast and analyzed as soon as possible after collection. Laboratory assays, including serum creatinine, fasting glucose, uric acid, alanine aminotransferase, total cholesterol, and triglycerides, were performed by certified personnel in the hospital's central laboratory according to standard operating procedures. Serum total 25(OH)D concentrations were measured by two-dimensional ultra-high-performance liquid chromatography (2D-UHPLC). Plasma TMAO concentrations were measured using a TMAO assay kit by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Changsha Duxact Biotech Co., Ltd.). TMAO concentrations are reported in ng/mL. Estimated glomerular filtration rate (eGFR) was calculated using the 2021 CKD-EPI equation without race[42].

2.3. Definition of vitamin D status

The primary outcome was continuous 25(OH)D (ng/mL), a recognized biomarker of overall vitamin D status.

Vitamin D deficiency was defined as 25(OH)D < 20 ng/mL, in accordance with the 2011 Endocrine Society clinical practice guideline[4]; severe deficiency was prespecified as < 10 ng/mL (25 nmol/L) for descriptive purposes. 25(OH)D was categorized as severe deficiency (<10 ng/mL), deficiency (10 to <20 ng/mL), insufficiency (20 to <30 ng/mL), and sufficiency (≥30 ng/mL).

2.4. Covariates

Age (years, continuous), sex (female/male), BMI (kg/m², continuous), and season (spring: March–May; summer: June–August; autumn: September–November; winter: December–February). Season was included because cutaneous vitamin D synthesis depends strongly on solar exposure and 25(OH)D concentrations vary seasonally in Chinese populations[43–45]. eGFR was treated as a sensitivity covariate rather than a core confounder, because TMAO is renally cleared and may lie on the causal pathway[22,23]. TMAO was natural-log transformed (skewness decreased from 7.0 to 0.3; Supplementary Figure S2) and standardized (per 1-SD increment). BMI was modeled both continuously and categorically using Chinese cutoffs: normal weight (<24), overweight (24–<28), and obesity (≥28 kg/m²)[46].

2.5. Statistical analysis

Skewed continuous variables were summarized as median (interquartile range [IQR]), approximately symmetric continuous variables as mean ± SD, and categorical variables as n (%). Between-group comparisons used the Wilcoxon rank-sum test (continuous variables) and the χ^2 test (categorical variables; Fisher's exact test when expected frequencies were small). Baseline characteristics were tabulated by vitamin D deficiency status.

The primary analysis used multivariable linear regression with continuous 25(OH)D as the outcome. Differences in 25(OH)D (β, 95% CI) were reported per 1-SD increment in logTMAO and by TMAO quartile (Q1 lowest = reference) across three nested models: unadjusted; model 1 (age, sex); and model 2 (plus BMI and season). β and 95% CI

were computed from the fitted regression models, with confidence intervals based on the t distribution. A cumulative adjustment ladder (M1–M5) is provided in Supplementary Table S1. Dose–response was assessed by (i) mean 25(OH)D across six TMAO percentile bands (<P10 to ≥P90), reporting both mean 25(OH)D and the prevalence of vitamin D deficiency within each band (unadjusted linear trend tests), and (ii) restricted cubic splines with 3 interior knots (4 degrees of freedom) at the 25th, 50th, and 75th percentiles of log-transformed TMAO for 25(OH)D on logTMAO in multivariable linear models, with nonlinearity tested against the linear model by F test (Fig. 1).

Effect modification by BMI was assessed by likelihood ratio interaction tests comparing models with and without a logTMAO × BMI product term. The primary interaction test used continuous BMI (1 degree of freedom), as prespecified, because BMI is inherently continuous and the continuous test has greater power to detect graded trends. A secondary test used the three-category BMI parameterization (2 degrees of freedom) for clinical interpretability. Stratum-specific estimates were obtained by fitting 25(OH)D-on-logTMAO linear models separately within the normal-weight, overweight, and obesity strata (each adjusted for age, sex, and season); the continuous modification pattern was assessed with a pooled model containing an interaction between logTMAO and a restricted cubic spline of BMI (3 degrees of freedom), from which the conditional slope of 25(OH)D on logTMAO was computed and plotted as a function of BMI over its 2nd–98th percentiles (Fig. 2). The presentation of effect-modification analyses was informed by the recommendations of Knol and

VanderWeele[47].

Prespecified sensitivity analyses re-estimated the model 2 association after (i) excluding participants with obesity, (ii) excluding high-leverage/influential observations identified by Cook distance (>4/n), and (iii) excluding participants with eGFR < 60 mL/min/1.73 m², and, post hoc, (iv) excluding participants with 25(OH)D >50 ng/mL (a range suggestive of vitamin D supplement use). The ordinal association of TMAO with 25(OH)D severity categories is tabulated in Supplementary Table S3.

All analyses were performed in R version 4.5.3. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Participant characteristics

Baseline demographic and biochemical characteristics of the 621 participants, stratified by vitamin D deficiency status (Table 1). The median age was 52.0 (40.0–63.0) years, 327 (52.7%) were men, and median BMI was 23.7 (21.8–25.9) kg/m². Median TMAO was 170.3 (116.1–247.1) ng/mL and mean ± SD 25(OH)D was 22.1 ± 11.7 ng/mL; 313 participants (50.4%) had vitamin D deficiency, including 37 (6.0%) with severe deficiency. Compared with participants without vitamin D deficiency, those with deficiency had lower TMAO (median 158.0 vs 188.8 ng/mL) and were younger (median 49.0 vs 56.0 years).

Table 1. Baseline characteristics of the 621 participants by vitamin D deficiency status.

Variable	Overall	VD deficient	VD non-deficient	*P*
25(OH)D (ng/mL)	19.9 (14.7, 25.8)	14.7 (12.0, 17.3)	25.9 (22.6, 32.1)	<0.001
TMAO (ng/mL)	170.3 (116.1, 247.1)	158.0 (109.8, 228.5)	188.8 (121.9, 276.9)	0.001
Sex (male, %)	327 (52.7)	176 (56.2)	151 (49.0)	0.086
Female	294 (47.3)	137 (43.8)	157 (51.0)	
Season, spring, n (%)	377 (60.7)	214 (68.4)	163 (52.9)	<0.001
Summer	140 (22.5)	63 (20.1)	77 (25.0)	
Winter	104 (16.7)	36 (11.5)	68 (22.1)	
Age (years)	52.0 (40.0, 63.0)	49.0 (38.0, 61.0)	56.0 (42.8, 63.0)	<0.001
BMI (kg/m²)	23.7 (21.8, 25.9)	24.1 (22.2, 26.5)	23.3 (21.4, 25.4)	0.001
Height (cm)	163.0 (158.0, 169.0)	164.0 (158.5, 170.0)	162.0 (157.5, 168.1)	0.013
Weight (kg)	63.5 (55.3, 72.6)	65.5 (57.1, 73.8)	61.2 (54.2, 70.4)	<0.001
Waist circumference (cm)	81.0 (73.0, 90.0)	82.5 (74.8, 91.0)	80.0 (72.0, 88.8)	0.015
Hip circumference (cm)	93.0 (90.0, 97.0)	94.0 (91.0, 98.0)	92.0 (89.0, 96.0)	<0.001
Systolic BP (mmHg)	121.0 (111.0, 131.0)	120.0 (109.0, 130.0)	121.0 (113.0, 132.0)	0.313
Diastolic BP (mmHg)	73.0 (66.0, 80.0)	73.0 (66.0, 80.0)	73.0 (66.0, 80.2)	0.882
Pulse rate (beats/min)	78.0 (70.0, 85.0)	79.0 (71.0, 87.0)	76.0 (69.0, 84.0)	0.001
ALT (U/L)	17.0 (11.0, 24.0)	16.0 (11.0, 24.0)	17.0 (11.0, 24.0)	0.769
Total bilirubin (μmol/L)	12.6 (9.9, 16.4)	12.6 (9.9, 17.0)	12.6 (9.9, 16.1)	0.708
Serum total protein (g/L)	70.2 (67.9, 72.8)	70.4 (68.0, 72.9)	70.1 (67.7, 72.5)	0.409

Serum albumin (g/L)	43.9 (42.3, 45.6)	43.9 (42.3, 45.7)	43.8 (42.4, 45.5)	0.763
Serum globulin (g/L)	26.3 (24.4, 28.2)	26.4 (24.4, 28.2)	26.1 (24.3, 28.1)	0.537
Albumin-to-globulin ratio	1.7 (1.6, 1.8)	1.7 (1.6, 1.8)	1.7 (1.6, 1.8)	0.766
Urea nitrogen (mmol/L)	5.4 (4.6, 6.4)	5.1 (4.4, 6.1)	5.6 (4.9, 6.6)	<0.001
Creatinine (μmol/L)	68.0 (57.0, 81.0)	68.0 (57.0, 82.0)	67.5 (58.0, 79.0)	0.945
Uric acid (μmol/L)	322.0 (263.0, 379.0)	327.0 (260.0, 390.0)	316.0 (264.0, 371.2)	0.157
Fasting glucose (mmol/L)	5.0 (4.7, 5.5)	5.0 (4.6, 5.3)	5.1 (4.8, 5.6)	0.002
Total cholesterol (mmol/L)	4.9 (4.3, 5.6)	4.9 (4.3, 5.6)	4.9 (4.3, 5.5)	0.662
Triglycerides (mmol/L)	1.3 (0.9, 1.9)	1.3 (0.9, 2.0)	1.2 (0.8, 1.7)	0.002
HDL-C (mmol/L)	1.4 (1.2, 1.6)	1.4 (1.2, 1.6)	1.5 (1.3, 1.7)	<0.001
LDL-C (mmol/L)	2.8 (2.2, 3.4)	2.8 (2.2, 3.4)	2.8 (2.2, 3.3)	0.692
eGFR (mL/min/1.73m²)	101.5 (92.4, 112.6)	104.1 (94.3, 114.3)	99.7 (90.2, 110.0)	<0.001

Values are median (interquartile range) for continuous variables and n (%) for categorical variables.

Vitamin D deficiency defined as 25(OH)D < 20 ng/mL. P-values from Wilcoxon rank-sum test (continuous) or chi-square test (categorical); Fisher's exact test was used when any expected cell count was < 5.

25(OH)D is the variable used to define the groups; its between-group difference is definitional.

Season was derived from the examination date (spring: March–May; summer: June–August; autumn: September–November; winter: December–February); no participants were examined during autumn. Missing values were excluded from the corresponding analyses (waist and hip circumference, n = 3 each, comprising two blank records and one entry recorded as "/"; fasting glucose, n = 1).

Abbreviations: TMAO, trimethylamine N-oxide; VD, vitamin D; BMI, body mass index; BP, blood pressure; ALT, alanine aminotransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate.

3.2. Association of plasma TMAO with 25(OH)D

In multivariable linear regression, plasma TMAO was positively associated with 25(OH)D (Table 2). In unadjusted analysis, each 1-SD increment in logTMAO was associated with a 2.53 ng/mL higher 25(OH)D (95% CI, 1.63 – 3.43; $P < 0.001$); the estimate decreased to 2.07 ng/mL (1.16 – 2.98) after adjustment for age and sex (model 1) and was 2.18 ng/mL (1.29 – 3.07; $P < 0.001$) after further adjustment for BMI and season (model 2). In quartile analysis, the highest (Q4) vs lowest (Q1) TMAO quartile differed by 4.56 ng/mL (95% CI, 2.05 – 7.07; $P < 0.001$; P for trend < 0.001). Point estimates increased in a dose – response pattern across quartiles (Q2, Q3, and Q4 vs Q1: −0.03, 0.57, and 4.56 ng/mL), with the difference concentrated at Q4. Estimates remained stable across the cumulative adjustment ladder M1 – M5 (β per 1-unit increment in logTMAO, 3.21 – 3.97; all $P < 0.001$; model R^2 increasing from 0.047 to 0.144; Supplementary Table S1).

Table 2. Linear regression of serum 25(OH)D (ng/mL) on plasma TMAO, by TMAO quartile and per 1-SD increment in logTMAO.

Model	Q1	Q2	Q3	Q4	*P* for trend	Per 1-SD	*P*
Unadjusted	Ref	0.23 (-2.34, 2.79)	1.69 (-0.88, 4.26)	5.27 (2.71, 7.84)	<0.001	2.53 (1.63, 3.43)	<0.001

Model 1	Ref	-0.08 (-2.58, 2.42)	0.45 (-2.12, 3.02)	4.12 (1.56, 6.69)	0.002	2.07 (1.16, 2.98)	<0.001
Model 2	Ref	-0.03 (-2.47, 2.41)	0.57 (-1.94, 3.08)	4.56 (2.05, 7.07)	<0.001	2.18 (1.29, 3.07)	<0.001

β (95% CI) = mean difference in 25(OH)D (ng/mL) with 95% confidence interval.

Q1 = lowest TMAO quartile (reference). P for trend from modeling TMAO quartile as a continuous ordinal variable (1-4).

Per 1-SD = per 1-SD increment in log-transformed TMAO.

Unadjusted: no covariates. Model 1: adjusted for age and sex. Model 2: further adjusted for BMI and season.

Abbreviations: TMAO, trimethylamine N-oxide; Q, quartile; SD, standard deviation; BMI, body mass index.

Consistent with the regression results, mean 25(OH)D across TMAO percentile bands increased from 20.1 ng/mL in the lowest decile (<P10) to 29.8 ng/mL in the highest (≥ P90) (*P* for trend < 0.001; Fig. 1A), with the corresponding vitamin D deficiency prevalence decreasing from 60.3% to 33.9% (Supplementary Table S2). Restricted cubic spline analysis suggested some departure from linearity (*P* for nonlinearity = 0.018), with predicted 25(OH)D remaining relatively flat at low TMAO concentrations and increasing progressively at higher concentrations, without a discrete threshold (Fig. 1B). In the reverse direction, median TMAO increased across 25(OH)D severity categories, from 138.2 ng/mL in severe deficiency to 208.7 ng/mL in sufficiency (*P* for trend < 0.001; Supplementary Table S3).

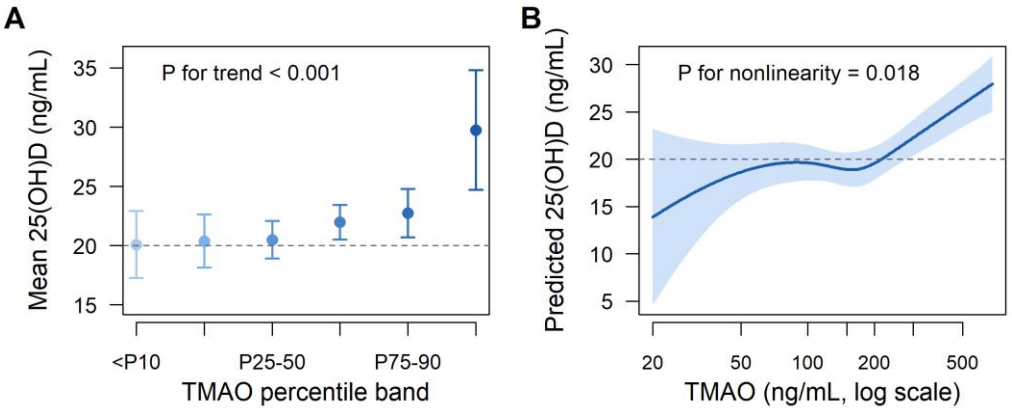


Figure 1. Dose–response association between plasma TMAO and 25(OH)D. (A) Mean 25(OH)D (ng/mL) with 95% CIs across six TMAO percentile bands (<P10, P10–25, P25–50, P50–75, P75–90, ≥P90); P for trend from unadjusted linear regression of 25(OH)D on the ordinal band index. (B) Restricted cubic spline (4 degrees of freedom; 3 interior knots) prediction of 25(OH)D on logTMAO adjusted for age, sex, BMI, and season; shaded areas are 95% CIs; P for nonlinearity from an F test against the linear model. In both panels, the dashed line marks the vitamin D deficiency threshold (20 ng/mL); in (B), TMAO is displayed on the log scale (ng/mL).

3.3. Effect modification by BMI

The TMAO–25(OH)D association was modified by BMI (*P* for interaction = 0.018, 1 df, continuous). In stratified analyses adjusted for age, sex, and season, the association was positive in normal-weight (β = 3.85 ng/mL per 1-unit increment in logTMAO; 95% CI, 1.69–6.01; $P < 0.001$; n = 332) and overweight (β = 3.01; 1.10–4.93; $P = 0.002$; n = 218) participants but was attenuated, with a reversed point estimate, among participants with obesity (β = −1.34; 95% CI, −5.50 to 2.83; $P = 0.524$; n = 71; Fig. 2A). The three-category parameterization was not statistically significant ($P = 0.146$, 2 df), likely owing to the limited sample size of the obesity subgroup. In the pooled spline model,

the conditional slope of 25(OH)D on logTMAO decreased monotonically with increasing BMI. The slope crossed zero at a BMI of approximately 28.4 kg/m², close to the Chinese obesity cutoff of 28 kg/m², and was negative at higher BMI values (Fig. 2B).

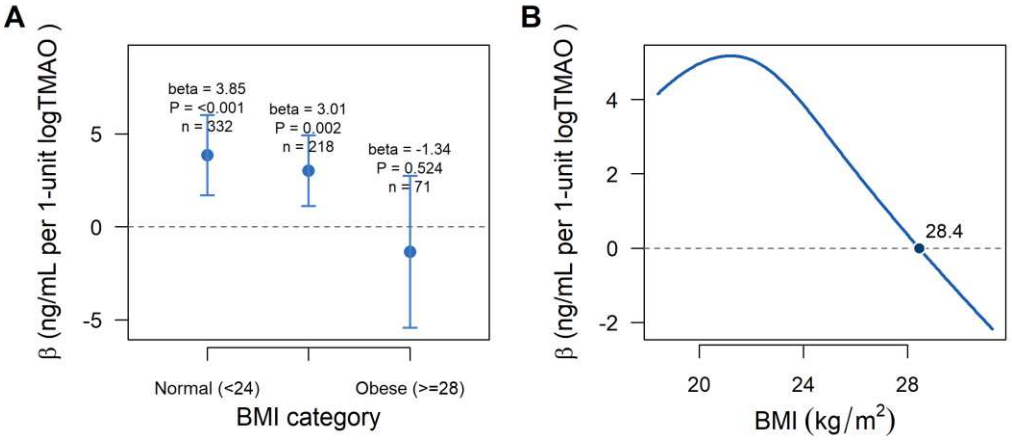


Figure 2. BMI effect modification of the TMAO–25(OH)D association. (A) Stratum-specific β (ng/mL) per 1-unit increment in logTMAO (95% CI) by Chinese BMI categories (normal weight, <24 kg/m², n = 332; overweight, 24–<28, n = 218; obesity, ≥28 kg/m², n = 71). (B) Restricted cubic spline (3 degrees of freedom) of the conditional slope of 25(OH)D on logTMAO as a continuous function of BMI (2nd–98th percentile range); the zero line marks no association. All estimates are adjusted for age, sex, and season.

3.4. Sensitivity analyses

The direction and statistical significance of the association were robust in prespecified sensitivity analyses (Table 3). The estimate strengthened after exclusion of participants with obesity (β per 1-SD increment = 2.40, consistent with the BMI modification in Section 3.3; $P < 0.001$); remained positive and statistically significant, although attenuated (β = 1.44; $P < 0.001$), after exclusion of the 27 high-leverage observations

identified by Cook distance (>4/n); and was essentially unchanged after exclusion of participants with eGFR < 60 mL/min/1.73 m² (n = 9; β = 2.19; $P < 0.001$). All point estimates retained the same direction and nominal significance. Similarly, excluding 18 participants with 25(OH)D >50 ng/mL (a range suggestive of vitamin D supplement use) attenuated the estimate to β = 1.18 (95% CI, 0.52–1.84) per 1-SD increment, without altering the direction or significance of the association.

Table 3. Sensitivity analyses: adjusted association of 25(OH)D with TMAO after prespecified and post hoc exclusions.

Scenario	n	Q4−Q1 β	β (95% CI) per 1-SD increment	*P* for trend
Main (n=621)	621	4.56 (2.05, 7.07)	2.18 (1.29, 3.07)	*P* < 0.001
Exclude obese (BMI≥28)	550	5.71 (2.98, 8.44)	2.40 (1.45, 3.34)	*P* < 0.001
Exclude Cook's distance >4/n (n=594)	594	3.24 (1.44, 5.04)	1.44 (0.75, 2.13)	*P* < 0.001
Exclude eGFR<60	612	4.65 (2.12, 7.18)	2.19 (1.29, 3.10)	*P* < 0.001
Excluding 25(OH)D >50 ng/mL (post hoc; n = 18)	603	3.23 (1.40, 5.06)	1.18 (0.52, 1.84)	*P* < 0.001

Linear regression adjusted for age, sex, BMI and season.

Q4-Q1 β = adjusted mean 25(OH)D difference (ng/mL) between the highest and lowest TMAO quartiles. Per 1-SD = per 1-SD increment in log-transformed TMAO. *P* for trend from the ordinal quartile term, as in Table 2.

Cook's distance threshold = 4/n. eGFR computed by 2021 CKD-EPI race-free equation. The exclusion of 25(OH)D >50 ng/mL was post hoc.

Abbreviations: TMAO, trimethylamine N-oxide; eGFR, estimated glomerular filtration rate; BMI, body mass index.

4. Discussion

4.1. Principal findings

In this cross-sectional study of 621 Chinese adults undergoing health examinations, lower plasma TMAO was associated with lower circulating 25(OH)D independent of age, sex, BMI, and season: each 1-SD increment in logTMAO corresponded to a 2.18 ng/mL higher 25(OH)D, and mean 25(OH)D increased from 20.1 ng/mL in the lowest TMAO percentile band to 29.8 ng/mL in the highest. The association was nonlinear, modified by BMI, and remained positive and statistically significant across four sensitivity analyses, although attenuated in magnitude after exclusion of influential points and of probable supplement users ($\beta = 1.18$). To our knowledge, this is the first study of the TMAO–vitamin D association in a health examination cohort and the first to demonstrate that it is positive in non-obese adults.

4.2. Comparison with previous studies

The TMAO literature summarized in the Introduction has concentrated almost exclusively on high concentrations, with meta-analytic[18] and narrative[48] syntheses consolidating the cardio-renal-metabolic evidence. Few studies have addressed the low end of the distribution. Our finding that low TMAO tracks with low vitamin D adds a previously overlooked dimension, suggesting that both ends of the TMAO distribution may carry health relevance. The BMI-dependent directionality of our association also helps reconcile the inverse TMAO–25(OH)D association reported by Barrea et al. in Italian endocrinology outpatients[41], which did not persist after adjustment for the fatty liver index: in our data, normal-weight and overweight participants (together 89%

of the sample) showed a positive association, whereas the association was attenuated, with a reversed point estimate, in the obesity subgroup (n = 71).

This convergence across metabolically distinct populations argues against a simple contradiction and supports BMI modification of the TMAO–vitamin D axis that should be modeled rather than averaged over. Our larger sample size and prespecified formal interaction testing extend these preliminary observations.

4.3. A U-shaped TMAO − health hypothesis

More broadly, our findings motivate a U-shaped TMAO–health hypothesis. High circulating TMAO has been consistently linked to cardiovascular and renal harm[17,19,22,31], whereas the present study characterizes the other end of the distribution—the low end—as a correlate of vitamin D deficiency. This perspective was anticipated by early debate over whether TMAO is friend or foe and by experimental evidence that seafood-derived TMAO improved diuresis and survival in heart failure rats[49,50]. The two extremes of plasma TMAO may thus carry different health risks, with a presumed optimum in the intermediate range. This cross-domain U-shape must be distinguished from a U-shaped dose–response of TMAO with vitamin D itself: in our cohort the TMAO–25(OH)D association was positive and essentially monotonic (Fig. 1), and the two arms of the hypothesized U correspond to different health endpoints (vitamin D status vs cardio-renal outcomes) drawn from different populations. Direct evaluation of the U-shaped TMAO–health hypothesis will require prospective cohorts with concurrent measurement of vitamin D status and cardio-renal endpoints.

4.4. Possible mechanisms

Several non-exclusive mechanisms could underlie the low-TMAO–low-25(OH)D association. First, the dietary precursors of TMAO—choline, carnitine, and betaine—are abundant in fish, seafood, eggs, and red meat, and fish is also one of the few natural vitamin D sources[34]. Low TMAO concentrations may reflect a dietary pattern low in these foods, jointly limiting TMAO biosynthetic substrates and dietary vitamin D intake. Consistent with a shared-source pathway, median TMAO rose monotonically across 25(OH)D severity categories, from severe deficiency to sufficiency (138.2 to 208.7 ng/mL), and the 18 participants with 25(OH)D >50 ng/mL had nearly twice the cohort-average TMAO concentration (408.3 vs 217.6 ng/mL), a pattern compatible with marine-source supplementation or habitual fatty-fish intake. Second, TMAO production depends on gut microbiota composition, which is itself shaped by host vitamin D status through vitamin D receptor (VDR)-mediated and bile acid signaling pathways[51,52]; dysbiosis could thus couple low TMAO production with impaired vitamin D homeostasis, and vitamin D-dependent reshaping of the microbiome has demonstrated functional immune consequences in experimental models[53]. Consistent with a microbiome-mediated arm, probiotic interventions lower circulating TMAO in humans[54]. Third, both TMAO and 25(OH)D undergo hepatic and renal handling: circulating TMAO rises as kidney function declines[22,23], kidney function exerts a causal and modifiable influence on circulating TMAO[55], and shared metabolic determinants cannot be excluded as residual confounding. Obesity-related changes in vitamin D metabolism and bioavailability may contribute in parallel[56]. The

attenuation, with a reversed point estimate, in obesity may also reflect obesity-specific alterations in gut microbial ecology, an expanded bile acid pool, and a chronic inflammatory milieu that may decouple TMAO from its dietary sources[57]. The reported associations between TMAO and nonalcoholic fatty liver disease[58,59] echo the covariate that abolished the association in the study of Barrea et al.[41].

4.5. Strengths and limitations

Strengths include the systematic, prespecified evaluation of nonlinearity, BMI-dependent effect modification, and directional robustness to influential-point and subgroup exclusions, as well as the use of a real-world health examination population reflective of routine vitamin D testing. Main limitations include the cross-sectional design, which precludes causal inference; single measurements of TMAO and 25(OH)D (TMAO reflects recent intake over hours to days, whereas 25(OH)D integrates exposure over weeks to months, so single measurements would tend to attenuate rather than inflate the association); the absence of data on sun exposure, dietary intake, and vitamin D supplementation, although excluding the 18 participants with 25(OH)D >50 ng/mL (probable supplement users) attenuated the adjusted estimate to 1.18 ng/mL per 1-SD (95% CI, 0.52–1.84) without abolishing significance; unrecorded medication information (including lipid-lowering, antihypertensive, and glucose-lowering agents), which may confound the TMAO–25(OH)D association; and the single-center, Chinese composition of the cohort, which limits generalizability. Sampling was concentrated in spring (60.7%), followed by summer (22.5%) and winter (16.7%), with no autumn representation; however, the TMAO–25(OH)D association

was essentially unchanged with and without season adjustment (β 2.18 vs 2.11). The BMI modification finding rests on the continuous-BMI interaction test and a small obesity subgroup (n = 71), with the categorical interaction not reaching significance (interaction tests require approximately four-fold the sample size of main effects, and the minimum detectable interaction in our data, $|\beta| = 0.42$ per kg/m², exceeded the observed −0.36), and should be considered hypothesis-generating. Residual and unmeasured confounding cannot be excluded.

5. Conclusions

In Chinese adults undergoing health examinations, lower plasma TMAO was associated with lower circulating 25(OH)D independent of age, sex, BMI, and season, and with a higher prevalence of vitamin D deficiency; the association was nonlinear and modified by BMI. These findings extend the clinical relevance of TMAO from its established harms at high concentrations to the lower end of the distribution. These data, together with the cardio-renal harms of high TMAO established in other populations, motivate a U-shaped TMAO–health hypothesis, whereby the two ends of the TMAO distribution carry distinct risks. This hypothesis warrants evaluation in prospective cohorts, alongside in-depth investigation of the low-TMAO phenotype, its dietary and microbial determinants, and its implications for skeletal and cardiometabolic health.

Data availability

Subject to relevant ethical and regulatory approvals, the de-identified data underlying the results of this article are available from the corresponding author upon reasonable request.

Authors' contributions

N.H.X. conceived and designed the study, and performed the analysis and interpretation of the results. Y.L. contributed to the drafting of the manuscript and the empirical work. Y.H.L and P.T.Y. contributed to data collection. X.J.D. contributed to data curation. J.G.W. had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Declaration of competing interest

The authors declare no conflicts of interest.

References

[1] Cui A, Zhang T, Xiao P, Fan Z, Wang H, Zhuang Y. Global and regional prevalence of vitamin D deficiency in population-based studies from 2000 to 2022: A pooled analysis of 7.9 million participants. Front Nutr 2023;10:1070808. https://doi.org/10.3389/fnut.2023.1070808.

[2] Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266–81. https://doi.org/10.1056/NEJMra070553.

[3] Chevalley T, Brandi ML, Cashman KD, Cavalier E, Harvey NC, Maggi S, et al. Role of vitamin D supplementation in the management of musculoskeletal diseases: update from an European Society of Clinical and Economical Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) working group. Aging Clin Exp

Res 2022;34:2603–23. https://doi.org/10.1007/s40520-022-02279-6.

[4] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911–30. https://doi.org/10.1210/jc.2011-0385.

[5] Jayedi A, Daneshvar M, Jibril AT, Sluyter JD, Waterhouse M, Romero BD, et al. Serum 25(OH)D Concentration, Vitamin D Supplementation, and Risk of Cardiovascular Disease and Mortality in Patients with Type 2 Diabetes or Prediabetes: a Systematic Review and Dose-Response Meta-Analysis. Am J Clin Nutr 2023;118:697–707. https://doi.org/10.1016/j.ajcnut.2023.07.012.

[6] Luo W, Xu D, Zhang J, Zhou Y, Yang Q, Lv Q, et al. Low serum 25-hydroxyvitamin D levels are associated with increased cardiovascular morbidity and mortality. Postgrad Med 2023;135:93–101. https://doi.org/10.1080/00325481.2022.2161250.

[7] Ismailova A, White JH. Vitamin D, infections and immunity. Rev Endocr Metab Disord 2022;23:265–77. https://doi.org/10.1007/s11154-021-09679-5.

[8] Bikle DD. Vitamin D Regulation of Immune Function. Curr Osteoporos Rep 2022;20:186–93. https://doi.org/10.1007/s11914-022-00732-z.

[9] Zhang Y, Li Y, Liu J, Wei X, Tan N, Zhang J, et al. Association of Vitamin D or Calcium Supplementation with Cardiovascular Outcomes and Mortality: A Meta-Analysis with Trial Sequential Analysis. J Nutr Health Aging 2021;25:263–70.

444 https://doi.org/10.1007/s12603-020-1551-9.

445 [10] Ruiz-García A, Pallarés-Carratalá V, Turégano-Yedro M, Torres F, Sapena V,
446 Martin-Gorgojo A, et al. Vitamin D Supplementation and Its Impact on Mortality and
447 Cardiovascular Outcomes: Systematic Review and Meta-Analysis of 80 Randomized
448 Clinical Trials. Nutrients 2023;15:1810. https://doi.org/10.3390/nu15081810.

449 [11] Liu W, Hu J, Fang Y, Wang P, Lu Y, Shen N. Vitamin D status in Mainland of
450 China: A systematic review and meta-analysis. eClinicalMedicine 2021;38:101017.
451 https://doi.org/10.1016/j.eclinm.2021.101017.

452 [12] Chan Y, Cai D, Guo R, Zhou X, He G, Li H, et al. Evaluation of the deficiency
453 status of 25-hydroxyvitamin D and associated factors in Southwest China: A hospital-
454 based retrospective cross-sectional analysis of a low-latitude, high-altitude, multiracial
455 region. Nutr Bull 2023;48:535–45. https://doi.org/10.1111/nbu.12645.

456 [13] Giustina A, Bilezikian JP, Adler RA, Banfi G, Bikle DD, Binkley NC, et al.
457 Consensus Statement on Vitamin D Status Assessment and Supplementation: Whys,
458 Whens, and Hows. Endocr Rev 2024;45:625–54.
459 https://doi.org/10.1210/endrev/bnae009.

460 [14] Demay MB, Pittas AG, Bikle DD, Diab DL, Kiely ME, Lazaretti-Castro M, et al.
461 Vitamin D for the Prevention of Disease: An Endocrine Society Clinical Practice
462 Guideline. J Clin Endocrinol Metab 2024;109:1907–47.
463 https://doi.org/10.1210/clinem/dgae290.

464 [15] Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora

metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57–63. https://doi.org/10.1038/nature09922.

[16] Canyelles M, Borràs C, Rotllan N, Tondo M, Escolà-Gil JC, Blanco-Vaca F. Gut Microbiota-Derived TMAO: A Causal Factor Promoting Atherosclerotic Cardiovascular Disease? Int J Mol Sci 2023;24:1940. https://doi.org/10.3390/ijms24031940.

[17] Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 2013;368:1575–84. https://doi.org/10.1056/NEJMoa1109400.

[18] Li D, Lu Y, Yuan S, Cai X, He Y, Chen J, et al. Gut microbiota-derived metabolite trimethylamine-N-oxide and multiple health outcomes: an umbrella review and updated meta-analysis. Am J Clin Nutr 2022;116:230–43. https://doi.org/10.1093/ajcn/nqac074.

[19] Tang WH, Wang Z, Fan Y, Levison B, Hazen JE, Donahue LM, et al. 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. https://doi.org/10.1016/j.jacc.2014.02.617.

[20] Tang WHW, Lemaitre RN, Jensen PN, Wang M, Wang Z, Li XS, et al. Trimethylamine N-Oxide and Related Gut Microbe-Derived Metabolites and Incident Heart Failure Development in Community-Based Populations. Circ Heart Fail 2024;17:e011569. https://doi.org/10.1161/CIRCHEARTFAILURE.124.011569.

[21] Crisci G, Israr MZ, Cittadini A, Bossone E, Suzuki T, Salzano A. Heart failure and trimethylamine N-oxide: time to transform a “gut feeling” in a fact? ESC Heart Fail

2023;10:1–7. https://doi.org/10.1002/ehf2.14205.

[22] Tang WH, Wang Z, Kennedy DJ, Wu Y, Buffa JA, Agatisa-Boyle B, et al. 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. https://doi.org/10.1161/CIRCRESAHA.116.305360.

[23] Zeng Y, Guo M, Fang X, Teng F, Tan X, Li X, et al. Gut Microbiota-Derived Trimethylamine N-Oxide and Kidney Function: A Systematic Review and Meta-Analysis. Adv Nutr 2021;12:1286–304. https://doi.org/10.1093/advances/nmab010.

[24] Wang M, Tang WHW, Li XS, de Oliveira Otto MC, Lee Y, Lemaitre RN, et al. The Gut Microbial Metabolite Trimethylamine N -oxide, Incident CKD, and Kidney Function Decline. J Am Soc Nephrol 2024;35:749–60. https://doi.org/10.1681/ASN.0000000000000344.

[25] Shan Z, Sun T, Huang H, Chen S, Chen L, Luo C, et al. Association between microbiota-dependent metabolite trimethylamine-N-oxide and type 2 diabetes. Am J Clin Nutr 2017;106:888–94. https://doi.org/10.3945/ajcn.117.157107.

[26] Mohammadi S, Eslami M, Pourghazi F, Ejtahed HS, Shahrestanaki E, Qorbani M, et al. Gut Microbiota-Derived Trimethylamine N-Oxide and the Risk of Diabetes: An Updated Systematic Review and Meta-Analysis. Obes Rev 2025;26:e13963. https://doi.org/10.1111/obr.13963.

[27] Yu PS, Wu PH, Hung WW, Lin MY, Zhen YY, Hung WC, et al. Association Between Trimethylamine N-oxide and Adverse Kidney Outcomes and Overall

Mortality in Type 2 Diabetes Mellitus. J Clin Endocrinol Metab 2024;109:2097–105. https://doi.org/10.1210/clinem/dgae009.

[28] Chen G, He L, Dou X, Liu T. Association of Trimethylamine-N-Oxide Levels with Risk of Cardiovascular Disease and Mortality among Elderly Subjects: A Systematic Review and Meta-Analysis. Cardiorenal Med 2022;12:39–54. https://doi.org/10.1159/000520910.

[29] Wang M, Peng H, Yang R, Yu H, Hou T, Wu Y, et al. Serum trimethylamine N-oxide and long-term mortality risk in rural northern China: a family-based cohort study. Br J Nutr 2025;134:462–9. https://doi.org/10.1017/S0007114525104224.

[30] Senthong V, Kiatchoosakun S, Wongvipaporn C, Phetcharaburanin J, Sritara P, Phrommintikul A, et al. Trimethylamine-N-oxide and 5-year mortality: the role of gut microbiota-generated metabolite from the CORE-Thailand cohort. Sci Rep 2024;14:21264. https://doi.org/10.1038/s41598-024-71479-z.

[31] Li XS, Wang M, Wang Z, Fan B, Tian MY, Filippazzo J, et al. Gut microbe-generated metabolite trimethylamine N-oxide and risk of abdominal aortic aneurysm: a cohort study. Eur Heart J 2026. https://doi.org/10.1093/eurheartj/ehag489.

[32] Han JM, Guo L, Chen XH, Xie Q, Song XY, Ma YL. Relationship between trimethylamine N-oxide and the risk of hypertension in patients with cardiovascular disease: A meta-analysis and dose-response relationship analysis. Medicine (Baltimore) 2024;103:e36784. https://doi.org/10.1097/MD.0000000000036784.

[33] Chen P, Guo Z. Plasmatic trimethylamine N-oxide and its relation to stroke: A

528 systematic review and dose-response meta-analysis. Medicine (Baltimore)
529 2022;101:e29512. https://doi.org/10.1097/MD.0000000000029512.

530 [34] Li X, Wang C, Yanagita T, Xue C, Zhang T, Wang Y. Trimethylamine N-Oxide in
531 Aquatic Foods. J Agric Food Chem 2024;72:14498–520.
532 https://doi.org/10.1021/acs.jafc.4c01974.

533 [35] Wang Z, Tang WHW, O'Connell T, Garcia E, Jeyarajah EJ, Li XS, et al.
534 Circulating trimethylamine N-oxide levels following fish or seafood consumption. Eur
535 J Nutr 2022;61:2357–64. https://doi.org/10.1007/s00394-022-02803-4.

536 [36] Lu Z, Chen TC, Zhang A, Persons KS, Kohn N, Berkowitz R, et al. An evaluation
537 of the vitamin D3 content in fish: Is the vitamin D content adequate to satisfy the
538 dietary requirement for vitamin D? J Steroid Biochem Mol Biol 2007;103:642–4.
539 https://doi.org/10.1016/j.jsbmb.2006.12.010.

540 [37] James KL, Gertz ER, Cervantes E, Bonnel EL, Stephensen CB, Kable ME, et al.
541 Diet, Fecal Microbiome, and Trimethylamine N-Oxide in a Cohort of Metabolically
542 Healthy United States Adults. Nutrients 2022;14:1376.
543 https://doi.org/10.3390/nu14071376.

544 [38] Obeid R, Awwad HM, Kirsch SH, Waldura C, Herrmann W, Graeber S, et al.
545 Plasma trimethylamine-N-oxide following supplementation with vitamin D or D plus B
546 vitamins. Mol Nutr Food Res 2017;61:1600358.
547 https://doi.org/10.1002/mnfr.201600358.

548 [39] Ozorowski M, Wiciński M, Wróbel Ł, Fajkiel-Madajczyk A. Cholecalciferol

549 supplementation lowers leptin and TMAO but increases NO and VEGF-A levels in
550 obese vitamin D deficient patients: Is it one of the potential cardioprotective
551 mechanisms of vitamin D? Nutr Metab (Lond) 2022;19:31.
552 https://doi.org/10.1186/s12986-022-00666-4.

553 [40] Wang X, Li X, Dong Y. Vitamin D Decreases Plasma Trimethylamine-N-oxide
554 Level in Mice by Regulating Gut Microbiota. Biomed Res Int 2020;2020:9896743.
555 https://doi.org/10.1155/2020/9896743.

556 [41] Barrea L, Muscogiuri G, Annunziata G, Laudisio D, de Alteriis G, Tenore GC, et
557 al. A New Light on Vitamin D in Obesity: A Novel Association with Trimethylamine-
558 N-Oxide (TMAO). Nutrients 2019;11:1310. https://doi.org/10.3390/nu11061310.

559 [42] Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New
560 Creatinine- and Cystatin C-Based Equations to Estimate GFR without Race. N Engl J
561 Med 2021;385:1737–49. https://doi.org/10.1056/NEJMoa2102953.

562 [43] Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous
563 synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not
564 promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab 1988;67:373–8.
565 https://doi.org/10.1210/jcem-67-2-373.

566 [44] Wang C, Li H, Huo L, Wang Q, Zhang T, He X, et al. Serum 25-Hydroxyvitamin
567 D Levels in Type 2 Diabetes Patients in North China: Seasonality and the Association
568 between Vitamin D Status and Glycosylated Hemoglobin Levels. Int J Clin Pract
569 2023;2023:4151224. https://doi.org/10.1155/2023/4151224.

570 [45] Wang X, Lu K, Shen J, Xu S, Wang Q, Gong Y, et al. Correlation between
571 meteorological factors and vitamin D status under different season. Sci Rep
572 2023;13:4762. https://doi.org/10.1038/s41598-023-31698-2.

573 [46] Zhou BF, Cooperative Meta-Analysis Group of the Working Group on Obesity in
574 China. Predictive values of body mass index and waist circumference for risk factors of
575 certain related diseases in Chinese adults--study on optimal cut-off points of body mass
576 index and waist circumference in Chinese adults. Biomed Environ Sci 2002;15:83–96.

577 [47] Knol MJ, VanderWeele TJ. Recommendations for presenting analyses of effect
578 modification and interaction. Int J Epidemiol 2012;41:514–20.
579 https://doi.org/10.1093/ije/dyr218.

580 [48] Kim SH, Yoon MY, Yoon SS. TMAO and the gut microbiome: implications for the
581 CVD-CKD-IBD axis. Ann Med 2025;57:2522324.
582 https://doi.org/10.1080/07853890.2025.2522324.

583 [49] Cho CE, Caudill MA. Trimethylamine-N-Oxide: Friend, Foe, or Simply Caught in
584 the Cross-Fire? Trends Endocrinol Metab 2017;28:121–30.
585 https://doi.org/10.1016/j.tem.2016.10.005.

586 [50] Gawrys-Kopczynska M, Konop M, Maksymiuk K, Kraszewska K, Derzsi L,
587 Sozanski K, et al. TMAO, a seafood-derived molecule, produces diuresis and reduces
588 mortality in heart failure rats. eLife 2020;9:e57028.
589 https://doi.org/10.7554/eLife.57028.

590 [51] Bellerba F, Muzio V, Gnagnarella P, Facciotti F, Chiocca S, Bossi P, et al. The

591 Association between Vitamin D and Gut Microbiota: A Systematic Review of Human
592 Studies. Nutrients 2021;13:3378. https://doi.org/10.3390/nu13103378.

593 [52] Sukik A, Alalwani J, Ganji V. Vitamin D, Gut Microbiota, and Cardiometabolic
594 Diseases-A Possible Three-Way Axis. Int J Mol Sci 2023;24:940.
595 https://doi.org/10.3390/ijms24020940.

596 [53] Giampazolias E, Pereira da Costa M, Lam KC, Lim KHJ, Cardoso A, Piot C, et al.
597 Vitamin D regulates microbiome-dependent cancer immunity. Science 2024;384:428–
598 37. https://doi.org/10.1126/science.adh7954.

599 [54] Sohouli MH, Ozovanu OD, Fatahi S, Hekmatdoost A. Impact of probiotic
600 supplementation on trimethylamine N-oxide (TMAO) in humans: A systematic review
601 and meta-analysis of randomized controlled trials. Clin Nutr ESPEN 2022;50:56–62.
602 https://doi.org/10.1016/j.clnesp.2022.06.006.

603 [55] Andrikopoulos P, Aron-Wisnewsky J, Chakaroun R, Myridakis A, Forslund SK,
604 Nielsen T, et al. Evidence of a causal and modifiable relationship between kidney
605 function and circulating trimethylamine N-oxide. Nat Commun 2023;14:5843.
606 https://doi.org/10.1038/s41467-023-39824-4.

607 [56] Karampela I, Sakelliou A, Vallianou N, Christodoulatos GS, Magkos F, Dalamaga
608 M. Vitamin D and Obesity: Current Evidence and Controversies. Curr Obes Rep
609 2021;10:162–80. https://doi.org/10.1007/s13679-021-00433-1.

610 [57] Zheng X, Huang Y, Yang M, Jin L, Zhang X, Zhang R, et al. Vitamin D is involved
611 in the effects of the intestinal flora and its related metabolite TMAO on perirenal fat

612 and kidneys in mice with DKD. Nutr Diabetes 2024;14:42.
613 https://doi.org/10.1038/s41387-024-00297-z.

614 [58] Theofilis P, Vordoni A, Kalaitzidis RG. Trimethylamine N-Oxide Levels in Non-
615 Alcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. Metabolites
616 2022;12:1243. https://doi.org/10.3390/metabo12121243.

617 [59] Ma R, Shi G, Li Y, Shi H. Trimethylamine N-oxide, choline and its metabolites are
618 associated with the risk of non-alcoholic fatty liver disease. Br J Nutr 2024;131:1915–
619 23. https://doi.org/10.1017/S0007114524000631.

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