Circulating trimethylamine *N*-oxide in association with diet and cardiometabolic biomarkers: an international pooled analysis

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ABSTRACT

Background: Trimethylamine *N*-oxide (TMAO), a diet-derived, gut microbial-host cometabolite, has been linked to cardiometabolic diseases. However, the relations remain unclear between diet, TMAO, and cardiometabolic health in general populations from different regions and ethnicities.

Objectives: To examine associations of circulating TMAO with dietary and cardiometabolic factors in a pooled analysis of 16 population-based studies from the United States, Europe, and Asia.

Methods: Included were 32,166 adults (16,269 white, 13,293 Asian, 1247 Hispanic/Latino, 1236 black, and 121 others) without cardiovascular disease, cancer, chronic kidney disease, or inflammatory bowel disease. Linear regression coefficients (β) were computed for standardized TMAO with harmonized variables. Study-specific results were combined by random-effects meta-analysis. A false discovery rate <0.10 was considered significant.

Results: After adjustment for potential confounders, circulating TMAO was associated with intakes of animal protein and saturated fat (β = 0.124 and 0.058, respectively, for a 5% energy increase) and with shellfish, total fish, eggs, and red meat (β = 0.370, 0.151, 0.081, and 0.056, respectively, for a 1 serving/d increase). Plant protein and nuts showed inverse associations (β = −0.126 for a 5% energy increase from plant protein and −0.123 for a 1 serving/d increase of nuts). Although the animal protein–TMAO association was consistent across populations, fish and shellfish associations were stronger in Asians (β = 0.285 and 0.578), and egg and red meat associations were more prominent in Americans (β = 0.153 and 0.093). Besides, circulating TMAO was positively associated with creatinine (β = 0.131 SD increase in log-TMAO), homocysteine (β = 0.065), insulin (β = 0.048), glycated hemoglobin (β = 0.048), and glucose (β = 0.023), whereas it was inversely associated with HDL cholesterol (β = −0.047) and blood pressure (β = −0.030). Each TMAO-biomarker association remained significant after further adjusting for creatinine and was robust in subgroup/sensitivity analyses.

Conclusions: In an international, consortium-based study, animal protein was consistently associated with increased circulating TMAO, whereas TMAO associations with fish, shellfish, eggs, and red meat varied among populations. The adverse associations of TMAO with certain cardiometabolic biomarkers, independent of renal function, warrant further investigation. *Am J Clin Nutr* 2021;113:1145–1156.

Keywords: trimethylamine *N*-oxide, diet, biomarker, cardiovascular disease, Consortium of Metabolomics Studies

Introduction

The gut microbiota and its metabolites are increasingly recognized as playing important roles in human nutrition and

cardiometabolic health (1, 2). Particularly, trimethylamine *N*-oxide (TMAO), a gut microbial-host cometabolite of dietary choline and carnitine, has received much attention (3, 4). Epidemiological studies have linked high circulating TMAO concentrations to multiple cardiometabolic diseases, including diabetes, cardiovascular disease (CVD), and chronic kidney disease (CKD) (4–7). Animal studies have shown that increasing TMAO promotes thrombosis, atherosclerosis, renal damage, and metabolic dysfunction (3–5, 8–10), whereas inhibiting TMAO reduces the formation of atherosclerotic lesions and improves glucose tolerance (8, 10, 11). TMAO has thus been proposed as a possible link between high intakes of dietary choline/carnitine or foods high in those nutrients (e.g., red meat and eggs) and poor cardiometabolic health (2, 3, 5, 12).

However, despite the existing evidence from animal models and epidemiological studies (mostly in patients or populations at high risk of CVD), the relations between diet, TMAO, and cardiometabolic health in general populations remain unclear (6, 13). The uncertainty could be due to variations in habitual diets that determine the TMAO precursors and gut microbial profiles, and also nondietary factors that might modulate TMAO concentrations and its cardiometabolic effects. Dietary sources of TMAO vary across populations. In typical Western diets, choline and carnitine mostly come from red meat, poultry, eggs, and dairy (14–16). Yet, choline is also abundant in fish, shellfish, legumes, and nuts (17), and TMAO is naturally abundant in fish and shellfish (no microbial metabolism required) (13). Studies have suggested that TMAO concentrations are associated with dairy and/or fish intakes in some European and Asian populations, rather than red meat in US populations (18–22). Studies have also reported associations of TMAO with choline from animal foods but not choline from plant foods, and with low adherence to the plant-based Mediterranean diet (23, 24), indicating that differences in habitual diets can result in variations in the food-TMAO associations. Besides dietary factors, increased TMAO has been linked to older age, male sex, obesity, dyslipidemia, inflammation, renal impairment, and history of diabetes, CVD, and CKD (18, 25–33), although those

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The data and materials that support the findings of this study are available upon request and committee approval.

Supplemental Tables 1–14 and Supplemental Figures 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: Airwave, Airwave Health Monitoring Study; CKD, chronic kidney disease; COMETS, COnsortium of METabolomics Studies; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FDR, false discovery rate; FHS, Framingham Heart Study; HbA1c, glycated hemoglobin; ICL-NPC, Imperial College London's National Phenome Centre; MESA, Multi-Ethnic Study of Atherosclerosis; NAFLD, nonalcoholic fatty liver disease; NHS, Nurses' Health Study; SMHS, Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study; TMAO, trimethylamine *N*-oxide; TMCS, Tsuruoka Metabolomics Cohort Study; WHR, waist-to-hip ratio.

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associations remain inconsistent and mostly unexplored in general populations. Collaborative epidemiological studies involving demographically diverse populations with different habitual diets and characteristics can further clarify the associations between diet and TMAO and cardiometabolic diseases.

To this end, we conducted an international pooled analysis to examine associations of circulating TMAO with demographics, diet, lifestyle, and cardiometabolic diseases and biomarkers in >32,000 participants from 16 studies conducted in the United States, Europe, and Asia.

Methods

Study populations and variables

This project is based on the COnsortium of METabolomics Studies (COMETS). Details of the design and studies within the COMETS have been described (34). Additionally, we reached out to studies not part of COMETS but with available blood TMAO data. A total of 16 studies joined the "TMAO Pooling Project," by alphabetical order, including 9 in the United States: the Coronary Artery Risk Development in Young Adults Study (CARDIA) (35), Framingham Heart Study (FHS) (36), Health Professionals Follow-Up Study (37), Insulin Resistance Atherosclerosis Family Study (38), Multi-Ethnic Study of Atherosclerosis (MESA) (39), Nurses' Health Study (NHS) (40), Nurses' Health Study II (NHS2) (40), Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (41), and Women's Health Initiative; 3 in Europe: the Airwave Health Monitoring Study (Airwave) (42), Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (43), and TwinsUK Registry (44); and 4 in Asia: the Guangzhou Nutrition and Health Study (45), Shanghai Men's Health Study (SMHS) (46), Shanghai Women's Health Study (SWHS) (47), and Tsuruoka Metabolomics Cohort Study (TMCS) (48). The TMAO Pooling Project was approved by the COMETS steering committee, committees of the participating studies, and the Institutional Review Board of Vanderbilt University Medical Center.

Participants with available data on blood TMAO, age (>18 y), and sex were included. To minimize the influence of certain diseases and their treatments on TMAO concentration, we excluded participants if they had a self-reported history of cancer (except nonmelanoma skin cancer), coronary artery disease, stroke, heart failure, inflammatory bowel disease, or CKD [self-reported diagnosis or if without the information, a 1-time estimated glomerular filtration rate (eGFR) <45 mL/min/1.73 m^2]. Moreover, participants above the study- and sex-specific 99th percentile of the TMAO concentration were excluded to minimize the influence of extreme values on linear regression results. Our final dataset included 32,166 adults, comprised of 16,269 white, 13,293 Asian, 1247 Hispanic/Latino, 1236 black, and 121 others (**Supplemental Figure 1**).

Survey-based study variables

A broad range of data, including demographics, lifestyle, dietary habits, anthropometrics, and disease history, were collected in each study at blood sample collection. A "data dictionary" was developed to harmonize variables across studies, including age at blood draw, sex, race and ethnicity (self-reported), BMI, waist-to-hip ratio (WHR), smoking status, alcohol consumption, physical activity, menopausal status and hormone therapy in women only, and history of diabetes, hypertension, dyslipidemia, and nonalcoholic fatty liver disease (NAFLD), which was defined by self-reported physician diagnosis or current use of medications to treat those conditions.

Habitual food intakes were assessed in 14 studies using FFQs that captured commonly consumed foods in the study population (Airwave and TMCS did not provide dietary information and were therefore excluded from all diet-related analyses). Intakes of total energy and nutrients were calculated using the country/region-specific food composition tables. Intakes of foods and nutrients were all standardized to intakes per 2000 kcal/d (49). Macronutrients, for example, carbohydrate, protein, and fat, were modeled as per 5% energy increment. Dietary fiber was modeled as 5 g/d increase. Food intakes were harmonized based on portion sizes (i.e., per 1 serving/d increase); included were: red meat, processed meat, poultry, total fish, and shellfish [1 serving = 4 oz (113.4 g)]; eggs (50 g); dairy foods—milk/cottage cheese [8 fluid oz (240 g)], firm cheese (50 g), and ice cream (100 g); soy products—soy milk [8 fluid oz (240 g)] and tofu/soybeans/soy meats [4 oz (113.4 g)]; legumes (50 g in dry weight); nuts (30 g in dry weight); vegetables (80 g); fruits (80 g); and whole grains (50 g in dry weight).

Metabolites and cardiometabolic biomarkers

Blood concentrations of TMAO were measured in each study using targeted or untargeted assays. Targeted assays were performed by the Broad Institute (50), Keio University (51), University of North Carolina Nutrition Obesity Research Center (52), and Sun Yat-sen University (45); untargeted assays were performed by Imperial College London's National Phenome Centre (ICL-NPC) (53) and Metabolon Inc. (54). The correlation of TMAO concentrations between different platforms was high (Spearman correlation $r = 0.93$ for the Broad Institute and Metabolon Inc. measures, and $r = 0.96$ for Metabolon Inc. and ICL-NPC measures) (34, 53). TMAO concentrations were log-transformed and normalized by study-specific mean and SD.

Cardiometabolic biomarkers were assessed per each study protocol. We harmonized data on glucose (milligrams per deciliter), insulin (microunits per milliliter), glycated hemoglobin (HbA1c; percentage of total hemoglobin), systolic blood pressure (millimeters of mercury), diastolic blood pressure (millimeters of mercury), total cholesterol (milligrams per deciliter), HDL cholesterol (milligrams per deciliter), LDL cholesterol (milligrams per deciliter), triglycerides (milligrams per deciliter), C-reactive protein (milligrams per liter), IL-6 (picograms per milliliter), creatinine (milligrams per deciliter), and homocysteine (micromoles per liter). Given usually skewed distributions of biomarkers, they were log-transformed and standardized by the mean and SD of each study. Additionally, we collected data on fasting status in all participating studies and recent use of antibiotics in 5 studies (i.e., FHS, MESA, SMHS, SWHS, and TMCS).

Statistical analysis

The analytic protocol was developed with inputs from all studies. A "standard statistical program" was developed based

on harmonized variables and sent to each study for data analysis. The final numbers of harmonized variables varied due to the difference in data availability across studies. Study-specific results were combined using random-effects meta-analyses, considering differences in the study time period, metabolomics platform, and participant characteristics. The degree of heterogeneity across studies was assessed by the Q and I^2 statistics; P for heterogeneity <0.10 was considered significant (55, 56). Linear regression was used to estimate β coefficients and 95% CIs for the associations of standardized log-TMAO with demographics (age, sex, and race and ethnicity), lifestyles (smoking, alcohol drinking, and physical activity), obesity and central obesity, metabolic conditions (diabetes, hypertension, dyslipidemia, and NAFLD), dietary intakes (per 5% energy increment for macronutrients and per 1 serving/d increment for foods), and cardiometabolic biomarkers (per 1 SD change in log-transformed value). A separate linear regression model was built for each variable of interest. Covariates were selected a priori and added to the model sequentially. The basic model included age (years), sex (men and women), race and ethnicity (white, black, Hispanic, Asian, and others), and fasting status (<6 h and ≥6 h). A second model further adjusted for education (less than high school, high-school graduation, post–high-school training or some college, and college graduation or higher), BMI (<18.5, 18.5–24.9, 25.0–29.9, and ≥30.0 kg/m^2), central obesity (normal, moderate, high, and very high defined per WHO criteria; WHR: <0.90, 0.90–0.94, 0.95–0.99, and ≥1.00 for non-Asian men; <0.85, 0.85–0.89, 0.90–0.94, and ≥0.95 for Asian men; and <0.75, 0.75–0.79, 0.80–0.84, and ≥0.85 for all women), tobacco smoking status (never, former, and current), total physical activity (study- and sex-specific tertiles of total physical activity level), alcohol consumption (none, >0 to ≤1, >1 to ≤2, and >2 drinks/d; 1 drink = 14 g ethanol), use of multivitamins (yes and no), menopausal status and hormone therapy in women (yes and no), and intakes of major TMAO-contributing foods: red meat, eggs, and total fish (study- and sex-specific quintiles). In the final model, we further adjusted for metabolic disease status, including diabetes, hypertension, dyslipidemia, and NAFLD (yes and no). Missing covariates were coded as an unknown category and included in the models. For diet-related analyses, we further excluded participants with extreme energy intake (beyond ±5 SDs of study- and sex-specific mean) and adjusted for total energy intake. To evaluate the possible variations in TMAO associations due to differences in habitual diet, we conducted stratified analyses by region (United States, Europe, and Asia). Sensitivity analyses were conducted by excluding participants who reported recent antibiotic use (data were available in 5 studies, mostly defined as antibiotic use in the last 7 d) and then comparing results with those using data from all participants of these 5 studies. For the biomarker-TMAO associations, further stratified analyses were conducted by age, sex, race and ethnicity, fasting status, obesity status, metabolic disease status, and intakes of red meat, fish, fiber, and vegetable/fruits. P values were corrected for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR); FDR-q <0.10 was considered significant to embrace all potentially meaningful results, because FDR-q <0.05 has been suggested as being too low for many clinical settings (57). All results described in the following section had FDR-q <0.10, unless specified. Analyses were conducted using SAS Enterprise Guide version 7.1 (SAS Institute Inc.) and Stata version 12 (StataCorp).

Results

Table 1 presents the characteristics of the participating studies. Among a total of 32,166 adults aged 19–84 y, 19,632 (61.0%) were women, 16,269 (50.6%) were white, 13,293 (41.3%) were Asian, 1247 (3.9%) were Hispanic/Latino, and 1236 (3.8%) were black. More details on baseline characteristics across studies are given in **Supplemental Tables 1** and **2**.

Circulating TMAO was associated with older age, male sex, and overweight (**Figure 1**). In our final model, each year of age was associated with a 0.015 SD increase in log-TMAO (95% CI: 0.011, 0.019). Women had lower TMAO concentrations than men (β: −0.095; 95% CI: −0.169, −0.021). We found no significant associations of TMAO with race or ethnicity, lifestyles, or metabolic disease history. Results of all 3 models can be found in **Supplemental Table 3**.

Circulating TMAO was positively associated with intakes of animal protein, saturated fat, monounsaturated fat, fish, shellfish, eggs, and red meat, whereas it was inversely associated with intakes of plant protein, carbohydrate, and nuts (**Figure 2**, **Supplemental Table 4**). In our final model, a 5% energy increase in animal protein or plant protein was associated with a 0.124 (95% CI: 0.093, 0.155) and a −0.126 (95% CI: −0.214, −0.039) SD increase in log-TMAO, respectively. With mutual adjustment for major TMAO-contributing foods (red meat, eggs, and fish), a 1 serving/d increase in total fish and shellfish was associated with a 0.151 (95% CI: 0.080, 0.222) and a 0.370 (95% CI: 0.037, 0.702) SD increase in log-TMAO, respectively, whereas a 1 serving/d increase in nuts was associated with a −0.123 (95% CI: −0.238, −0.008) SD increase in log-TMAO. The associations of TMAO with saturated fat, monounsaturated fat, eggs, and red meat were modest: β ranged from 0.05 to 0.08 for each 5% energy or 1 serving/d increment.

The positive association between animal protein and circulating TMAO was found in all regions (**Table 2**, **Supplemental Table 5**). However, associations of eggs, red meat, saturated fat, and plant protein with TMAO were only significant in US studies: β = 0.153 (95% CI: 0.068, 0.238) and 0.093 (95% CI: 0.021, 0.165) for 1 serving/d of eggs and red meat; 0.070 (95% CI: 0.028, 0.113) and −0.161 (95% CI: −0.248, −0.075) for a 5% energy increment from saturated fat and plant protein, respectively; all P-heterogeneity values by region <0.10. However, stronger associations of fish and shellfish with TMAO were found in Asian studies than in US or European studies (both P-heterogeneity by region <0.10). In Asian studies, a 1 serving/d of total fish and shellfish was associated with β = 0.285 (95% CI: 0.163, 0.407) and 0.578 (95% CI: 0.166, 0.990), respectively; whereas, no significant associations were found in US and European studies, except for total fish in US studies (β: 0.161; 95% CI: 0.081, 0.241).

Circulating TMAO was positively associated with creatinine, homocysteine, and glycemic control biomarkers, whereas it was inversely associated with HDL and blood pressure (**Figure 3**, **Supplemental Table 6**). After adjustment for dietary intakes and metabolic disease status, a 1 SD increase in log-creatinine was related to a 0.131 (95% CI: 0.089, 0.174) SD increase in

TABLE 1 Characteristics of the participating studies: the TMAO Pooling Project[1]

	Baseline[2]	No. of participants[3]	Age[4]	No. of women	No. of participants by race and ethnicity				Metabolomics platform[5]
					White	Black	Hispanic	Asian	
United States									
Coronary Artery Risk Development in Young Adults Study	2000–2001	784	33–55	373	400	384	0	0	UNC NORC
Framingham Heart Study	1971	2255	26–84	1200	2255	0	0	0	Broad Institute
Health Professionals Follow-Up Study	1993–1995	1179	40–75	0	1109	2	0	2	Broad Institute
Insulin Resistance Atherosclerosis Family Study	1999–2002	1630	28–70	961	0	548	1082	0	Metabolon Inc.
Multi-Ethnic Study of Atherosclerosis	2000–2002	734	44–84	360	255	187	165	127	ICL-NPC
Nurses' Health Study	1989–1990	2993	43–69	2993	2953	27	0	7	Broad Institute
Nurses' Health Study II	1996–1999	2990	32–54	2990	2910	29	0	38	Broad Institute
Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial	1993–2001	1123	55–74	1123	1018	59	0	10	Metabolon Inc.
Women's Health Initiative	1993–1998	320	62–72	320	320	0	0	0	Metabolon Inc.
Europe									
Airwave Health Monitoring Study	2004	1938	19–65	666	1938	0	0	0	Metabolon Inc.
Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study	1985–1988	2072	50–69	0	2072	0	0	0	Metabolon Inc.
UK Adult Twin Registry	1992	1039	32–73	1039	1039	0	0	0	Metabolon Inc.
Asia									
Guangzhou Nutrition and Health Study	2008–2010	1732	40–80	1186	0	0	0	1732	Sun Yat-sen University
Shanghai Men's Health Study	2002–2006	699	40–75	0	0	0	0	699	Metabolon Inc.
Shanghai Women's Health Study	1996–2000	1423	40–71	1423	0	0	0	1423	Metabolon Inc.
Tsuruoka Metabolomics Cohort Study	2012–2015	9255	34–75	4996	0	0	0	9255	Keio University
Total		32,166	19–84	19,632	16,269	1236	1247	13,293	

[1] ICL-NPC, Imperial College London's National Phenome Centre; TMAO, trimethylamine *N*-oxide; UNC NORC, University of North Carolina Nutrition Obesity Research Center.

[2] The time period of blood collection used for metabolomics study.

[3] Included were only participants who were eligible for the TMAO Pooling Project.

[4] Age range at blood collection.

[5] Targeted assays included the Broad Institute (United States), Keio University (Japan), University of North Carolina Nutrition Obesity Research Center (United States), and Sun Yat-sen University (China); untargeted assays included the ICL-NPC (United Kingdom) and Metabolon Inc. (Unites States).

Subgroup	No. of Subjects	β (95% CI)	*Q-value*	I^2
Asian vs. White, US Cohorts only	184/11,220	0.104 (-0.128, 0.336)	0.572	0.0%
Diabetes vs. Non-diabetes	1,886/30,280	0.060 (-0.020, 0.140)	0.362	69.2%
High WHR vs. Normal WHR	3,766/4,461	0.037 (-0.017, 0.092)	0.362	28.2%
Current smokers vs. Never smokers	6,162/18,444	0.033 (-0.010, 0.075)	0.362	33.3%
Overweight vs. Normal weight	8,310/16,373	0.030 (0.011, 0.048)	0.012	0.0%
>1 to ≤2 alcohol drinks/d vs. None	3,708/13,137	0.028 (-0.012, 0.068)	0.362	23.6%
Very high WHR vs. Normal WHR	3,829/4,461	0.019 (-0.008, 0.046)	0.362	2.7%
>2 alcohol drinks/d vs. None	4,217/13,137	0.019 (-0.030, 0.067)	0.608	37.4%
Hypertension vs. Normotension	8,057/24,107	0.015 (-0.017, 0.047)	0.558	41.3%
Age, years	32,166	0.015 (0.011, 0.019)	<0.001	76.6%
NAFLD vs. Non-NAFLD	1,114/12,121	0.014 (-0.082, 0.110)	0.852	17.6%
Menopause vs. Pre-menopause	7,299/5,228	0.009 (0.001, 0.017)	0.156	0.0%
Obese vs. Normal weight	3,292/16,373	0.009 (-0.008, 0.026)	0.497	0.0%
Former smokers vs. Never smokers	7,438/18,444	0.006 (-0.022, 0.033)	0.829	13.4%
Dyslipidemia vs. Non-dyslipidemia	6,981/21,472	0.004 (-0.006, 0.013)	0.608	0.0%
>0 to ≤1 alcohol drink/d vs. None	9,487/13,137	0.003 (-0.016, 0.022)	0.852	0.0%
Hormone therapy vs. None	2,591/14,137	0.000 (-0.006, 0.006)	0.985	0.0%
Hispanic vs. White, US Cohorts only	1,247/11,220	-0.004 (-0.418, 0.410)	0.985	90.7%
Underweight vs. Normal weight	4,166/16,373	-0.008 (-0.042, 0.025)	0.796	11.0%
Moderate WHR vs. Normal WHR	4,121/4,461	-0.013 (-0.032, 0.007)	0.362	0.0%
Moderate physical activity vs. Low	10,238/10,614	-0.016 (-0.034, 0.001)	0.226	0.0%
High physical activity vs. Low	10,349/10,614	-0.037 (-0.074, 0.000)	0.196	49.6%
Women vs. Men	19,632/12,534	-0.095 (-0.169, -0.021)	0.096	65.2%
Black vs. White, US Cohorts only	1,236/11,220	-0.120 (-0.233, -0.007)	0.182	0.0%

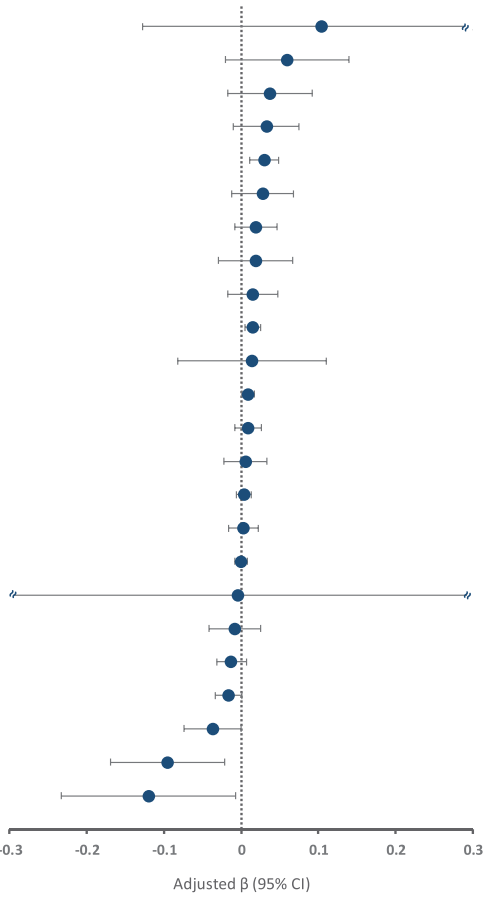

FIGURE 1 Circulating trimethylamine *N*-oxide (TMAO) in relation to demographics, lifestyle, and medical history. Linear regression coefficients (β) and 95% CIs were adjusted for age, sex, race and ethnicity, education, fasting time, obesity, central obesity, smoking status, alcohol consumption, physical activity, use of multivitamins, menopausal status and hormone therapy in women, intakes of red meat, egg, and fish, and history of diabetes, hypertension, dyslipidemia, and nonalcoholic fatty liver disease. β indicates the increase or decrease in SD units of TMAO on the log-scale. *Q*-values represent corrected *P* values for multiple comparisons by controlling the false discovery rate. I^2 represents the degree of heterogeneity. NAFLD, nonalcoholic fatty liver disease; WHR, waist-to-hip ratio.

log-TMAO. Increasing TMAO concentrations were also related to homocysteine (0.065; 95% CI: 0.019, 0.112), insulin (0.048; 95% CI: 0.024, 0.072), HbA1c (0.048; 95% CI: 0.019, 0.076), and glucose (0.023; 95% CI: 0.007, 0.039). Meanwhile, TMAO was associated with lower HDL (−0.047; 95% CI: −0.067, −0.027) and blood pressure (−0.030; 95% CI: −0.049, −0.011, for systolic blood pressure). The biomarker-TMAO associations seemed similar across the US, European, and Asian studies (**Table 2**, **Supplemental Table 7**).

To evaluate whether the biomarker-TMAO associations are independent of, or confounded by, renal function (given a strong TMAO-creatinine association), in 5 studies (2 US and 3 Asian studies), we further adjusted for creatinine and found no appreciable changes in results, whereas associations for insulin/HbA1c/glucose became even stronger (**Supplemental Table 8**). In addition, we conducted stratified analyses by renal function—reduced (eGFR <90) compared with normal (eGFR ≥90)—and found no significant interactions (**Supplemental Table 9**; all *P*-interaction >0.15). The TMAO-HbA1c association was significant in individuals with normal or reduced renal function: β = 0.069 (95% CI: 0.040, 0.098) and 0.085 (95% CI: 0.045, 0.125), respectively. All biomarker-TMAO associations remained robust in stratified analyses by other baseline characteristics, including metabolic disease history and dietary intakes of meat, fish, fiber, and fruits/vegetables (**Supplemental Tables 10** and **11**).

Results did not change after excluding recent antibiotic users (**Supplemental Tables 12**, **13**, and **14**). Study-specific results are shown in forest plots (**Supplemental Figures 2** and **3**). Results remained robust if any study was excluded from the meta-analysis one at a time (data not shown).

Discussion

In this international collaborative project including 16 population-based studies from the United States, Europe, and Asia, circulating TMAO was positively associated with intakes of animal protein, saturated fat, monounsaturated fat, fish, shellfish, eggs, and red meat, and inversely associated with intakes of plant

Subgroup	No. of Subjects	β (95% CI)	*Q*-value	I^2
Shellfish, per 1 serving/day	16,955	0.370 (0.037, 0.702)	0.063	39.2%
Total fish, per 1 serving/day	17,800	0.151 (0.080, 0.222)	<0.001	81.4%
Animal protein, per 5% energy	15,725	0.124 (0.093, 0.155)	<0.001	8.5%
Eggs, per 1 serving/day	17,800	0.081 (0.019, 0.144)	0.033	58.8%
Saturated fat, per 5% energy	18,589	0.058 (0.012, 0.104)	0.037	49.2%
Red meat, per 1 serving/day	17,800	0.056 (0.017, 0.094)	0.018	73.9%
Monounsaturated fat, per 5% energy	18,589	0.052 (0.019, 0.084)	0.011	0.0%
Polyunsaturated fat, per 5% energy	18,589	0.028 (-0.022, 0.079)	0.438	0.0%
Processed meat, per 1 serving/day	16,955	0.025 (-0.081, 0.131)	0.747	52.5%
Poultry, per 1 serving/day	16,955	0.007 (-0.030, 0.043)	0.789	18.1%
Vegetables, per 1 serving/day	16,955	0.001 (-0.006, 0.008)	0.857	17.4%
Fruits, per 1 serving/day	16,955	0.001 (0.000, 0.001)	0.063	0.0%
Dairy foods, per 1 serving/day	16,955	-0.001 (-0.011, 0.009)	0.859	12.9%
Whole grains, per 1 serving/day	15,099	-0.001 (-0.003, 0.000)	0.215	0.0%
Wheat, per 1 serving/day	16,955	-0.005 (-0.017, 0.007)	0.526	23.3%
Fiber, per 5g increase	18,589	-0.007 (-0.023, 0.009)	0.526	0.0%
Legume, per 1 serving/day	16,635	-0.017 (-0.065, 0.031)	0.591	27.6%
Carbohydrate, per 5% energy	18,589	-0.033 (-0.043, -0.022)	<0.001	0.0%
Soy products, per 1 serving/day	16,955	-0.070 (-0.216, 0.076)	0.524	23.0%
Nuts, per 1 serving/day	15,099	-0.123 (-0.238, -0.008)	0.071	60.6%
Plant protein, per 5% energy	15,725	-0.126 (-0.214, -0.039)	0.018	24.3%

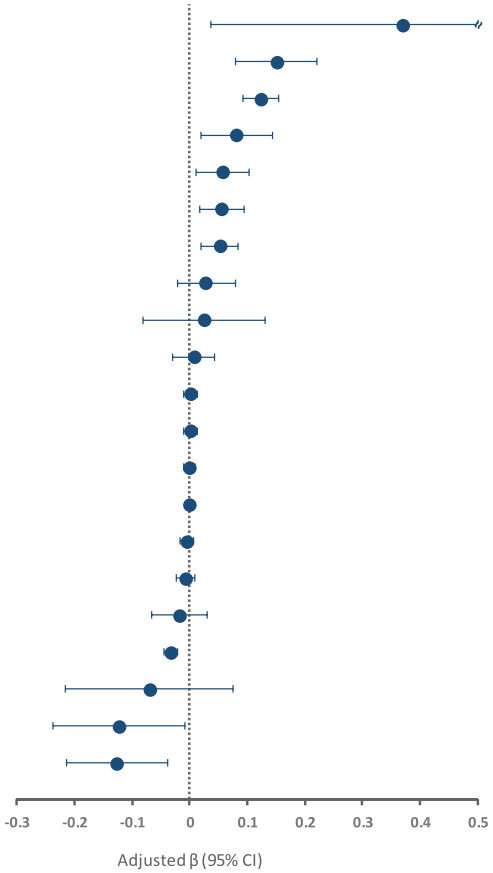

FIGURE 2 Circulating trimethylamine *N*-oxide (TMAO) in relation to dietary factors. Linear regression coefficients (β) and 95% CIs were adjusted for age, sex, race and ethnicity, education, fasting time, obesity, central obesity, smoking status, alcohol consumption, physical activity, use of multivitamins, menopausal status and hormone therapy in women, intakes of red meat, egg, and fish, and history of diabetes, hypertension, dyslipidemia, and nonalcoholic fatty liver disease. β indicates the increase or decrease in SD units of log-TMAO by dietary changes. *Q*-values represent corrected *P* values for multiple comparisons by controlling the false discovery rate. I^2 represents the degree of heterogeneity.

protein, carbohydrate, and nuts. Whereas the animal protein–TMAO association was consistent, TMAO associations with eggs, red meat, saturated fat, and plant protein were mainly observed in US studies, and associations with fish and shellfish were more evident in Asian studies. Furthermore, circulating TMAO was associated with multiple cardiometabolic risk factors after comprehensive adjustments, including biomarkers of renal function (creatinine), glycemic control (insulin/HbA1c/glucose), methylation (homocysteine), and dyslipidemia (HDL), although the effect sizes were generally small. Meanwhile, TMAO was not associated with inflammatory markers and other blood lipids.

TMAO is a gut microbial-host cometabolite derived from dietary choline and carnitine, abundant in red meat, eggs, fish, dairy, and some nuts and legumes. Habitual diets determine not only the amount of TMAO precursors from different foods but also gut microbial composition and possibly the ability of the gut microbiome to generate TMAO (5, 11, 22, 58), which in turn could affect host cardiometabolic health. In the current study, we found a positive association between animal protein intake and TMAO concentrations, consistent across ethnic groups and geographic regions. This finding agrees with results from previous studies that high intakes of animal foods (i.e., red meat, eggs, dairy, or fish) and animal-sourced choline were associated with increased TMAO concentrations (18–22, 24). Although TMAO was related to overall animal food intakes, we found varied associations between specific animal foods and TMAO concentrations by population/region (e.g., US compared with Asian studies). Variations in the consumption of TMAO-contributing foods have been reported across populations. For most Americans, the main sources of choline/carnitine were red meat, poultry, eggs, and dairy (14–16), although Hispanic/Latino Americans, on average, consumed more choline from legumes than non-Hispanic white Americans (59). For many Asians, most dietary choline was from eggs and soy foods (60). TMAO concentrations have been associated with intakes of dairy and/or fish in some European and Asian populations (18–21), rather than meat in US populations (19, 22). A recent international study—the INTERMAP study, which measured urinary TMAO concentrations in 4680 adults from Japan, China, the United States, and the United Kingdom—also reported population-specific associations between foods and TMAO: Japanese showed stronger associations of TMAO with

TABLE 2 Circulating TMAO in relation to dietary factors and cardiometabolic biomarkers: stratified analysis by region[1]

	United States		Europe[2]		Asia[2]	
	β (95% CI)[3]	Q-value[4]	β (95% CI)[3]	Q-value[4]	β (95% CI)[3]	Q-value[4]
Macronutrients, per 5% energy increase						
Animal protein	0.110 (0.079, 0.141)	<0.001	0.208 (0.053, 0.362)	0.051	0.194 (0.102, 0.286)	<0.001
Plant protein[5]	−0.161 (−0.248, −0.075)	<0.001	0.190 (−0.086, 0.467)	0.378	−0.107 (−0.295, 0.081)	0.559
Saturated fat[5]	0.070 (0.028, 0.113)	0.004	−0.049 (−0.107, 0.009)	0.240	0.112 (0.007, 0.217)	0.117
Monounsaturated fat	0.057 (0.020, 0.094)	0.006	0.034 (−0.126, 0.194)	0.778	0.033 (−0.041, 0.107)	0.655
Carbohydrate	−0.035 (−0.047, −0.023)	<0.001	−0.002 (−0.050, 0.046)	0.933	−0.031 (−0.055, −0.008)	0.048
Food items, per 1 serving/d increase						
Red meat[5]	0.093 (0.021, 0.165)	0.029	0.011 (−0.014, 0.036)	0.677	−0.013 (−0.166, 0.141)	0.884
Eggs[5]	0.153 (0.068, 0.238)	<0.001	−0.008 (−0.045, 0.030)	0.778	0.120 (0.010, 0.231)	0.117
Total fish[5]	0.161 (0.081, 0.241)	<0.001	0.040 (−0.038, 0.117)	0.595	0.285 (0.163, 0,407)	<0.001
Shellfish	0.290 (−0.122, 0.703)	0.294	NA		0.578 (0.166, 0.990)	0.038
Nuts	−0.123 (−0.238, −0.008)	0.086	NA		NA	
Biomarkers, per 1 SD log-biomarker increase						
Creatinine, mg/dL	0.129 (0.068, 0.189)	<0.001	0.216 (0.058, 0.374)	0.063	0.123 (0.066, 0.181)	<0.001
Homocysteine, μmol/L	0.065 (0.019, 0.112)	0.020	NA		NA	
Insulin, uU/mL	0.036 (0.009, 0.063)	0.023	NA		0.094 (0.042, 0.146)	<0.001
HbA1c, % of total hemoglobin	0.049 (0.014, 0.084)	0.020	0.007 (−0.037, 0.052)	0.847	0.076 (0.051, 0.101)	<0.001
Glucose, mg/dL	0.040 (0.006, 0.075)	0.050	0.032 (0.000, 0.063)	0.117	0.012 (−0.010, 0.034)	0.346
Systolic blood pressure, mmHg	−0.015 (−0.047, 0.017)	0.459	−0.058 (−0.117, 0.000)	0.117	−0.024 (−0.045, −0.004)	0.038
Diastolic blood pressure, mmHg	−0.028 (−0.057, 0.002)	0.117	−0.037 (−0.101, 0.026)	0.445	−0.027 (−0.047, −0.007)	0.024
HDL cholesterol, mg/dL	−0.056 (−0.083, −0.030)	<0.001	−0.042 (−0.124, 0.039)	0.462	−0.031 (−0.052, −0.010)	0.009

[1]HbA1c, glycated hemoglobin; NA, not available; TMAO, trimethylamine *N*-oxide.

[2]Dietary data were not available in the Airwave Health Monitoring Study (Europe) and the Tsuruoka Metabolomics Cohort Study (Asia).

[3]Linear regression coefficients (β) and 95% CIs were adjusted for total energy, age, sex, race and ethnicity, education, fasting time, obesity, central obesity, smoking status, alcohol consumption, physical activity level, use of multivitamins, menopausal status and hormone therapy in women, intakes of red meat, egg, and fish, and history of diabetes, hypertension, dyslipidemia, and nonalcoholic fatty liver disease. β indicates the increase or decrease in SD units of log-TMAO.

[4]Q-value represents corrected P value for multiple comparisons by controlling the false discovery rate.

[5]P values for heterogeneity across the United States, Europe, and Asia: 0.058 for plant protein, 0.002 for saturated fat, 0.099 for red meat, 0.001 for eggs, and 0.003 for total fish.

Subgroup	No. of Subjects	β (95% CI)	*Q*-value	I^2
Creatinine, 1SD increase	20,544	0.131 (0.089, 0.174)	<0.001	80.4%
Homocysteine, 1SD increase	4,620	0.065 (0.019, 0.112)	0.010	44.3%
Insulin, 1SD increase	8,263	0.048 (0.024, 0.072)	<0.001	0.0%
HbA1c, 1SD increase	16,611	0.048 (0.019, 0.076)	0.003	35.3%
Glucose, 1SD increase	20,539	0.023 (0.007, 0.039)	0.009	0.0%
Interleukin-6, 1SD increase	7,217	0.017 (-0.030, 0.063)	0.523	65.7%
C-reactive protein, 1SD increase	17,822	0.013 (-0.003, 0.028)	0.157	0.0%
LDL cholesterol, 1SD increase	18,353	0.000 (-0.026, 0.026)	0.985	46.0%
Triglycerides, 1SD increase	18,518	-0.012 (-0.043, 0.019)	0.523	52.2%
Total cholesterol, 1SD increase	26,222	-0.013 (-0.038, 0.012)	0.391	62.6%
SBP, 1SD increase	21,736	-0.030 (-0.049, -0.011)	0.004	19.5%
DBP, 1SD increase	21,736	-0.031 (-0.047, -0.015)	<0.001	8.8%
HDL cholesterol, 1SD increase	23,753	-0.047 (-0.067, -0.027)	<0.001	30.2%

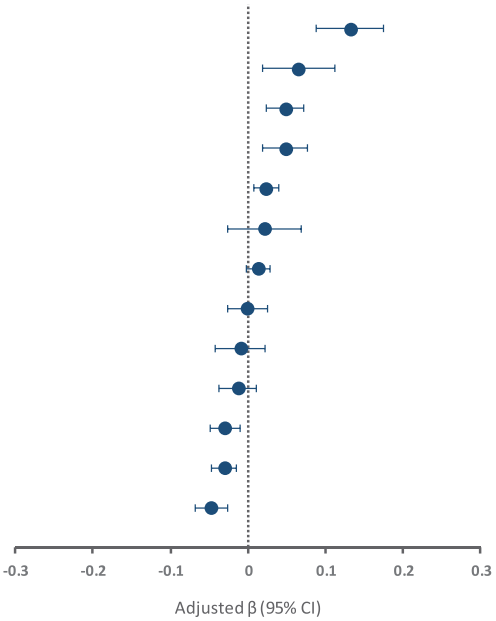

FIGURE 3 Circulating trimethylamine *N*-oxide (TMAO) in relation to cardiometabolic biomarkers. Linear regression coefficients (β) and 95% CIs were adjusted for age, sex, race and ethnicity, education, fasting time, obesity, central obesity, smoking status, alcohol consumption, physical activity, use of multivitamins, menopausal status and hormone therapy in women, intakes of red meat, egg, and fish, and history of diabetes, hypertension, dyslipidemia, and nonalcoholic fatty liver disease. β indicates the increase or decrease in SD units of log-biomarkers by per SD change in log-TMAO. *Q*-values represent corrected *P* values for multiple comparisons by controlling the false discovery rate. I^2 represents the degree of heterogeneity. DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; SBP, systolic blood pressure.

fish and shellfish than Westerners (19). Overall, findings from our and other population-based studies have supported that high animal food intakes increased TMAO concentrations, but the impacts of different animal foods on TMAO concentrations vary among populations. The TMAO pathway could be one of the mechanisms underlying the associations of red meat/eggs with CVD risk in US populations (3, 5, 12, 22), but this is less likely in European or Asian populations.

We also observed significant inverse associations of plant protein, carbohydrate, and nuts with TMAO concentrations, even though nuts and legumes (a major source of plant protein) contain considerable amounts of choline. These findings were in line with previous reports that TMAO concentrations were lower in individuals with a habitual plant-based Mediterranean diet (23) and were decreased after a nut-supplemented diet (61). The seemingly incongruous association of nuts with reduced TMAO concentrations implies that the gut microbiota can convert choline to trimethylamine at different rates under different habitual diets (e.g., animal- compared with plant-based diets). So far, a few microbial genes have been identified for choline-trimethylamine conversion, for example, choline utilization C/D (CutC/D) (62, 63), and some taxa have been associated with TMAO concentration, for example, *Clostridium* and *Fusobacterium* (58, 64), although their contributions to TMAO production in humans remain unclear. Meanwhile, accumulating evidence has shown distinct gut microbial profiles in individuals with animal- compared with plant-based diets, including taxa that could be related to TMAO production (23, 65). For the current analysis, microbiome data were not available. Nevertheless, a few of our participating cohorts have recently collected stool samples from participants. Future studies will enable further investigations of the relations between diet, gut microbiota, microbial metabolite TMAO, and cardiometabolic health and potential diet–microbiota interactions that might affect TMAO concentrations and risk of cardiometabolic diseases.

Among biomarkers, we found significant associations of TMAO with creatinine, homocysteine, insulin, HbA1c, glucose, HDL, and blood pressure, but null associations for inflammatory markers and other blood lipids. Many studies have reported elevated TMAO concentrations in patients with diabetes, CKD, or CVD (4–7, 26–29, 66, 67) and associations of baseline TMAO or changes in TMAO concentrations with the development of those diseases (24, 25, 68–70). Evidence from animal models has indicated a causal role for TMAO in the development and/or progression of diabetes and CKD (9), in addition to CVD. For example, knockdown of Flavin Containing Dimethylaniline Monoxygenase 3 (FMO3) in mice, the host gene for generating TMAO, led to improved insulin sensitivity and glycemic control (10, 71, 72); conversely, supplementation with TMAO or choline promoted renal fibrosis and functional impairment (9). Yet, some studies suggested that elevated TMAO is an indication of renal impairment and/or underlying diabetes, which could confound the associations of TMAO with other diseases (28, 73, 74). In the current study, we found a strong association between TMAO and creatinine. However, the associations between TMAO and other cardiometabolic biomarkers remained similar after further controlling for creatinine or in stratified

analyses by renal function. Notably, the TMAO associations with insulin/HbA1c/glucose became even stronger after the creatinine adjustment, and the TMAO-HbA1c association was significant in individuals with normal renal function. Consistent with our findings, the Multiethnic Cohort Study recently reported a significant association of circulating TMAO with HOMA-IR in 1653 US adults without severe diseases (58). The overall evidence so far seems to support bidirectional associations between elevated TMAO concentrations and cardiometabolic disorders. Given our cross-sectional design, prospective studies and intervention studies are needed to investigate the potential role of reducing TMAO [e.g., via plant-based or Mediterranean-style diets or supplementation with nuts or other bioactive compounds (64)] in the prevention or treatment of diabetes, CKD, or CVD. Finally, the inverse association of TMAO with blood pressure seems contradictory with the adverse cardiometabolic effect of TMAO and with previous findings in US adults (19); however, this association was weak and only significant in Asian studies ($\beta = -0.03$ SD), and thus would have minimal clinical significance.

Our current study has several limitations besides a cross-sectional design. First, this is a secondary analysis of existing data collected in studies using different instruments. To address this issue, we developed a data dictionary for variable harmonization and a standard analytic program for statistical analyses. We applied random-effects meta-analysis to combine study-specific results and found no substantial changes in overall results if any study was excluded from the meta-analysis. Second, a one-time measurement of TMAO might not reflect its usual concentration, and other measurement errors could affect our findings, in spite of all participating studies using validated questionnaires and metabolite/biomarker assays. Generally, measurement errors are likely to attenuate the exposure-outcome association. Third, we cannot rule out residual confounding due to uncontrolled or imperfectly controlled variables. For example, due to a lack of data, we could not control the potential effect of refined grains, which are often consumed with TMAO-contributing foods and have adverse cardiovascular effects; although in an exploratory analysis with additional adjustments for total carbohydrates or wheat products (mostly refined), the overall results remained virtually the same (data not shown). Finally, our large sample size resulted in high precision for detecting small effects, which could be statistically significant but with unclear clinical or public health interpretation. Future intervention studies are needed to determine to what extent TMAO concentrations can be reduced by certain dietary or microbial changes and whether a reduction in TMAO concentrations confers clinically meaningful results.

Nevertheless, to our knowledge, this is the first large-scale, epidemiological study evaluating associations between diet, TMAO, and cardiometabolic biomarkers in ethnically and geographically diverse populations. Comprehensive data on demographics, diet, lifestyle, and blood concentrations of TMAO and biomarkers were harmonized among >32,000 participants from 16 studies in 3 continents. A series of adjustments for potential confounders and stratified analyses by individual characteristics allowed for in-depth analyses to provide insights into relations between diet, TMAO, and cardiometabolic health.

In summary, in a large-scale, international pooled analysis, we observed associations of circulating TMAO with high intakes of animal protein, saturated fat, monounsaturated fat, fish, shellfish, eggs, and red meat, and with low intakes of plant protein, carbohydrate, and nuts. The association of TMAO with animal protein was consistent across populations, but its associations with fish, shellfish, eggs, and red meat varied among the US, European, and Asian populations. Circulating TMAO was associated with multiple cardiometabolic risk factors, including impaired renal function and poor glycemic control (independent of renal function), but not with inflammation and blood lipids except HDL. Whereas prospective studies and mechanistic investigations are needed to elucidate potential causality and underlying mechanisms further, our study shows that certain dietary and cardiometabolic factors are linked to TMAO metabolism, supporting the role of diet–microbiota–host interactions in human cardiometabolic health.

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The authors' responsibilities were as follows—DY, X-OS: designed the study; DMH, SCM, KAM, JO, CM, NDP, HE, SH, IT, HZ, DA, TJW, WZ, HC, CMU, MG-F, IK, MF, QC, CEM, LEW, PE, REG, X-OS: provided essential reagents or essential materials; DY, JJY: performed statistical analysis and drafted the manuscript; DY: had primary responsibility for final content; and all authors: reviewed and approved the final manuscript.

The authors report no conflicts of interest.

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