ORIGINAL RESEARCH

Urinary Levels of Trimethylamine-N-Oxide and Incident Coronary Heart Disease: A Prospective Investigation Among Urban Chinese Adults

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Background—Trimethylamine-N-oxide (TMAO), a diet-derived, gut microbial–host cometabolite, has been associated with adverse cardiovascular outcomes in patient populations; however, evidence is lacking from prospective studies conducted in general populations and non-Western populations.

Methods and Results—We evaluated urinary levels of TMAO and its precursor metabolites (ie, choline, betaine, and carnitine) in relation to risk of coronary heart disease (CHD) among Chinese adults in a nested case–control study, including 275 participants with incident CHD and 275 individually matched controls. We found that urinary TMAO, but not its precursors, was associated with risk of CHD. The odds ratio for the highest versus lowest quartiles of TMAO was 1.91 (95% CI, 1.08–3.35; P_{trend}=0.008) after adjusting for CHD risk factors including obesity, diet, lifestyle, and metabolic diseases and 1.75 (95% CI, 0.96–3.18; P_{trend}=0.03) after further adjusting for potential confounders or mediators including central obesity, dyslipidemia, inflammation, and intake of seafood and deep-fried meat or fish, which were associated with TMAO level in this study. The odds ratio per standard deviation increase in log-TMAO was 1.30 (95% CI, 1.03–1.63) in the fully adjusted model. A history of diabetes mellitus modified the TMAO–CHD association. A high TMAO level (greater than or equal to versus lower than the median) was associated with odds ratios of 6.21 (95% CI, 1.64–23.6) and 1.56 (95% CI, 1.00–2.43), respectively, among diabetic and nondiabetic participants ($P_{interaction}$=0.02). Diabetes mellitus status also modified the associations of choline, betaine, and carnitine with risk of CHD; significant positive associations were found among diabetic participants, but null associations were noted among total and nondiabetic participants.

Conclusions—Our study suggests that TMAO may accelerate the development of CHD, highlighting the importance of diet–gut microbiota–host interplay in cardiometabolic health. **(*J Am Heart Assoc*. 2019;8:e010606. DOI: 10.1161/JAHA.118.010606)**

Key Words: cardiovascular disease risk factors • Chinese • gut microbiota • metabolomics • nested case-control study • nutrition • prospective cohort study • trimethylamine-N-oxide

Trimethylamine-N-oxide (TMAO) has gained much attention recently because of its potential adverse effects on atherosclerosis and cardiovascular disease (CVD).[1,2] TMAO is a gut microbial–host cometabolite of dietary trimethylamines (mainly choline and carnitine). Its metabolism involves 3 steps: (1) gut microbial degradation of dietary trimethylamines, (2) conversion of trimethylamine to TMAO by host liver enzymes, and (3) kidney excretion of circulating TMAO in urine. Studies from the Cleveland Clinic have shown that TMAO promotes atherosclerosis in mice by inhibiting reverse cholesterol transport, activating macrophages, and increasing platelet activity and thrombosis,[3–6] whereas inhibiting TMAO production reduces the formation of atherosclerotic lesions.[7] Their studies have also shown that elevated TMAO levels

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Clinical Perspective

What Is New?

- This study is the first conducted in a general population showing that trimethylamine-N-oxide (TMAO), a diet-derived, gut microbial–host cometabolite, is positively associated with risk of developing coronary heart disease (CHD), after adjustment for traditional CHD risk factors.
- The TMAO–CHD association was found among participants with or without existing metabolic conditions but was more pronounced among diabetic participants.
- Urinary TMAO level was significantly associated with consumption of deep-fried meat or fish, suggesting a potential interaction between meat cooking methods and the gut microbiota.

What Are the Clinical Implications?

- Elevated TMAO may be a novel risk factor for CHD in general and in high-risk populations.
- Possible interactions of TMAO with diabetes mellitus history and dietary intake imply potential applications of the diet–gut microbiota–host interplay in preventing cardiovascular disease.

predict worse CVD and death outcomes among cardiology patients, independent of traditional risk factors.[5,6]

Given those promising findings, an increasing number of studies have investigated the associations of TMAO and its precursor metabolites with CVD outcomes, such as major adverse cardiovascular events (MACE; including myocardial infarction, stroke, heart failure, other CVDs, and death).[8–11] A recent meta-analysis reported a relative risk of 1.62 (95% CI, 1.45–1.80) for MACE, comparing the highest and lowest levels of TMAO.[2] However, as the authors pointed out, MACE is a composite of clinical events, the definition of which varied across studies and often included death due to any cause. Furthermore, all published prospective studies were clinical cohorts of high-risk individuals, such as patients with prior CVDs or chronic kidney disease (CKD). Reverse causality and confounding bias caused by existing diseases and their treatments are of major concern.[12–15] To our knowledge, no studies have examined, in general populations, whether TMAO is associated with risk of developing CVD, especially coronary heart disease (CHD)—the leading cause of death worldwide.[16]

In addition, TMAO food sources vary across populations. For most Americans, dietary choline and carnitine come mostly from red meat, poultry, eggs, and dairy.[17,18] However, choline is also abundant in fish, shellfish, and legumes,[19] and TMAO is naturally abundant in seafood (no microbial metabolism required).[20,21] It has been found that Hispanic/Latino people consume more choline from legumes than non-Hispanic white people[22]; TMAO level is associated with intake of fish and dairy, but not meat, in Europeans; and among Chinese adults, nearly 50% of dietary choline is plant-based, mostly from soy foods.[23] All published prospective studies on the TMAO–CVD association, thus far, have been from Western populations (mostly white Americans). Data from populations with different habitual diets and potentially differentiated microbial production of TMAO would help advance our understanding of the nature of the TMAO–CVD association.

To address the research gap regarding whether TMAO is associated with incident CHD in general-risk populations and in nonwhite populations, we conducted a case–control study nested within 2 cohorts of Chinese adults: the SWHS (Shanghai Women's Health Study) and the SMHS (Shanghai Men's Health Study). Baseline urinary concentrations of TMAO, choline, betaine, and carnitine were quantified, providing an integrated measure of the diet–microbial host cometabolism of TMAO. We evaluated the associations of these metabolites with dietary intake, lifestyle factors, CVD biomarkers (eg, blood lipids and high-sensitivity C-reactive protein [hs-CRP]), and risk of CHD, with adjustments for potential confounders.

Methods

Study Population

The SWHS and SMHS are 2 population-based, prospective cohorts of 74 941 women and 61 480 men (aged 40–74 years) who lived in Shanghai, China, between 1996 and 2006.[24,25] At baseline (December 1996–May 2000 for the SWHS [response rate: 93%]; January 2002–September 2006 for the SMHS [response rate: 74%]), an in-person interview was conducted to collect information on sociodemographics, diet, lifestyle, medical history, and anthropometrics. Biological samples, including peripheral blood and spot urine, were collected from willing study participants. Overall, blood samples were available from 75% of the participants, and urine samples were available from 88%. All samples were transported in a Styrofoam box with ice packs (0–4°C), processed within 6 hours of collection, and stored at −80°C until laboratory assays. Participants have been followed-up for chronic disease outcomes via home visits every 2 to 4 years (overall follow-up rates >92%) and for death outcomes via linkages to the Shanghai Vital Statistic Registry annually (complete rates >99%). Both cohorts are approved by the institutional review boards of the Shanghai Cancer Institute and Vanderbilt University. All participants provided informed consent. The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure. Information regarding the data sharing policy for the SWHS and SMHS can be found at https://www.mc.vanderbilt.edu/swhs-smhs/.

A Nested Case-Control Study of CHD

We drew participants with CHD, including nonfatal myocardial infarction and fatal CHD, and controls from cohort participants who provided a blood and urine sample and reported no history of CHD, stroke, or cancer at baseline. Details on the study design were published previously.[26] In brief, potential cases of incident myocardial infarction were identified during follow-up visits, and those participants' medical records were reviewed by physicians to adjudicate the diagnosis. Fatal CHD cases were identified by reviewing participants' medical records and death certificates with CHD listed as the underlying cause of death (*International Classification of Diseases, Ninth Revision* [*ICD-9*] codes 410–414). A total of 377 cases of confirmed, incident CHD were included. For each case, 2 controls were selected from eligible cohort participants who were free of CHD at the time of case diagnosis, matched by sex, age (±2 years), date of sample collection (±1 month), time of sample collection (morning or afternoon), use of antibiotics in past 7 days (yes or no), and menopausal status (for women). For the current study, we further excluded participants who reported extreme calorie intake (n=12; <500 or >3500 kcal/day for women; <800 or >4200 kcal/day for men) or history of kidney disease (n=59) or who had blood or protein in their urine based on a dipstick test (n=31). After exclusion, 275 cases remained, and we randomly selected 1 matched control for each case for the current metabolomics study given limited study funding.

Laboratory Assays

Urinary concentrations of metabolites were quantified at the Vanderbilt University Mass Spectrometry Research Center with an Acquity ultraperformance liquid chromatography system (Waters) interfaced with a TSQ Quantum triple-stage quadrupole mass spectrometer (Thermo Scientific) using heated electrospray ionization operating in multiple reaction monitoring mode. Briefly, a 20-μL urine sample was added to an 80-μL acetonitrile:water (2:1) solution containing stable isotope–labeled internal standards: TMAO-d_9, choline-d_4, betaine-d_3, and carnitine-d_3. After centrifuging at 10 000*g* for 10 minutes, 90 μL of supernatant was transferred and injected into the ultraperformance liquid chromatography system. Chromatographic separation was performed with a Zic-cHILIC column (3 μm, 150×2.1 mm; Merck SeQuant) at a flow rate of 300 μL/min. The mobile phases were (1) 15 mmol/L ammonium acetate with 0.2% acetic acid in water:acetonitrile (90:10, vol/vol) and (2) 15 mmol/L ammonium acetate with 0.2% acetic acid in acetonitrile:water:methanol (90:5:5, vol/vol). The gradient was as follows in mobile phase B: 0 minute, 85%; 2 minutes, 85%; 5 minutes, 30%; 9 minutes, 30%; 11 minutes, 85%; 20 minutes, 85%. Spray voltage was set to 5 kV. Capillary and vaporizer temperatures were 300°C and 185°C, with sheath and auxiliary gases set to 60 and 45 psi, respectively. The skimmer offset was set to −10 V, and collision pressure was 1.8×10^{-3} mm Hg. Optimized multiple reaction monitoring parameters are shown in Table 1. Calibration curves were created for each metabolite with detection limits for TMAO (5 μmol/L), choline and betaine (10 μmol/L), and carnitine (500 nmol/L). Interassay coefficients of variation were found for TMAO (4.5%), choline (3.0%), betaine (5.9%), and carnitine (8.5%).

Urinary creatinine was measured using a Roche-Cobas MiraPlus chemistry analyzer. Plasma concentrations of total cholesterol, HDL (high-density lipoprotein) cholesterol, triglycerides, and hs-CRP were measured, as described previously.[26] To control for batch variation and potential bias, samples of each case–control pair were analyzed in the same assay run with a random arrangement, and laboratory personnel were blind to the case–control status of the samples.

Covariate Assessments

Detailed information was collected on participants' sociodemographics (eg, age, sex, and education), lifestyle (eg, cigarette smoking, alcohol drinking, and physical activity), medical history (eg, diabetes mellitus, hypertension, and dyslipidemia), and diet (both habitual and recent food intake) at study enrollment. Specifically, food intake during the past 12 months was assessed using validated semiquantitative food-frequency questionnaires.[27,28] Total caloric and common nutrient intakes were calculated based on the Chinese Food Composition Tables 2002.[29] Choline and betaine intakes were calculated based on the US Department of Agriculture database for the Choline Content of Common Foods (release 2).[19] Details

Table 1. Optimized Multiple Reaction Monitoring Parameters for TMAO, Choline, Betaine, and Carnitine

Metabolite	Parent Mass (m/z)	Product Mass (m/z)	Collision Energy (V)	Tube Lens (V)
TMAO	76	76	8	56
TMAO-d_9	85	85	2	56
Choline	104	104	8	69
Choline-d_4	108	108	2	56
Betaine	118	118	2	69
Betaine-d_3	121	121	2	62
Carnitine	162	103	16	62
Carntine-d_3	165	103	20	83

TMAO indicates trimethylamine-N-oxide.

on estimation of trimethylamine nutrient intake among our cohorts were described previously.[23] Total trimethylamine intake was the sum of choline, betaine, and carnitine intake. Trimethylamine intake from animal- or plant-based foods was estimated. We also evaluated participants' overall dietary quality by their adherence to Dietary Approaches to Stop Hypertension (DASH),[30] which has been associated with ≈40% reduced CVD mortality among our cohorts.[31] Furthermore, participants were asked questions regarding intake of selected foods in the past 7 days and the past 24 hours before biospecimen collection, including the number of times that soy foods, deep-fried wheat or rice products, and deep-fried or stir-fried meat or fish (including red meat, poultry, and fish) were consumed.

Statistical Analyses

A series of data-processing procedures was performed before analysis. Undetected metabolite concentrations (n=3 for TMAO and n=5 for betaine) were assigned half of the minimum of nonmissing values. Metabolite concentrations were standardized by urinary creatinine concentration and expressed as nanomoles per milligram of creatinine. Levels of metabolites, blood lipids, and hs-CRP were log-transformed to improve normality of their distributions. Nutrient and food intakes were adjusted for total caloric intake and standardized to intakes per 2000 kcal. Missing data on covariates (all found in <10 participants) were imputed by the median for continuous variables and the mode for categorical variables.

Baseline characteristics and metabolite levels were compared among cases and controls using a paired *t* test for continuous variables and the Cochran–Mantel–Haenszel test for categorical variables. Associations of trimethylamine metabolites with baseline characteristics, CVD biomarkers, and dietary intakes were evaluated using a general linear model with adjustment for age, sex, time since last meal (hours), recent use of antibiotics (yes or no), and total calorie intake (diet-related analyses only). In the general linear model, metabolites, biomarkers, and dietary intakes were standardized by mean±SD. Conditional logistic regression was used to estimate odds ratios (ORs) and 95% CIs for CHD risk related to levels of trimethylamine metabolites by quartiles and per SD increase. Quartiles were based on sex-specific distributions among controls. *P* for trend was obtained by modeling the median value of each quartile as a continuous variable. Covariates in model 1 included age, obesity (body mass index ≥27.5 according to World Health Organization [WHO] criteria for Asians),[32] DASH diet score, leisure-time exercise (metabolic equivalents), smoking pack-years, history of hypertension (including use of antihypertensive medications), history of diabetes mellitus, and total menstruation years in women. Model 2 further included central obesity (waist–hip ratio ≥0.85 for women and ≥0.95 for men, according to WHO criteria for Asians),[33] dyslipidemia (self-reported history or measured total cholesterol ≥240 mg/dL, LDL ≥160 mg/dL, HDL <50/40 mg/dL for women/men, or triglycerides ≥200 mg/dL, according to the US National Cholesterol Educational Program),[34] and low-grade inflammation (hs-CRP >1 mg/L, median among controls, similar to suggested optimal cutoff for hs-CRP in general Chinese populations).[35] Model 3 further included intake of saltwater fish and shellfish (sex-specific quartile) and deep-fried meat or fish (times per week). Covariates were chosen based on our prior knowledge of major CHD risk factors and their associations with TMAO level and CHD risk in our cohorts.

Stratified analyses were conducted to evaluate the TMAO–CHD association by CHD risk factors and TMAO food sources (eg, meat versus fish versus plant foods). A product term of metabolite levels (dichotomized by sex-specific median among controls or as a continuous variable) with the stratified variable was added to the fully adjusted model. *P* value for interaction was obtained from the product term; meanwhile, OR and 95% CI in each stratum were obtained by specifying the level of stratified variable. Sensitivity analyses were conducted among participants without recent use of antibiotics (241 case–control pairs). Restricted cubic splines were used to test potential nonlinear associations. Three knots at the 5th, 50th, and 95th percentiles were chosen based on the goodness of model fit (Akaike information criterion). Data analyses were performed using SAS (version 9.4; SAS Institute). A 2-sided $P<0.05$ was considered statistically significant.

Results

Compared with controls, men and women who developed CHD had significantly higher waist–hip ratios, prevalence of hypertension, and hs-CRP levels at baseline (Table 2). Women with CHD had a higher prevalence of diabetes mellitus but lower DASH diet scores and shorter total years of menstruation; men with CHD had higher body mass index and total and LDL cholesterol, and triglyceride levels. No significant case–control difference was found in cigarette smoking, self-reported history of dyslipidemia, intake of trimethylamine nutrients, or urinary levels of TMAO and other metabolites.

We observed significant positive associations of TMAO level (β reflects SD changes in log-TMAO) with female sex (β=0.19; Table 3), central obesity (β=0.21), dyslipidemia (lipid-defined, β=0.24), and high hs-CRP (β=0.19) and negative associations with hypertension or antihypertensive medication (β=−0.19) and antibiotics (β=−0.34). We also observed significant associations of choline and betaine with history of diabetes mellitus (β=0.60 and β=1.21, respectively), betaine with central obesity (β=0.22) and history of dyslipidemia (β=0.30), and carnitine with menstruation years in women (β=−0.27). TMAO and related metabolites generally showed no associations with lifestyles including smoking, alcohol drinking, exercise, and

Table 2. Baseline Characteristics and Metabolite Concentrations by Incident Coronary Heart Disease Status in the SWHS and SMHS*

	Women, n=148 Pairs			Men, n=127 Pairs		
Baseline Characteristics	Cases	Controls	*P* Value	Cases	Controls	*P* Value
Age, y	62.3±7.3	61.9±7.3	0.001	62.2±8.7	62.2±8.7	0.91
BMI, kg/m^2	25.7±3.9	25.2±3.5	0.21	24.8±3.4	23.8±3.3	0.01
WHR	0.85±0.06	0.83±0.06	0.004	0.92±0.06	0.90±0.06	0.008
DASH diet score	45.1±7.5	46.8±6.5	0.04	43.8±8.3	43.4±8.6	0.76
Cigarette smoking, pack-year	2.0±7.5	1.0±4.8	0.15	21.6±23.4	18.3±20.6	0.25
Exercise, MET-h/wk	1.4±3.4	1.1±1.5	0.25	1.4±2.3	1.3±2.0	0.90
Years of menstruation	29.9±6.7	31.4±4.7	0.02	…	…	…
History of diabetes mellitus	24 (16.2)	12 (8.1)	0.03	16 (12.6)	16 (12.6)	0.99
History of hypertension	75 (50.7)	42 (28.4)	<0.0001	77 (60.6)	44 (34.7)	<0.0001
History of dyslipidemia	17 (11.5)	13 (8.8)	0.44	18 (14.2)	12 (9.5)	0.24
Dietary TMA intakes†						
Total TMA, mg/d	401±119	415±121	0.31	433±118	429±124	0.79
Animal-sourced TMA, mg/d	189±93	200±86	0.32	208±92	216±97	0.50
Plant-sourced TMA, mg/d	212±71	215±73	0.68	226±69	214±69	0.19
Choline, mg/d	321±106	338±100	0.13	343±118	338±116	0.76
Betaine, mg/d	66±43	61±35	0.36	82±47	83±47	0.92
Carnitine, mg/d	15±9	16±11	0.32	20±12	20±12	0.75
Blood CVD biomarkers						
Total cholesterol, mg/dL	187.7 (181.9, 193.6)	181.2 (175.7, 186.9)	0.13	185.3 (179.5, 191.2)	169.9 (164.6, 175.4)	<0.0001
LDL cholesterol, mg/dL	103.9 (99.1, 108.8)	99.2 (94.7, 103.9)	0.17	105.8 (100.0, 111.8)	92.2 (87.2, 97.5)	0.0006
HDL cholesterol, mg/dL	41.6 (40.1, 43.1)	43.4 (41.9, 45.0)	0.10	35.8 (34.3, 37.4)	37.4 (35.8, 39.0)	0.14
Triglycerides, mg/dL	189.0 (172.7, 206.7)	169.1 (154.5, 185.0)	0.07	209.9 (188.5, 233.7)	171.3 (153.9, 190.7)	0.006
hs-CRP, mg/L	1.16 (0.90, 1.50)	0.77 (0.59, 0.99)	0.01	1.99 (1.53, 2.60)	1.03 (0.79, 1.34)	0.0001
Urinary TMA metabolites‡						
TMAO, nmol/mg creatinine	391.1 (326.8, 468.1)	351.3 (293.3, 420.7)	0.24	334.6 (286.9, 390.2)	283.6 (242.7, 331.4)	0.20
Choline, nmol/mg creatinine	70.4 (63.1, 78.7)	68.5 (61.3, 76.4)	0.65	69.6 (62.4, 77.6)	65.5 (58.7, 73.0)	0.40
Betaine, nmol/mg creatinine	115.8 (100.2, 133.9)	101.8 (88.1, 117.7)	0.19	130.3 (109.5, 155.1)	122.9 (103.3, 146.3)	0.64
Carnitine, nmol/mg creatinine	38.0 (31.9, 45.2)	38.5 (32.4, 45.8)	0.90	35.5 (29.5, 42.6)	35.8 (29.8, 42.9)	0.95

BMI indicates body mass index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MET, metabolic equivalent; SMHS, Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study; TMA, trimethylamine; TMAO, trimethylamine N-oxide; WHR, waist–hip ratio.

*Data were mean±SD, number (percentage), or geometric mean (95% CI). *P* values were obtained by using a paired *t* test for continuous variables and the Cochran–Mantel–Haenszel test for categorical variables.

†Dietary intakes were adjusted for total energy intake and standardized to intakes per 2000 kcal.

‡Urinary metabolite concentration was standardized by creatinine concentration and expressed as nmol/mg creatinine. TMAO concentration was shown after excluding participants who reported recent use of oral antibiotics.

DASH score. Among dietary intakes, TMAO was associated with animal-based trimethylamine (β=0.09) but not plant-based trimethylamine and with saltwater fish (β=0.19) and shellfish (β=0.08) but not freshwater fish, red meat, poultry, eggs, dairy, soy foods, or legumes. TMAO was strongly associated with deep-fried meat and fish consumption (β=0.36 for once or more per week versus none, β=0.25 for consumption 1 additional time per week, and β=0.41 for consumption 1 additional time in past 24 hours). In contrast, TMAO was not associated with stir-fried meat or fish or deep-fried wheat or rice products, and other metabolites were not associated with deep-fried meat or fish.

Urinary TMAO, but not its precursor metabolites, was associated with risk of CHD (Table 4). The OR for the highest versus lowest quartiles of TMAO was 1.91 (95% CI, 1.08–

Table 3. Associations of Urinary TMA Metabolites With Baseline Characteristics, Cardiometabolic Biomarkers, and Dietary Intakes*

	TMAO		Choline		Betaine		Carnitine	
Baseline Characteristics	β	*P* Value	β	*P* Value	β	*P* Value	β	*P* Value
Age, y	−0.005	0.38	0.009	0.09	0.008	0.14	−0.008	0.12
Sex, female vs male	0.19†	0.03†	−0.04	0.66	−0.16	0.06	−0.06	0.50
BMI, 1 kg/m^2	0.02	0.17	−0.02	0.14	0.004	0.97	−0.007	0.56
Obesity‡	0.07	0.51	−0.08	0.45	−0.15	0.16	−0.23†	0.02†
WHR, 0.1 U	0.11	0.12	0.10	0.15	0.15†	0.03†	−0.09	0.20
Central obesity‡	0.21†	0.02†	0.16	0.08	0.22†	0.01†	−0.06	0.48
DASH diet score	0.003	0.54	−0.007	0.20	−0.005	0.34	0.009	0.11
Ever smoked cigarettes	−0.11	0.30	−0.09	0.40	−0.15	0.17	−0.04	0.71
Smoking, 10 pack-years	−0.04	0.15	−0.04	0.19	−0.04	0.13	−0.004	0.87
No leisure-time exercise	−0.09	0.34	−0.08	0.37	−0.10	0.25	0.05	0.55
Exercise, MET-h/wk	0.01	0.52	0.04†	0.01†	0.02	0.17	−0.01	0.45
Alcohol, drink/d	−0.03	0.36	−0.04	0.12	−0.0003	0.99	0.02	0.45
Postmenopause	−0.07	0.83	−0.45	0.14	−0.22	0.42	0.05	0.88
Years of menstruation	0.10	0.49	0.07	0.61	0.14	0.31	−0.27†	0.05†
History of diabetes mellitus	0.15	0.24	0.60†	<0.0001†	1.21†	<0.0001†	−0.18	0.17
History of hypertension	−0.18†	0.04†	−0.12	0.15	−0.05	0.55	−0.01	0.90
Antihypertensive medication	−0.19†	0.04†	−0.11	0.25	−0.09	0.33	0.009	0.93
History of dyslipidemia, self-reported	−0.14	0.32	0.15	0.28	0.30†	0.03†	−0.03	0.84
Recent use of NSAID	−0.19	0.15	−0.26	0.05	−0.30†	0.03†	−0.12	0.39
Time since last meal, h	−0.02	0.18	−0.02	0.11	−0.01	0.45	−0.03	0.03
Recent use of oral antibiotics	−0.34†	0.05†	0.26	0.14	−0.10	0.54	0.23	0.19
CVD biomarkers								
Total cholesterol	0.05	0.27	−0.02	0.60	0.07	0.09	−0.002	0.96
LDL cholesterol	0.04	0.37	−0.04	0.38	0.01	0.82	0.03	0.49
HDL cholesterol	−0.10†	0.03†	−0.01	0.76	−0.03	0.58	0.06	0.16
Triglyceride	0.09†	0.04†	0.05	0.25	0.14†	0.002†	−0.07	0.09
Dyslipidemia, lipids-defined‡	0.24†	0.02†	−0.004	0.97	0.11	0.33	−0.10	0.36
hs-CRP	0.05	0.25	0.05	0.25	0.01	0.82	0.006	0.89
High hs-CRP‡	0.19†	0.03†	0.03	0.71	0.004	0.96	0.005	0.95
TMA nutrients								
Total TMA	0.08	0.07	0.07	0.08	0.19†	<0.0001†	−0.008	0.85
Animal-based TMA	0.09†	0.03†	0.05	0.23	0.15†	0.0004†	−0.007	0.88
Plant-based TMA	0.01	0.78	0.06	0.15	0.12†	0.004†	−0.005	0.91
Total choline	0.09†	0.04†	0.08	0.07	0.18†	<0.0001†	−0.04	0.33
Animal-based choline	0.10†	0.02†	0.06	0.17	0.16†	0.0003†	−0.01	0.77
Plant-based choline	0.03	0.51	0.06	0.13	0.12†	0.007†	−0.07	0.11
Betaine	−0.01	0.83	0.01	0.76	0.07	0.11	0.09†	0.04†
Carnitine	−0.03	0.50	0.01	0.77	0.08	0.06	0.05	0.27
Usual food intakes								
Red meat	−0.06	0.17	−0.02	0.68	0.04	0.36	−0.01	0.82

Continued

Table 3. Continued

Baseline Characteristics	TMAO		Choline		Betaine		Carnitine	
	β	*P* Value	β	*P* Value	β	*P* Value	β	*P* Value
Poultry	0.03	0.48	−0.02	0.60	0.01	0.73	0.02	0.65
Egg	0.07	0.08	0.10†	0.02†	0.14†	0.001†	−0.05	0.28
Dairy products	0.02	0.59	0.02	0.57	0.10†	0.02†	0.16†	0.0001†
Total fish	0.17†	<0.0001†	0.006	0.88	0.06	0.14	−0.04	0.39
Saltwater fish	0.19†	<0.0001†	0.04	0.34	0.04	0.31	−0.02	0.73
Freshwater fish	0.04	0.35	0.008	0.86	0.03	0.44	−0.009	0.83
Shell fish	0.08†	0.05†	−0.06	0.15	0.06	0.23	−0.07	0.12
Soy products	0.02	0.72	0.09†	0.03†	0.12†	0.004†	−0.08	0.07
Legumes	0.04	0.41	0.07	0.10	0.07	0.11	−0.02	0.68
Vegetables	0.05	0.23	0.10†	0.02†	0.12†	0.004†	−0.007	0.86
Fruits	0.07	0.11	−0.06	0.18	−0.08	0.09	0.05	0.24
Wheat products	−0.03	0.52	0.003	0.95	0.03	0.43	0.10†	0.02†
Stir-fried meat/fish, daily vs none	0.10	0.47	0.15	0.27	0.19	0.16	0.25	0.07
Deep-fried meat/fish, ≥1/week vs none	0.36†	0.02†	0.04	0.80	0.02	0.89	−0.02	0.92
Deep-fried meat/fish, time per week	0.25†	0.005†	0.04	0.62	0.12	0.18	0.05	0.59
Food intakes before sample collection								
Deep-fried meat/fish, times in past 7 d	0.11†	0.02†	−0.009	0.86	0.08	0.10	−0.009	0.85
Deep-fried meat/fish, times in past 24 h	0.41†	0.0008†	0.009	0.94	0.15	0.22	0.12	0.33
Stir-fried meat/fish, times in past 7 d	0.009	0.46	−0.0002	0.99	0.01	0.38	0.04	0.004
Stir-fried meat/fish, times in past 24 h	0.10	0.11	−0.03	0.61	−0.007	0.91	0.05	0.48
Deep-fried wheat/rice, times in past 7 d	−0.01	0.79	0.04	0.33	0.08	0.08	−0.001	0.98
Deep-fried wheat/rice, times in past 24 h	−0.14	0.29	0.16	0.20	0.12	0.33	−0.02	0.90
Soy products, times in past 7 d	−0.001	0.94	0.01	0.36	0.02	0.12	0.004	0.81
Soy products, times in past 24 h	−0.005	0.93	−0.0006	0.99	0.05	0.43	−0.03	0.62

BMI indicates body mass index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MET, metabolic equivalent; TMA, trimethylamine; TMAO, trimethylamine N-oxide; WHO, World Health Organization; WHR, waist–hip ratio.

*β coefficients and *P* values were obtained from general linear regression of log-transformed and standardized metabolites (nmol/mg creatinine) with adjustment for age, sex, time since last meal (h), and recent use of oral antibiotics (yes/no). For dietary intakes, total energy intake was further adjusted. Biomarkers were log-transformed and standardized by its mean±SD. Usual intakes of TMA nutrients and foods were standardized to 2000 kcal/d. The β coefficients reflect the associations of 1-SD increase in biomarkers or dietary intakes with β±SD increase in metabolites.

†Significant results.

‡Obesity was defined as BMI ≥27.5 according to WHO criteria for Asians. Central obesity was defined as WHR ≥0.85 for women and ≥0.95 for men, according to WHO criteria for Asians. Dyslipidemia was defined as total cholesterol ≥240 mg/dL, LDL ≥160 mg/dL, HDL <50/40 mg/dL for women/men, or triglycerides ≥200 mg/dL, according to the US National Cholesterol Educational Program definitions. High hs-CRP was defined as ≥1 mg/L, median among controls, similar to the suggested optimal cutoff for hs-CRP in general Chinese populations.

3.35) after adjustment for CHD risk factors, including obesity and history of hypertension and diabetes mellitus (model 1, P_{trend}=0.008). Given the significant associations of TMAO with central obesity, dyslipidemia, and high hs-CRP in our study, we further adjusted for them (model 2) and found a slightly attenuated OR of 1.75 (95% CI, 0.98–3.13) for the highest versus lowest quartiles (P_{trend}=0.03). Additional controlling for saltwater fish or shellfish intake slightly strengthened the association (OR: 1.80; 95% CI, 0.99–3.27), whereas additionally controlling for deep-fried meat or fish intake weakened the association (OR: 1.70; 95% CI, 0.94–3.07). In the fully adjusted model 3, the ORs were 1.75 (95% CI, 0.96–3.18) for the highest versus lowest quartiles of TMAO (P_{trend}=0.03) and 1.30 (1.03–1.63) for a 1-SD increase in log-TMAO. Restricted cubic spline analysis suggested a linear association between TMAO and CHD risk (linear, *P*= 0.02; nonlinear, *P*=0.46; overall, *P*=0.03).

No significant effect modifications were found by sex, overall or central obesity, hypertension, dyslipidemia, or hs-CRP level on the TMAO–CHD association. However, history of

Table 4. ORs (95% CIs) of Incident CHD by Levels of Urinary TMA Metabolites*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for Trend	Per SD Increase
TMAO						
Median, nmol/mg creatinine	92.0	202.9	308.5	594.1		
No. of cases/controls	62/70	56/69	75/67	82/69		
Model 1	1.00 (ref)	0.98 (0.56–1.71)	1.64 (0.92–2.90)	1.91 (1.08–3.35)	0.008	1.35 (1.09–1.67)
Model 2	1.00 (ref)	0.98 (0.56–1.72)	1.54 (0.86–2.74)	1.75 (0.98–3.13)	0.03	1.29 (1.04–1.60)
Model 3	1.00 (ref)	0.94 (0.53–1.67)	1.60 (0.89–2.90)	1.75 (0.96–3.18)	0.03	1.30 (1.03–1.63)
Choline						
Median, nmol/mg creatinine	37.8	45.4	62.8	77.5		
No. of cases/controls	72/68	60/69	57/69	86/69		
Model 1	1.00 (ref)	0.95 (0.55–1.63)	0.88 (0.51–1.52)	1.40 (0.81–2.43)	0.28	1.15 (0.92–1.44)
Model 2	1.00 (ref)	0.91 (0.52–1.57)	0.89 (0.51–1.54)	1.28 (0.73–2.25)	0.44	1.12 (0.89–1.40)
Model 3	1.00 (ref)	0.93 (0.53–1.61)	0.90 (0.52–1.57)	1.32 (0.75–2.35)	0.40	1.14 (0.90–1.44)
Betaine						
Median, nmol/mg creatinine	39.6	66.4	98.2	199.1		
No. of cases/controls	70/68	61/69	46/69	98/69		
Model 1	1.00 (ref)	0.78 (0.46–1.32)	0.62 (0.35–1.08)	1.46 (0.81–2.62)	0.18	1.15 (0.92–1.43)
Model 2	1.00 (ref)	0.77 (0.45–1.33)	0.61 (0.35–1.09)	1.37 (0.75–2.49)	0.29	1.13 (0.90–1.41)
Model 3	1.00 (ref)	0.75 (0.43–1.30)	0.61 (0.34–1.09)	1.31 (0.71–2.41)	0.32	1.13 (0.90–1.43)
Carnitine						
Median, nmol/mg creatinine	9.4	19.8	40.7	90.1		
No. of cases/controls	71/68	62/69	74/69	68/69		
Model 1	1.00 (ref)	0.94 (0.54–1.62)	1.19 (0.70–2.01)	0.98 (0.57–1.69)	0.86	1.02 (0.84–1.24)
Model 2	1.00 (ref)	1.01 (0.58–1.77)	1.21 (0.71–2.06)	1.08 (0.62–1.88)	0.67	1.05 (0.86–1.28)
Model 3	1.00 (ref)	0.99 (0.56–1.74)	1.16 (0.68–2.00)	1.09 (0.62–1.92)	0.66	1.05 (0.86–1.28)

CHD indicates coronary heart disease; DASH, Dietary Approaches to Stop Hypertension; OR, odds ratio; TMA, trimethylamine; TMAO, trimethylamine N-oxide.
*Sex-specific quartiles among controls. Conditional logistic regression was conducted by matched case–control pair. Model 1 was adjusted for age, obesity, DASH diet score, leisure-time exercise (metabolic equivalents), smoking pack-years, history of hypertension (including use of antihypertensive medications), history of diabetes mellitus, and total menstruation years in women. Model 2 further included central obesity, dyslipidemia, and low-grade inflammation. Model 3 further included intakes of saltwater fish and shellfish (sex-specific quartile) and deep-fried meat or fish (times/wk).

diabetes mellitus modified the associations of all TMAO-related metabolites with CHD (interaction, all $P<0.05$; Table 5). Strong positive associations were found among diabetic patients, and comparing greater than or equal to versus less than the median gave ORs of 6.21, 5.20, 5.32, and 3.79 for TMAO, choline, betaine, and carnitine, respectively. Among nondiabetic participants, only TMAO showed a significant association, with fully adjusted ORs of 1.56 (95% CI, 1.00–2.43) in comparing greater than or equal to versus less than the median and 1.26 (95% CI, 1.00–1.59) per SD increase (Table 6). The TMAO–CHD association appeared stronger among individuals with low intake of plant-based trimethylamine (OR: 2.20; 95% CI, 1.23–3.94) or low intake of fiber (OR: 2.06; 95% CI, 1.14–3.72) than their counterparts (both interactions, $P=0.10$). The TMAO–CHD association was not influenced by levels of precursor metabolites, but precursor metabolites showed trends of positive associations when TMAO level was high (greater than or equal to the median). If considered jointly, participants with a high TMAO level (high-TMAO producers) showed an OR of 1.66 (95% CI, 0.98–2.81) for CHD compared with those who had a low TMAO level despite a high precursor level (low-TMAO producers). Results were similar when participants who recently used antibiotics were excluded.

Discussion

Our study showed, for the first time, that TMAO, a diet-derived, gut microbial–host cometabolite, is associated with a higher risk of developing CHD in a general population. The positive association was observed after adjusting for major

Table 5. ORs (95% CI) of Incident CHD by Urinary TMA Metabolites Among Subgroups of Participants*

Participant Subgroups	No. of Cases/Controls	TMAO	Choline	Betaine	Carnitine
All participants	275/275	1.73 (1.12–2.66)	1.12 (0.74–1.70)	1.07 (0.72–1.59)	1.13 (0.76–1.70)
Women	148/148	1.98 (1.14–3.44)	1.12 (0.62–2.03)	1.08 (0.63–1.85)	0.99 (0.57–1.72)
Men	127/127	1.43 (0.76–2.68)	1.12 (0.62–2.02)	1.06 (0.60–1.87)	1.33 (0.73–2.42)
(Central) obesity	140/118	1.95 (1.08–3.52)	1.66 (0.92–2.99)	1.48 (0.83–2.63)	1.04 (0.59–1.81)
No (central) obesity	135/157	1.54 (0.86–2.77)	0.78 (0.44–1.37)	0.81 (0.47–1.40)	1.25 (0.72–2.19)
Diabetes mellitus	40/28	6.21 (1.64–23.6)†	5.20 (1.43–18.9)†	5.32 (1.01–28.0)†	3.79 (1.22–11.7)†
No diabetes mellitus	235/247	1.56 (1.00–2.43)	0.93 (0.60–1.45)	0.97 (0.64–1.46)	0.95 (0.61–1.46)
Hypertension	153/88	1.73 (0.94–3.19)	1.30 (0.72–2.34)	1.28 (0.69–2.40)	1.22 (0.66–2.25)
No hypertension	122/187	1.72 (1.00–2.96)	1.00 (0.60–1.69)	0.95 (0.58–1.56)	1.08 (0.65–1.78)
Dyslipidemia	228/214	1.61 (1.01–2.59)	1.07 (0.68–1.69)	0.97 (0.63–1.49)	1.11 (0.72–1.72)
No dyslipidemia	47/61	2.34 (0.90–6.09)	1.37 (0.54–3.49)	1.68 (0.68–4.16)	1.24 (0.51–3.03)
High hs-CRP	176/140	1.59 (0.89–2.82)	1.40 (0.80–2.44)	1.07 (0.65–1.78)	1.16 (0.69–1.93)
Normal hs-CRP	99/135	1.90 (1.02–3.55)	0.81 (0.42–1.58)	1.06 (0.56–1.98)	1.10 (0.60–2.03)
High meat-TMA intake‡	127/138	1.74 (0.93–3.26)	1.01 (0.56–1.83)	1.00 (0.56–1.80)	1.59 (0.89–2.84)
Low meat-TMA intake	148/137	1.72 (0.98–3.01)	1.24 (0.71–2.16)	1.11 (0.67–1.86)	0.83 (0.48–1.43)
High fish-TMA intake‡	147/138	1.75 (0.98–3.11)	1.01 (0.58–1.78)	1.18 (0.70–1.99)	1.32 (0.77–2.26)
Low fish-TMA intake	128/137	1.73 (0.94–3.17)	1.14 (0.66–1.98)	0.93 (0.53–1.63)	1.03 (0.59–1.83)
High plant-TMA intake‡	137/138	1.35 (0.75–2.42)	1.02 (0.58–1.81)	1.32 (0.73–2.37)	1.54 (0.86–2.74)
Low plant-TMA intake	138/137	2.20 (1.23–3.94)†	1.21 (0.68–2.15)	0.89 (0.52–1.53)	0.85 (0.49–1.49)
High deep-fried meat‡	101/103	1.89 (0.95–3.77)	1.37 (0.71–2.66)	1.23 (0.63–2.40)	1.77 (0.91–3.45)
Low deep-fried meat	174/172	1.69 (1.00–2.82)	1.02 (0.61–1.71)	1.01 (0.63–1.60)	0.90 (0.54–1.48)
High saltwater fish/shellfish‡	138/138	1.98 (1.11–3.53)	1.13 (0.64–1.99)	1.37 (0.81–2.34)	1.49 (0.88–2.54)
Low saltwater fish/shellfish	137/137	1.32 (0.75–2.35)	1.10 (0.64–1.91)	0.79 (0.45–1.37)	0.85 (0.49–1.48)
High dietary fiber‡	147/138	1.42 (0.80–2.53)	1.00 (0.56–1.77)	1.01 (0.57–1.79)	1.30 (0.74–2.28)
Low dietary fiber	128/137	2.06 (1.14–3.72)†	1.22 (0.71–2.11)	1.06 (0.61–1.86)	0.94 (0.53–1.67)
High precursor metabolites	147/138	1.99 (1.08–3.66)	…	…	…
Low precursor metabolites	128/137	1.49 (0.83–2.67)	…	…	…
High TMAO	157/136	…	1.05 (0.60–1.82)	1.37 (0.78–2.40)	1.24 (0.73–2.12)
Low TMAO	118/139	…	1.20 (0.68–2.12)	0.82 (0.47–1.45)	0.87 (0.46–1.63)

CHD indicates coronary heart disease; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; TMA, trimethylamine; TMAO, trimethylamine N-oxide.

*ORs for greater than or equal to vs less than sex-specific median among controls. Conditional logistic regression model was conducted by matched case–control pair, adjusting for the same covariates of model 3 in Table 4 and the interaction term between metabolites level and the stratified variable. ORs and 95% CIs were obtained for each stratum. *P* values for interaction term were not significant except that *P* for interactions between metabolites and history of diabetes mellitus were all significant (0.04 for TMAO, 0.01 for choline, 0.05 for betaine, and 0.02 for carnitine).

†Significant results with OR ≥2.

‡High and low dietary intakes were defined by energy-adjusted, sex-specific median consumption.

CVD risk factors and among individuals without metabolic conditions such as diabetes mellitus, hypertension, dyslipidemia, and low-grade inflammation. However, presence of diabetes mellitus significantly strengthened the TMAO–CHD association: high TMAO level was associated with a >6-fold risk of CHD among diabetic participants but a 1.6-fold risk among nondiabetic participants. This study is also the first conducted in a non-Western population, whose TMAO food sources differed from Western populations included in prior studies. We found that TMAO was not associated with plant-based trimethylamine intake, was weakly associated with animal-based trimethylamine, was moderately associated with seafood, and was strongly associated with deep-fried meat or fish. TMAO was also associated with central obesity, dyslipidemia, and low-grade inflammation. However, the TMAO–CHD association remained significant after controlling for these intermediate risk factors and dietary factors. In contrast, TMAO's precursor metabolites (choline, betaine, and

Table 6. ORs (95% CIs) of Incident CHD by Urinary TMA Metabolites (Per SD Increase)*

Participant Subgroups	No. of Cases/Controls	TMAO	Choline	Betaine	Carnitine
All participants	275/275	1.30 (1.03–1.63)	1.14 (0.90–1.44)	1.13 (0.90–1.43)	1.05 (0.86–1.28)
Women	148/148	1.30 (0.97–1.75)	1.13 (0.80–1.60)	1.17 (0.84–1.62)	1.07 (0.82–1.40)
Men	127/127	1.30 (0.94–1.79)	1.17 (0.84–1.56)	1.11 (0.83–1.49)	1.02 (0.76–1.37)
(Central) obesity	140/118	1.29 (0.94–1.76)	1.37 (1.00–1.89)	1.19 (0.89–1.59)	1.03 (0.79–1.34)
No (central) obesity	135/157	1.32 (0.98–1.77)	0.95 (0.70–1.30)	1.08 (0.79–1.47)	1.06 (0.80–1.40)
Diabetes mellitus	40/28	1.88 (0.99–3.68)†	1.76 (1.07–2.90)†	2.29 (1.27–4.13)†	1.57 (0.95–2.61)
No diabetes mellitus	235/247	1.26 (1.00–1.59)	1.02 (0.79–1.32)	1.01 (0.79–1.29)	0.96 (0.77–1.20)
Hypertension	153/88	1.21 (0.89–1.65)	1.10 (0.82–1.49)	1.08 (0.79–1.49)	1.13 (0.84–1.51)
No hypertension	122/187	1.38 (1.03–1.86)	1.17 (0.87–1.58)	1.18 (0.88–1.56)	0.99 (0.76–1.28)
Dyslipidemia	228/214	1.23 (0.97–1.57)	1.11 (0.86–1.43)	1.13 (0.88–1.44)	1.06 (0.85–1.32)
No dyslipidemia	47/61	1.67 (1.03–2.69)	1.28 (0.76–2.13)	1.19 (0.70–2.03)	1.97 (0.63–1.50)
High hs-CRP	176/140	1.23 (0.93–1.62)	1.22 (0.91–1.64)	1.14 (0.87–1.49)	1.12 (0.87–1.44)
Normal hs-CRP	99/135	1.43 (1.00–2.04)	1.03 (0.75–1.44)	1.12 (0.78–1.61)	0.94 (0.70–1.28)
High meat-TMA intake‡	127/138	1.50 (1.09–2.08)†	1.20 (0.87–1.64)	1.15 (0.86–1.53)	1.12 (0.85–1.49)
Low meat-TMA intake	148/137	1.16 (0.87–1.54)	1.08 (0.79–1.48)	1.14 (0.82–1.57)	0.97 (0.74–1.28)
High fish-TMA intake‡	147/138	1.25 (0.96–1.64)	1.18 (0.87–1.60)	1.12 (0.85–1.48)	1.03 (0.78–1.35)
Low fish-TMA intake	128/137	1.50 (1.04–2.16)†	1.05 (0.76–1.44)	1.15 (0.81–1.63)	1.09 (0.82–1.44)
High plant-TMA intake‡	137/138	1.33 (0.97–1.83)	1.09 (0.78–1.50)	1.12 (0.81–1.53)	1.20 (0.91–1.58)
Low plant-TMA intake	138/137	1.28 (0.96–1.70)	1.18 (0.87–1.60)	1.15 (0.84–1.56)	0.90 (0.68–1.20)
High deep-fried meat‡	101/103	1.28 (0.93–1.77)	1.23 (0.85–1.77)	1.44 (1.01–2.06)	1.35 (0.97–1.89)
Low deep-fried meat	174/172	1.34 (1.00–1.79)	1.10 (0.84–1.44)	1.00 (0.76–1.32)	0.90 (0.70–1.17)
High saltwater fish/shellfish‡	138/138	1.37 (1.03–1.81)	1.28 (0.93–1.75)	1.23 (0.91–1.65)	1.14 (0.86–1.51)
Low saltwater fish/shellfish	137/137	1.15 (0.81–1.61)	1.02 (0.76–1.38)	1.02 (0.75–1.40)	0.98 (0.75–1.27)
High dietary fiber‡	147/138	1.27 (0.94–1.71)	1.07 (0.78–1.46)	1.09 (0.82–1.45)	1.08 (0.83–1.40)
Low dietary fiber	128/137	1.34 (1.01–1.81)	1.19 (0.87–1.63)	1.18 (0.85–1.66)	0.99 (0.74–1.32)
High precursor metabolites	147/138	1.34 (1.01–1.78)	…	…	…
Low precursor metabolites	128/137	1.20 (0.89–1.61)	…	…	…
High TMAO	157/136	…	1.15 (0.84–1.57)	1.44 (1.04–1.99)	1.07 (0.81–1.42)
Low TMAO	118/139	…	1.11 (0.81–1.51)	0.89 (0.65–1.20)	0.98 (0.74–1.30)

CHD indicates coronary heart disease; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; TMA, trimethylamine; TMAO, trimethylamine N-oxide.

*ORs for per SD increase in log-transformed metabolites. Conditional logistic regression model was conducted by matched case–control pair, adjusting for the same covariates of model 3 in Table 4 and the interaction term between metabolites and the stratified variable. ORs and 95% CIs were obtained for each stratum. *P* values for interaction term were not significant except that $P_{interaction}$=0.04 for choline with history of diabetes mellitus, $P_{interaction}$=0.01 for betaine with history of diabetes mellitus, and $P_{interaction}$=0.02 for betaine with high/low TMAO.

†Significant results with OR ≥1.5.

‡High and low dietary intakes were defined by energy-adjusted, sex-specific median consumption.

carnitine) were not associated with risk of CHD except among diabetic individuals.

The gut microbiota has been recognized as playing an important role in human cardiovascular health.[36–38] Disturbed gut microbiota, common in industrialized countries and becoming common in fast-developing countries, has been linked with chronic diseases, including CHD.[39] Long-term diet is one of the most important determinants of the gut microbiota.[36,38,40] Diet not only shapes the composition and functionality of gut microbiota but also provides substrates for microbial metabolism. The gut microbial metabolites are able to trigger host signaling pathways and thus affect human cardiovascular health.[41,42] Specifically, TMAO, a gut microbial metabolite of dietary choline and carnitine, has been shown recently to promote atherosclerosis and to predict CVD and death events in clinical cohorts.[1,2] In 2011–2013, Cleveland Clinic investigators first reported that elevated plasma TMAO (the highest versus lowest quartiles) was associated with a ≈50% increased risk of MACE (myocardial infarction, stroke, or death) among cardiology patients, after adjusting for a wide

arrange of CVD biomarkers.[3–6] Their findings have been confirmed in several independent cohorts of CVD, CKD, and diabetic patients.[8–11] In 2017, a meta-analysis summarized that high levels of TMAO predicted relative risk of 1.62 (95% CI, 1.45–1.80) for MACE that includes death outcome (19 studies) and 1.66 (95% CI, 1.35–2.05) for MACE without all-cause death in the definition (6 studies).[2] However, results from patient cohorts might not be held true in general populations. TMAO level has been shown to be significantly elevated in patients with CVD, CKD, or diabetes mellitus.[9,10,43–46] In particular, circulating TMAO level in CKD patients were 5 to 10 times higher than those of general populations (eg, 20 versus 1–4 μmol/L).[9,10,45] Treatment for diseases and potential diet and lifestyle changes after disease diagnosis may have also changed TMAO level, raising concern about reverse causation and confounding bias. Studies have found strong effects of a wide range of medications (eg, antidiabetic, antihypertensive, and NSAIDs) on modulating the gut microbiota and its metabolites.[12–15] In this study, we observed inverse associations of TMAO with antihypertensive medications, NSAIDs, and self-reported dyslipidemia (possibly suggesting the use of lipid-lowering medications) and positive associations of TMAO with blood lipids–defined dyslipidemia and hs-CRP–defined chronic inflammation. It would be difficult to delineate potential influence of diseases and treatments in patient-based cohorts when a majority of participants were taking multiple medications. Therefore, the significant TMAO–CHD association observed in our study, after adjusting for metabolic conditions and medications and in subgroups of disease-free individuals, provides an important piece of epidemiologic evidence to support a potentially causal role of the TMAO pathway in the development of CVD.

As a potential, novel risk factor and target for CVD prevention, it is appealing to investigate factors that influence TMAO level, especially modifiable factors such as diet. Dietary TMAO precursors choline and carnitine are abundant in red meat, poultry, eggs, dairy, and soy products.[19] However, TMAO is also a naturally occurring compound that is abundant in seafood.[20,21] Blood and urinary levels of TMAO reflect an integrated measure of dietary intake, microbial production, and host metabolism but cannot distinguish microbial-generated TMAO from naturally occurring seafood TMAO. Therefore, the food–TMAO associations may vary depending on individuals' habitual diets (determining TMAO food sources and the gut microbiota) and recently consumed foods that contain choline, carnitine, and/or TMAO. Evidence regarding the food–TMAO association remains limited; a few observational studies reported generally weak and possibly population-specific associations.[46–51] Higher blood or urinary TMAO was associated with fish but not meat intake in studies conducted in France, the United Kingdom, Sweden, and Canada[46,47,50,51]; with dairy but not fish, meat, or eggs in a Germany study[49]; and in contrast, with red meat and eggs in US studies (including the Framingham Heart Study, Yang et al., unpublished data, 2019). Among Chinese adults in Shanghai (a coastal city), we found no associations of urinary TMAO with intake of red meat, poultry, eggs, or dairy but weak associations with intake of saltwater fish and shellfish. We also found no association of TMAO with plant-based choline, despite a relatively large amount of consumption (≈300 mg/day, mostly from soy foods).[23] Of note, we observed a strong association of TMAO with deep-fried meat or fish but not with stir-fried meat or fish or deep-fried wheat or rice, suggesting that cooking methods for meat and fish may influence the TMAO level. Fried meat and fish intake has been linked with increased risks of CVD, diabetes mellitus, and cancers.[52–56] Our findings suggest a potential novel mechanistic explanation for the harmful health effects of fried meat and fish via the gut microbiota–related TMAO pathway.

We observed that diabetes mellitus modified the associations of all TMAO-related metabolites with CHD risk. This is in line with results from some prior studies showing stronger associations of these metabolites with MACE among diabetic participants than nondiabetic participants.[5,8] As discussed earlier, diabetes mellitus and antidiabetic medications can alter the gut microbiota, and diabetes mellitus is an established CVD risk factor.[12,15] Thus, it is important to consider diabetes mellitus status and treatments while investigating microbial-related mechanisms in CVD. Our present study included only 68 patients with diabetes mellitus and did not collect treatment information at baseline. Future investigations into the role of TMAO in CVD development among diabetic populations are needed. We also observed that the TMAO–CHD association appeared stronger among individuals with low fiber intake (or the positive association seemed to be attenuated if fiber intake was high). Dietary fiber is a key factor in maintaining a healthy microbial community and influencing its metabolic activities.[57,58] Studies have shown that soluble fiber, especially fermented soluble fiber, reduced TMAO production in mice fed with red meat,[59] and vegans and vegetarians (presumably having high fiber intakes) produced less TMAO than omnivores after consuming carnitine.[4] Total fiber intake was not associated with TMAO level in the current study. However, our observation of a potential diet–gut microbiota interaction on sequential risk of CHD is intriguing and worthy of future research.

A major strength of our study is its prospective and population-based design. Biopecimens were collected, on average, 4 years before CHD diagnosis, and results were similar in stratified analysis by follow-up years (data not shown). All participants were free of CVD, CKD, and cancer at baseline, and the TMAO–CHD association was also significant among individuals without a metabolic condition. Thus, potential reverse causality and confounding due to diseases and treatments are minimized in our study. Furthermore, detailed information on lifestyles and diet (both usual and recent food intake) was available to the study and has rarely

been considered (usually unavailable) in prior clinical studies. A major weakness of our study is a single measurement of metabolites in spot urine samples. We cannot compare urinary TMAO with previous reports that mostly measured circulating TMAO. However, plasma and creatinine-adjusted urinary TMAO are highly correlated (r=0.91),[5] and most participants in this study had no major kidney impairment (based on a urine dipstick test), so we would expect similar findings on the TMAO–CHD association if circulating TMAO were measured. Furthermore, TMAO is a product of dynamic interactions among diet, gut microbiota, and host, which may vary over time.[48,60] Unfortunately, most population-based epidemiological studies collect only biological samples once at baseline. Repeated collections and measurements may be conducted in a subset of participants and used to calibrate biomarkers with large within-person variation.[61] Stronger associations might be observed if repeated samples were available, and measurement errors would be reduced. Finally, although our analyses were based on a priori hypotheses, chance findings due to multiple comparisons cannot be ruled out. Future replication studies are needed.

In summary, elevated urinary level of TMAO is associated with a higher risk of CHD in our study among urban Chinese adults, even after adjustment for traditional CVD risk factors and among those without existing metabolic conditions. Possible interactions were observed between TMAO and diabetes mellitus history and dietary fiber intake, implying potential applications of the diet–gut microbiota–host interplay in CVD prevention.

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Author Contributions

Yu has full access to all the data in the study and takes responsibility for the integrity and the accuracy of the data analysis. Yu, Shu, Zhang, Wang, and Zheng contributed to the study concept and design. Yu, Shu, River, Zhang, Cai, Calcutt, Xiang, Li, Gao, and Zheng contributed to the collection and management of data. Yu analyzed the data and drafted the article. All authors contributed to results interpretation, article revision, and approved the final version of the article.

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Disclosures

None.

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