REVIEW

The Gut Microbial Metabolite Trimethylamine *N*-Oxide and Hypertension Risk: A Systematic Review and Dose–Response Meta-analysis

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ABSTRACT

The gut microbial metabolite trimethylamine *N*-oxide (TMAO) is increasingly regarded as a novel risk factor for cardiovascular events and mortality. However, little is known about the association between TMAO and hypertension. This meta-analysis was conducted to quantitatively assess the relation between the circulating TMAO concentration and hypertension prevalence. The PubMed, Cochrane Library, and Embase databases were systematically searched up to 17 June 2018. Studies recording the hypertension prevalence in members of a given population and their circulating TMAO concentrations were included. A total of 8 studies with 11,750 individuals and 6176 hypertensive cases were included in the analytic synthesis. Compared with low circulating TMAO concentrations, high TMAO concentrations were correlated with a higher prevalence of hypertension (RR: 1.12; 95% CI: 1.06, 1.17; $P < 0.0001$; $I^2 = 64\%$; *P*-heterogeneity = 0.007; random-effects model). Consistent results were obtained in all examined subgroups as well as in the sensitivity analysis. The RR for hypertension prevalence increased by 9% per 5-μmol/L increment (RR: 1.09; 95% CI: 1.05, 1.14; $P < 0.0001$) and 20% per 10-μmol/L increment of circulating TMAO concentration (RR: 1.20; 95% CI: 1.11, 1.30; $P < 0.0001$) according to the dose–response meta-analysis. To our knowledge, this is the first systematic review and meta-analysis demonstrating a significant positive dose-dependent association between circulating TMAO concentrations and hypertension risk. *Adv Nutr* 2020;11:66–76.

Keywords: hypertension, microbial metabolite, trimethylamine *N*-oxide (TMAO), risk factor, meta-analysis

Introduction

It is estimated that the number of hypertensive patients will increase to 1.56 billion by 2025 (1). Despite recent advances in diagnosis and treatment, hypertension, as a common health problem, remains the foremost cause of premature death and disability (2). However, the causes and pathogenesis of this disease are still mysterious, and many risk factors for hypertension remain unexplored.

Recent studies in animal models and human subjects have demonstrated a close relation between the gut microbiota and hypertension (3–5). Alterations in the gut microbiome were found in hypertension (5). Further reports showed that microbial dysbiosis could elicit hypertension (6, 7). In a gut microbiota transplantation experiment, significant increase in blood pressure was found in the Dahl salt-sensitive rats that received gut microbiota from Dahl salt-resistant rats (8). Moreover, a meta-analysis further analyzed the overall combined findings of trials and suggested protective efficacy of probiotics against raised blood pressure (9). Therefore, alterations in the gut microbiota might contribute to the occurrence and development of hypertension.

Subsequent studies suggested that metabolites derived from the gut microbiota play important roles in the pathophysiological changes in the cardiovascular system and kidney (10, 11). Microbial metabolites, including short-chain fatty acids and hydrogen sulfide, have been confirmed to regulate blood pressure in animal models (12, 13). Trimethylamine *N*-oxide (TMAO) is another notable gut microbiota metabolite generated from the oxidation of its precursor

trimethylamine (TMA) by hepatic flavin-dependent monooxygenases (14, 15). Recent studies have highlighted the close relation between high blood concentrations of TMAO and the increased risk of atherosclerosis and major adverse cardiovascular events (16, 17). A high concentration of TMAO influences the metabolism of steroids and bile acid (18), exacerbates vascular dysfunction (19), and facilitates macrophage foam cell maturity and hyperreactivity (17, 20). In addition, TMAO is speculated to enhance hypertension susceptibility because it can prolong the hypertensive effect in an angiotensin II–induced hypertensive model (21). However, no study to date has comprehensively analyzed the potential association between circulating TMAO and hypertension. Hence, we performed the present dose–response meta-analysis of published articles to quantitatively evaluate the relation between circulating TMAO concentrations and the prevalence of hypertension.

Methods

The current meta-analysis was performed in accordance with the recommendations of the PRISMA (Preferred Reporting Items for Systematic Reviews) statement (22). The review protocol is registered in PROSPERO, number CRD42018102714.

Search strategy

The electronic databases PubMed (MEDLINE), Embase, and the Cochrane Library were systematically searched by 2 authors (XG and RZ) up to 19 June 2018. Indexing terms included ("Trimethylamine N-oxide" or "TMAO") and ("patient" or "subject" or "participant") without language restrictions. Additional records were identified by assessing the reference lists of reviews, original reports, and case reports. The initial screening of eligible studies was based on the titles and abstracts. The final determination was made after examination of the full text in terms of the inclusion criteria.

Eligibility criteria

Studies recording or analyzing the proportion of hypertensive patients in a certain population and their circulating TMAO concentrations were included in the meta-analysis. Exclusion criteria were as follows: *1*) duplications or conference abstracts; *2*) missing data and data that were impossible to extract or calculate from the published results; and *3*) animal experiments, review articles, or case reports. The eligibility of the included studies was assessed by 2 reviewers (XG and LZ) independently. Any disputes were resolved by consensus with all the authors.

Data retrieval

Data extraction was completed by 2 reviewers (XG and RZ) independently. A third reviewer (XZ) was invited to examine and resolve the conflicting data. Information extracted from each included study comprised the name of the first author, publication year, country or region where the study was performed, number of participants, hypertension definition, primary characteristics, detailed information on the participants—e.g., sex ratio; age of study subjects; BMI; circulating TMAO concentration; estimated glomerular filtration rate (eGFR); smoking states; proportions of patients with hypertension, diabetes, or dyslipidemia; and different drug use, blood sampling, TMAO measurement, study design, and study period (**Tables 1** and **2**).

Qualitative assessment

Two reviewers (XG and LZ) independently assessed the methodological quality of the included studies. The risk of bias of cohort studies was estimated according to the Newcastle–Ottawa quality assessment scale (NOS) (23). Three major aspects were evaluated using this scale, including selection, comparability, and exposure/outcome, with 8 detailed questions (**Supplemental Table 1**). Studies with ≥6 stars were deemed to be high quality. An 11-item checklist recommended by the Agency for Healthcare Research and Quality (AHRQ) was used to evaluate the methodological quality of the cross-sectional study (**Supplemental Table 2**). An item would be scored "1" if it was answered "YES," and "0" if the answer was "NO" or "UNCLEAR." The final quality assessments were as follows: low quality = 0–3; moderate quality = 4–7; high quality = 8–11.

Statistical analysis

The pooled hypertension prevalence was compared between individuals with high TMAO concentrations (above the median TMAO concentration) and those with low TMAO concentrations (below the median TMAO concentration). If primary studies reported the outcomes per tertile in TMAO concentrations, we compared the hypertension prevalence in the top (high TMAO) compared with the remaining (low TMAO) tertiles of TMAO distribution to harmonize the different presentations of data. RRs and 95% CIs were used to estimate the combined effects. The overall effect was calculated by a Z-test, and $P < 0.05$ (2-tailed) was deemed statistically significant. Potential heterogeneity was assessed by Cochran Q and I^2 statistics, and statistical heterogeneity was defined as $P < 0.05$ and/or $I^2 > 50\%$. A fixed-effects model would be used if $I^2 \leq 50\%$; otherwise, a random-effects model would be employed to calculate the pooled RR estimates.

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Supplemental Tables 1–3, Supplemental Figure 1, and Supplemental References 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/advances/.
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Abbreviations used: AHRQ, Agency for Healthcare Research and Quality; eGFR, estimated glomerular filtration rate; NOS, Newcastle–Ottawa quality assessment scale; TMA, trimethylamine; TMAO, trimethylamine *N*-oxide.

TABLE 1 Characteristics of included studies[1]

Reference	Year	Country	Participants, *n*	Study population	Hypertension definition	Blood sample	TMAO measure method	Study design	Study period
Tang-1 (24)	2017	USA	1216	Patients with T2DM	Hypertension history	Fasting plasma	LC-MS/MS	Single-center, prospective cohort	2010–2017
Senthong-1 (25)	2016	USA	2235	Patients with stable CAD	Hypertension history	Fasting plasma	HPLC-MS/MS	Single-center, prospective cohort	2001–2007
Liu (26)	2018	China	90	Patients with CAD (including ACS and stable angina)	Hypertension history	Fasting plasma	RRLC-QTOF/MS	Single-center, prospective cohort study	June 2012 to June 2014
Tang-2 (27)	2014	USA	720	Patients with stable cardiac disease with a history of HF	Hypertension history	Fasting plasma	LC-MS/MS	Single-center, prospective cohort	2001–2007
Gruppen (28)	2017	The Netherlands	5469	Patients with modestly impaired renal function (urinary albumin concentration ≥10 mg/L)	SBP >140 mmHg, DBP >90 mmHg, and/or medication with antihypertensive agents	Fasting plasma	1D-1H-CPMG	PREVEND cohort	1997–2011
Senthong-2 (29)	2016	USA	821	Patients with PAD	Hypertension history	Fasting plasma	HPLC-MS/MS	Single-center, prospective cohort study	2001–2007
Suzuki (30)	2016	UK	972	Patients with AHF	Hypertension history	N/A	HPLC-MS/MS	Single-center, prospective cohort study	Feb 2006 to Aug 2011
Mafune (31)	2016	Japan	227	Patients who underwent cardiovascular surgery (CAD, valvular heart disease, aortic disease)	SBP ≥140 mmHg, DBP ≥90 mmHg, or medication with antihypertensive agents	Fasting serum	HPLC-APCI-MS/MS	Cross-sectional study	28 Jan 2010 to 29 Oct 2010

[1] ACS, acute coronary syndrome; AHF, acute heart failure; CAD, coronary artery disease; DBP, diastolic blood pressure; HF, heart failure; HPLC-APCI-MS/MS, high-performance liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry; LC-MS/MS, stable isotope dilution liquid chromatography with online tandem mass spectrometry; N/A, not applicable; PAD, peripheral artery disease; PREVEND, Prevention of Renal and Vascular End-Stage Disease; RRLC-QTOF/MS, rapid-resolution liquid chromatography quadrupole time-of-flight mass spectrometry; SBP, systolic blood pressure; TMAO, trimethylamine *N*-oxide; T2DM, type 2 diabetes mellitus; 1D-1H-CPMG, 1-dimensional (1D) proton (1H) Carr–Purcell–Meiboom–Gill (CPMG) spectra using a deconvolution assay.

TABLE 2 Characteristics of study population in the included studies[1]

Reference	Age, *y*	Male, *n* (%)	BMI, kg/m^2	TMAO, μmol/L	eGFR, mL/(min · 1.73 m^2)	Smoking, *n* (%)	Hypertension, *n* (%)	Diabetes, *n* (%)	Dyslipidemia, *n* (%)	ACEI or ARB use, *n* (%)	β-Blocker use, *n* (%)	Loop diuretics use, *n* (%)	GLD use, *n* (%)	LLD use, *n* (%)	Aspirin use, *n* (%)
Tang-1 (24)	64.4 ± 10.2	705 (58.0)	N/A	4.4 [2.8–7.7]	82 [62–94]	766 (63.0)	961 (79.0)	1216 (100.0)	N/A	717 (59.0)	803 (66.0)	N/A	669 (55.0)	778 (64.0)[2]	912 (75.0)
Senthong-1 (25)	63 ± 11	1587 (71.0)	N/A	3.8 [2.5–6.5]	98.7 [74.4–125]	1565 (70.0)	1497 (67.0)	782 (35.0)	N/A	1229 (55.0)	1565 (70.0)	N/A	N/A	1587 (71.0)[2]	1810 (81.0)
Liu (26)	57.9 ± 9.7	66 (73.3)	N/A	1.53 [1.04–2.43]	79.4 ± 14.8	33 (36.7)	52 (57.8)	35 (38.9)	N/A	15 (16.7)	22 (24.4)	N/A	N/A	68 (75.6)[2]	78 (86.7)
Tang-2 (27)	66 ± 10	425 (59.0)	28.4 [25.1–33.1]	5 [3.0–8.5]	72[56–87]	N/A	562 (78.1)	295 (41.0)	N/A	497 (69.0)	497 (69.0)	425 (59.0)	N/A	439 (61.0)[2]	461 (64.0)
Gruppen (28)	53.5 ± 12.0	2661 (48.6)	26.68 ± 4.38	3.2 [1.71–5.70]	96.7 ± 14.8	1511 (27.6)	1811 (33.1)	336 (6.1)	N/A		1169 (21.4)[3]		N/A	523 (9.6)	N/A
Senthong-2 (29)	66 ± 10	542 (66.0)	N/A	4.8 [2.9–8]	78.8 [59.4–90.9]	608 (74.1)	681 (83.0)	353 (43.0)	N/A	493 (60.0)	566 (68.9)	N/A	N/A	575 (70.0)[2]	624 (76.0)
Suzuki (30)	78 [69–84]	593 (61.0)	N/A	5.6 [3.4–10.5]	51 [39–67]	93 (9.6)	243 (25.0)	329 (33.8)	237 (24.4)	N/A	N/A	N/A	N/A	N/A	N/A
Mafune (31)	68 [61–74]	158 (69.6)	23 [21–25]	3.07 [0.09–141.2]	N/A	121 (53.3)	177 (78.0)	62 (27.3)	117 (51.5)	134 (59.0)	81 (35.7)	N/A	9 (4.0)[4]	79 (34.8)[2]	N/A

[1] Data are *n* (%); mean ± SD; or median [IQR] or (range). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; GLD, glucose-lowering drug; LLD, lipid-lowering drug; N/A: not applicable; TMAO, trimethylamine *N*-oxide.
[2] Only statins.
[3] All blood pressure–lowering drugs.
[4] Only insulin.

Subgroup analysis was performed to illuminate the heterogeneity according to the study characteristics, including study location, number of participants, and characteristics of the participants enrolled in each study.

Studies reporting hypertension prevalence with at least 3 TMAO exposure concentrations were included in a dose–response analysis. We presumed that the groups were equally divided if the number of exposed participants was not reported in each stratification of TMAO. The missing number of cases was calculated based on the total number and effect size available in the article. If the median or mean concentrations of circulating TMAO were not indicated in the study, we used the midpoint of each category instead. If the boundaries for the lowest and highest category were open-ended, the midpoint of this category was estimated by assuming the interval was the same as the closest category. For each study, we defined the lowest category of circulating TMAO concentration as a reference dose. Nonlinear and linear associations were examined with a random-effects dose–response meta-analysis. Restricted cubic splines with 3 knots were used to calculate study-specific RR estimates per 1-μmol/L increment in TMAO concentration.

A sensitivity analysis was performed to test the reliability of the results by sequentially eliminating each of the included studies. Potential publication bias was evaluated by the Egger test and Begg test. The funnel plot was provided for visual inspection of any bias. Statistical analyses were accomplished with Stata 13.0 (Stata Corp) and Review Manager (RevMan, Version 5.3; The Cochrane Collaboration).

Results

Literature flow

The initial electronic search of the literature yielded 381 potentially relevant citations. After duplicate removal and title/abstract screening, 76 full-text articles were retrieved for detailed assessment. Of these studies, 68 articles lacked usable data. Finally, 7 cohort studies (24–30) and 1 cross-sectional study (31) were included in the meta-analysis with 11,750 individuals (**Figure 1**).

Study characteristics and quality assessment

The specified characteristics of the included studies as well as their study populations are summarized in Tables 1 and 2. Studies included in this meta-analysis mainly explored the role of circulating TMAO in patients with a high cardiovascular risk, including diabetes mellitus (24), cardiac disease (25–27, 30, 31), kidney disease (28), and peripheral artery disease (29). The average circulating TMAO concentrations in the 7 included studies ranged from 1.53 to 5.6 μmol/L. Most of the 8 publications comprehensively reported the baseline information of the enrolled participants. Four studies (26, 27, 29, 31) with a small sample size (<900) were included. One study (28) tested the TMAO concentration in serum, and 1 study (27) did not clearly indicate whether fasting blood samples were tested in their methods. Overall, 4 studies were

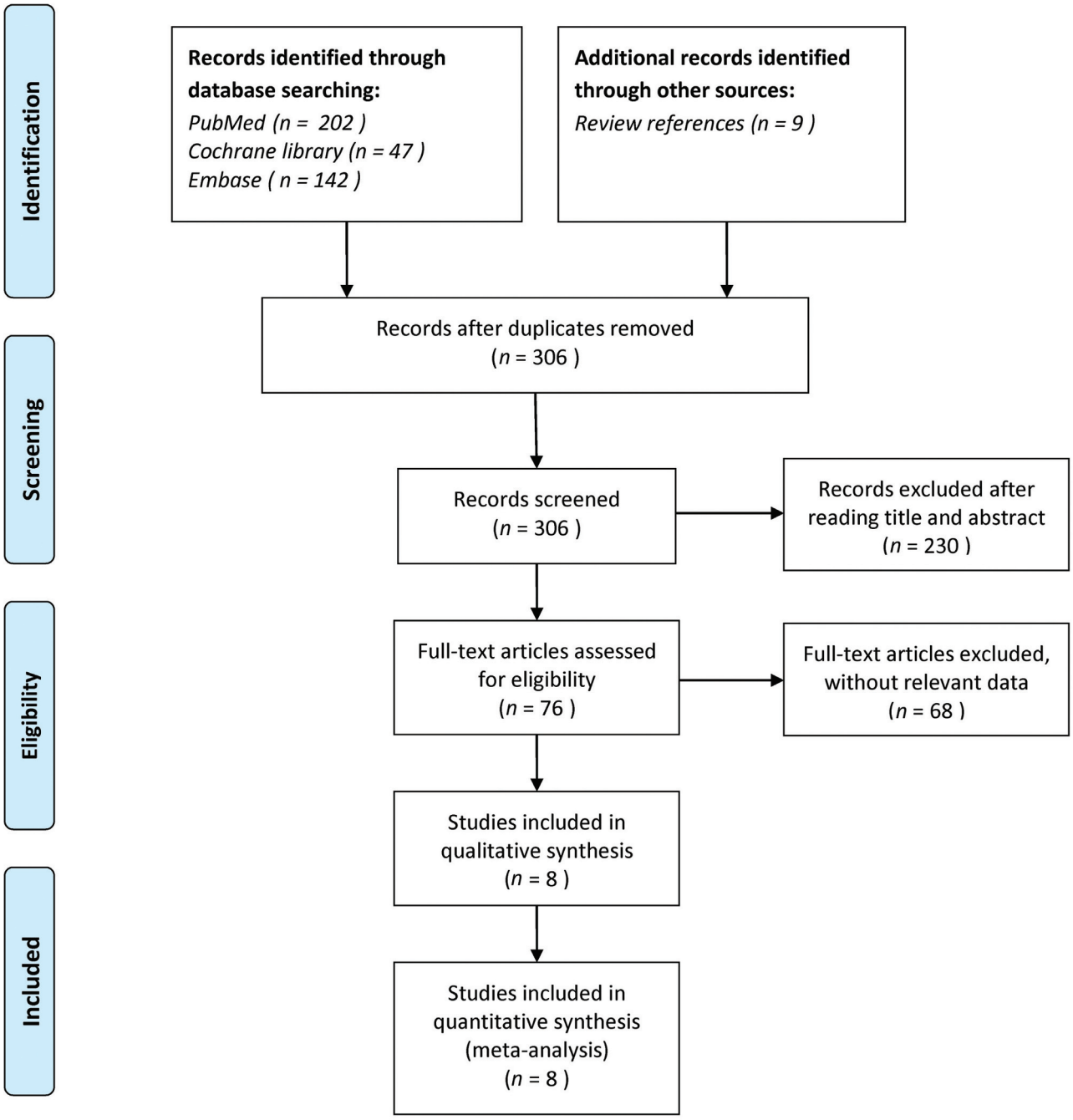

FIGURE 1 Flowchart of study selection for the meta-analysis.

performed in the United States (24, 25, 27, 29), 2 in Europe (28, 30), and 2 in Asia (26, 31).

Study quality was high in most of the included cohort studies, with an average NOS score of 6.7 points. One study (26) with a lower quality had 4 stars (Supplemental Table 1). The cross-sectional study (31) scored 7 points using an 11-item checklist recommended by the AHRQ (Supplemental Table 2).

Circulating TMAO concentrations and hypertension prevalence

The pooled analysis comparing the hypertension prevalence in participants with high and low circulating TMAO concentrations involved 6176 hypertension cases in 11,750 participants (RR: 1.12; 95% CI: 1.06, 1.17; $P < 0.0001$; $I^2 = 64\%$; P-heterogeneity = 0.007; random-effects model; **Figure 2**). The results indicated that a high

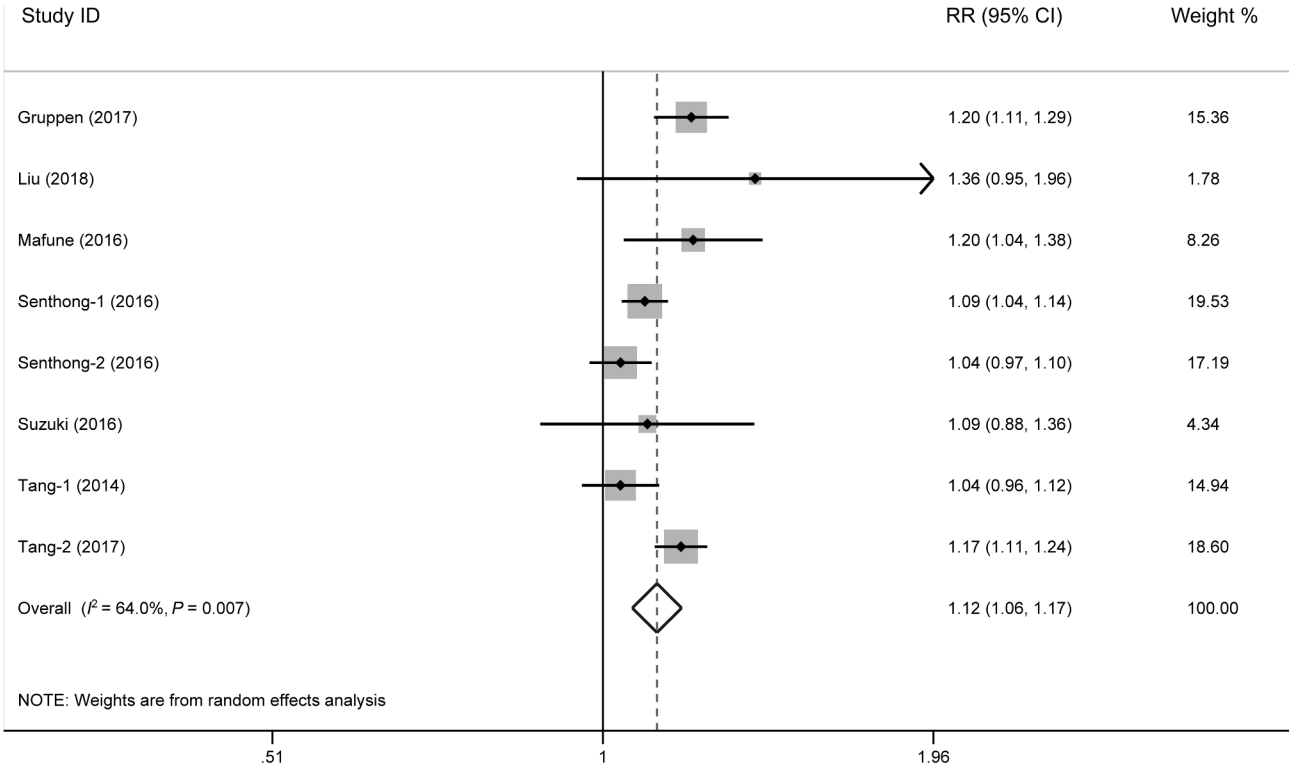

FIGURE 2 Pooled RR of high circulating trimethylamine *N*-oxide (TMAO) concentrations for the risk of hypertension prevalence. I^2 represents the degree of heterogeneity.

circulating TMAO concentration was associated with a higher prevalence of hypertension.

Given the significant heterogeneity, subgroup analysis was performed to explore potential heterogeneity according to different study characteristics, and to validate the effect size in different stratifications at the same time (**Table 3**). Fifteen items were stratified for subgroup analysis to assess the impact of study location, sample size, quality of the included studies, hypertension definition, and characteristics of the target population on heterogeneity. The major heterogeneity was potentially attributed to the target population and proportion of individuals with diabetes in each study because heterogeneity in the subgroups was largely decreased after stratification. In addition, potential residual heterogeneity was derived from the studies with younger participants (i.e., mean age <60 y) and those recruiting more current smokers (i.e., ≥70%) and fewer users of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (i.e., <60%). Generally, the results were not affected by these stratifications, which indicated that the results were robust to some degree.

Sensitivity analysis

Sensitivity analysis was conducted to confirm the association between circulating TMAO concentrations and hypertension prevalence. The pooled RRs were repeated by sequentially removing 1 of the included studies with a random-effects model (**Supplemental Table 3**). None of the studies changed the overall effect of high TMAO concentration on hypertension prevalence. Two studies (29, 31) were found to substantially increase the heterogeneity when we performed the sensitivity analysis. After excluding these studies, the heterogeneity dropped considerably (I^2 = 24%; P-heterogeneity = 0.26), and the pooled RR remained generally unchanged (RR: 1.08; 95% CI: 1.04, 1.12; $P < 0.0001$).

Dose–response association between TMAO and hypertension prevalence

A total of 5 articles (24, 25, 28, 29, 31) were further used to estimate whether there was a dose–response relation between circulating TMAO concentrations and hypertension prevalence. **Figure 3** shows the linear dose–response meta-analysis, because we did not find a significant nonlinear dose–response relation (P-nonlinearity = 0.24). The results demonstrated a pooled RR of 1.02 (95% CI: 1.01, 1.03) per 1-μmol/L increment in TMAO concentrations, 1.09 (95% CI: 1.05, 1.14) per 5-μmol/L increment in TMAO concentrations, and 1.20 (95% CI: 1.11, 1.30) per 10-μmol/L increment in TMAO concentrations, which implies that the hypertension risk could increase by 9% per 5-μmol/L and 20% per 10-μmol/L increment of circulating TMAO concentration.

TABLE 3 Subgroup analysis of high concentration TMAO for hypertension prevalence according to study characteristics[1]

Subgroups	Studies, *n* (references)	Effects model	Overall effect		Heterogeneity	
			RR (95% CI)	*P* value	I^2, %	*P* value
All	8 (24–31)	Random	1.12 (1.06, 1.17)	<0.0001	64	0.007
Study location						
United States	4 (24, 25, 27, 29)	Random	1.09 (1.03, 1.15)	0.004	73	0.01
Europe	2 (28, 30)	Fixed	1.18 (1.10, 1.27)	<0.00001	0	0.45
Asia	2 (26, 31)	Fixed	1.24 (1.08, 1.42)	0.002	0	0.51
Participants, *n*						
<900	4 (26, 27, 29, 31)	Fixed	1.07 (1.02, 1.12)	0.008	48	0.12
≥900	4 (24, 25, 28, 30)	Random	1.14 (1.08, 1.21)	<0.00001	55	0.08
Study quality						
High	6 (24, 25, 27–30)	Random	1.10 (1.05, 1.17)	0.0003	70	0.005
Low/moderate	2 (26, 31)	Fixed	1.24 (1.08, 1.42)	0.002	0	0.15
Target population						
Patients with cardiovascular impairments	6 (25–27, 29–31)	Fixed	1.08 (1.04, 1.12)	<0.0001	24	0.26
Patients with renal impairments	1 (28)	Fixed	1.20 (1.11, 1.29)	<0.00001	—	—
Patients with T2DM	1 (24)	Fixed	1.17 (1.11, 1.24)	<0.00001	—	—
Hypertension definition						
High BP and/or antihypertensive agents	2 (28, 31)	Fixed	1.20 (1.12, 1.28)	<0.00001	0	0.96
Hypertension history	6 (24–27, 29, 30)	Random	1.09 (1.04, 1.15)	0.001	60	0.03
Mean/median age, y						
<65	4 (24–26, 28)	Random	1.15 (1.09, 1.22)	<0.00001	61	0.05
≥65	4 (27, 29–31)	Fixed	1.06 (1.01, 1.12)	0.02	24	0.27
Male, %						
<60	3 (24, 27, 28)	Random	1.14 (1.05, 1.23)	0.003	77	0.01
≥60	5 (25, 26, 29–31)	Fixed	1.09 (1.05, 1.13)	<0.0001	29	0.23
Mean circulating TMAO, μmol/)						
<4	4 (25, 27, 28, 31)	Random	1.16 (1.07, 1.25)	0.0009	59	0.06
≥4	4 (24, 27, 29, 30)	Random	1.08 (1.00, 1.17)	0.04	73	0.01
Mean/median eGFR, mL/(min · 1.73 m^2)						
<90	6 (24, 26, 27, 29–31)	Random	1.11 (1.03, 1.19)	0.004	64	0.02
≥90	2 (25, 28)	Random	1.14 (1.02, 1.26)	0.02	82	0.02
Current smoker, %						
<70	5 (24, 26, 28, 30, 31)	Fixed	1.18 (1.13, 1.25)	<0.00001	0	0.85
≥70	2 (25, 29)	Fixed	1.07 (1.03, 1.12)	0.0003	35	0.21
N/A	1 (27)	Fixed	1.04 (0.96, 1.12)	0.37	—	—
Hypertension, %						
<60	3 (26, 28, 30)	Fixed	1.19 (1.11, 1.28)	<0.00001	0	0.57
≥60	5 (24, 25, 27, 29, 31)	Random	1.10 (1.04, 1.16)	0.0008	69	0.001
Diabetes, %						
<30	2 (28, 31)	Fixed	1.20 (1.12, 1.28)	<0.00001	0	0.96
30–70	5 (25–27, 29, 30)	Fixed	1.07 (1.04, 1.11)	0.0001	0	0.41
>70	1 (24)	Fixed	1.17 (1.11, 1.24)	<0.00001	—	—
ACEI/ARB use, %						
≤55	2 (25, 26)	Fixed	1.10 (1.05, 1.15)	0.0001	32	0.23
>55	4 (24, 27, 29, 31)	Random	1.10 (1.02, 1.19)	0.01	76	0.006
N/A	2 (29, 30)	Fixed	1.18 (1.10, 1.27)	<0.00001	0	0.45
β-blocker use, %						
<50	2 (26, 31)	Fixed	1.24 (1.08, 1.42)	0.002	0	0.51
≥50	4 (24, 25, 27, 29)	Random	1.09 (1.03, 1.15)	0.004	73	0.01
N/A	2 (28, 30)	Fixed	1.18 (1.10, 1.27)	<0.00001	0	0.45
Statins use, %						
<70	3 (24, 27, 31)	Random	1.13 (1.03, 1.24)	0.01	72	0.03
≥70	3 (25, 26, 29)	Fixed	1.08 (1.04, 1.12)	<0.0001	39	0.19
N/A	2 (28, 30)	Fixed	1.18 (1.10, 1.27)	<0.00001	0	0.45

[1] ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; eGFR, estimated glomerular filtration rate; N/A, not applicable; TMAO, trimethylamine *N*-oxide; T2DM, type 2 diabetes mellitus.

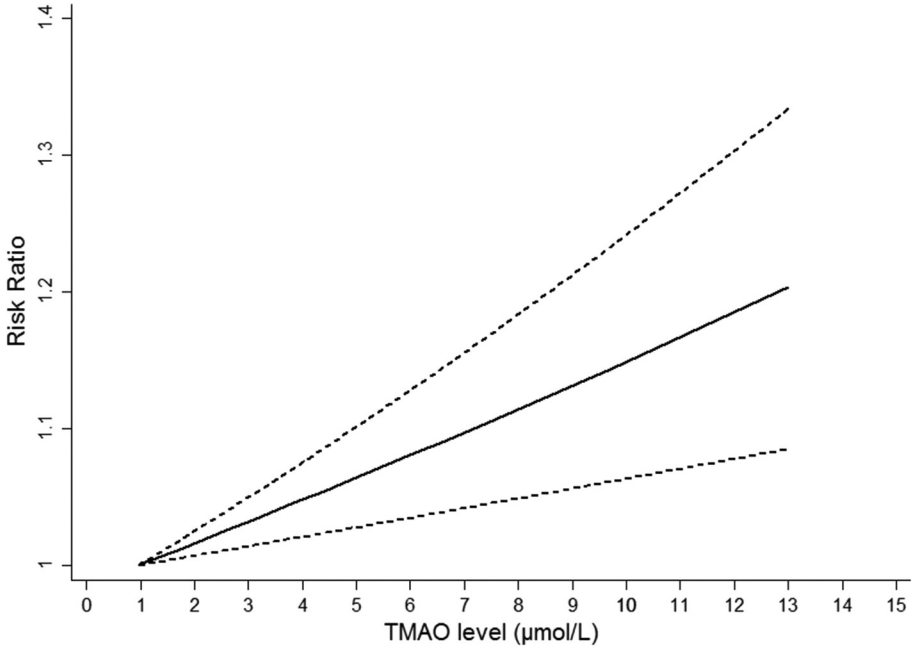

FIGURE 3 Dose–response association between the circulating trimethylamine *N*-oxide (TMAO) concentration and hypertension prevalence. Linear relation (solid line) and 95% CI (dashed lines) of pooled RR of hypertension prevalence by 1 μmol/L increment of circulating TMAO.

Publication bias

There was no evidence of publication bias according to the Begg test ($P = 0.90$) and Egger test ($P = 0.54$) for the meta-analysis of circulating TMAO concentration and hypertension prevalence. No evidence of asymmetry was shown in the funnel plot (**Supplemental Figure 1**).

Discussion

To our knowledge, this is the first meta-analysis to disclose the relation between circulating TMAO concentration and hypertension prevalence in a large population. Compared with people with low circulating TMAO concentrations, those with high TMAO concentrations had a 12% increased risk of hypertension. Subgroup analyses by different stratifications further authenticated the association between TMAO and hypertension. Moreover, a dose-dependent direct association was confirmed between circulating TMAO concentrations and hypertension risk. In general, our study revealed a positive relation between circulating TMAO concentration and increased risk of hypertension.

The high heterogeneity of populations involved in the meta-analysis was the major challenge to clarify the relation between circulating TMAO and hypertension prevalence. Meta-regression analysis was not conducted for heterogeneity detection because only 8 studies were included in the study (32). The present study involved a series of sensitivity and subgroup analyses to explore the potential sources of heterogeneity. As we predicted, several factors potentially contributed to the heterogeneity of this study, especially the sample size, smoking status, target population, and proportion of patients with diabetes in each study. Previous studies found that both smoking and diabetes lead to variations in gut microbiota (33–35). Moreover, the association between TMAO and diabetes has been confirmed in several independent studies (36, 37). It is possible that microbiota variation driven by smoking and diabetes might regulate the circulating TMAO concentrations and consequently contribute to the heterogeneity in the present meta-analysis. Dietary habit is known to significantly influence blood pressure. Previous research showed a positive effect of modest salt reduction and dietary fiber intake on blood pressure (38, 39). Moreover, recent evidence has demonstrated that diet not only affects the gut microbiota (40, 41) but also influences the TMAO concentrations in blood (42, 43). Dietary habits might affect blood pressure by altering the gut microbiota and its metabolites. Notably, variations in diet and gut microbiota rather than genes primarily influenced the TMAO concentration in mice and humans (44). These findings highlight the importance of dietary habits on blood TMAO concentration, and differences in dietary habits (e.g., Eastern compared with Western diets) could affect the variety in TMAO distributions in studies from different locations. However, stratification according to study location did not modify the association between TMAO and hypertension prevalence. It is known that TMAO is cleared by the kidneys. The stratification based on renal function (eGFR) did not change the results either. Subgroup analysis according to different populations also elicited consistent results although substantial heterogeneity of patient populations existed in the current study. Generally, the results remained consistent

across all the subgroups, which further authenticated the significant, positive correlation between circulating TMAO concentration and hypertension prevalence.

The rapid growth in the number of hypertensive patients has led to a heavy economic burden (45). Our dose–response meta-analysis suggested that the RR for hypertension prevalence increased by 9% per 5-μmol/L increment, and by 20% per 10-μmol/L increment in circulating TMAO concentration. It should be noted that even a small decline in blood pressure can have substantial public health benefits and improve cardiovascular health (46, 47). The present results encourage us to explore potential strategies to decrease circulating TMAO concentrations, which could be used as novel effective ways to reduce blood pressure and the future prevalence of hypertension. Foods rich in L-carnitine and phosphatidylcholine, such as eggs (48), red meat (49), and marine fish (50), are all common sources of dietary TMAO, which reminds nutritionists to balance the advantages of the nutrients in such foods with the disadvantages of their metabolites (e.g., TMAO) when they prescribe diets for their patients, especially those with cardiovascular disease, hypertension, and diabetes.

Recent and emerging evidence has revealed the key roles of TMAO in cardiovascular diseases (51). However, relevant scientific work has just begun, with only limited studies conducted to unravel the links between TMAO and hypertension. Major findings addressing the correlation between TMAO and hypertension are as follows: hypertensive patients have more gut microbial enzymes involved in TMA production than those without hypertension (5), and increased permeability of the colon to TMA has been confirmed in hypertensive rats (52). These findings suggest why hypertension is associated with a high concentration of TMAO. Previous reports have shown that TMAO infusion can prolong the hypertensive effect in a hypertensive rat model (21). In addition, TMAO could contribute to cardiovascular diseases by promoting inflammatory responses (53, 54), and the crucial role of immunity in hypertension has been firmly corroborated (55, 56). Therefore, TMAO is probably involved in the pathogenesis of hypertension through multiple pathways.

Because long-term monitoring of circulating TMAO concentrations in subjects before they develop hypertension is absent in existing studies, it is still difficult to determine whether a high circulating TMAO concentration is a triggering factor for hypertension in patients. The association between variation in gut microbiota (as well as its metabolites) and development of hypertension seems to be a classic chicken-and-egg mystery. The above results provide preliminary evidence for the involvement of gut microbial alterations and TMAO in the pathogenesis of hypertension. Our results, derived from clinical data, add new direct evidence that largely confirms the reliability of the association between a high concentration of TMAO and a high risk of hypertension. Further large-scale prospective cohorts are expected to characterize the association, especially the causality in the general population; also, interventional studies could help to determine the role of modulation of TMAO concentrations or its precursors as a novel therapeutic approach for hypertension.

Study strengths

The current meta-analysis firstly elucidated the latent relation between the gut microbe–generated metabolite TMAO and hypertension prevalence. Most of the original articles included in our meta-analysis were of high quality and reported detailed baseline characteristics of the participants. The elaborate information was a major advantage when we tried to explore the sources of heterogeneity. The influences of potential confounders were evaluated in the subgroup analysis. Consistent results were also obtained in the sensitivity analysis. In addition, the risk of hypertension prevalence related to specific, quantitative values of TMAO concentration was estimated in the dose–response analysis.

Study limitations

Several major limitations still warrant consideration. Due to limited reports, all studies included in the present meta-analysis enrolled participants with a high cardiovascular risk, and most of the participants were from the United States. These facts indicate that the current meta-analysis could have potential bias. Further explorations are needed to reveal the relation between TMAO and hypertension risk in more comprehensive populations with long-term follow-ups. Moreover, several important values, such as dietary intake, which might influence the production of TMAO, and the long-term concentrations of TMAO, which are more appropriate to confirm this relation than just a single measurement, were not available in the present included studies. Hypertension history was validated in the included studies, but the blood pressure values of the participants at the time of sample collection were not available in the included studies, thus we cannot evaluate the relation between TMAO concentrations and the severity of hypertension. However, the consistent results derived from multiple stratification analyses (including potential confounders) further authenticated the close correlation between TMAO and hypertension. Further studies are expected to confirm such an association given these limitations, and explore the association with other clinically significant end points such as incidence and severity of hypertension in the general population.

Conclusions

Our meta-analysis suggested a significant positive dose-dependent association between the circulating TMAO concentration and hypertension prevalence regardless of different stratifications. Further studies are expected to explore the causality of the association and determine the value of modulation of TMAO concentrations in hypertension prognosis.

Acknowledgments

The authors' contributions were as follows—HF, XZ: devised the study; XG, RZ: contributed to the acquisition, analysis, and interpretation of data; XG, LZ: conducted the quality assessment; XG: wrote the initial draft of the manuscript; LZ, RZ, PY, ZX, GL, XX: contributed to discussion and revision; and all authors: read and approved the final manuscript.

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