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Gut Microbiota Metabolites and Risk of Major Adverse Cardiovascular Disease Events and Death: A Systematic Review and Meta-Analysis of Prospective Studies

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***Background*—**Gut microbial metabolites have been implicated as novel risk factors for cardiovascular events and premature death. The strength and consistency of associations between blood concentrations of the gut microbial metabolites, trimethylamine-N-oxide (TMAO) and its precursors, with major adverse cardiovascular events (MACE) or death have not been comprehensively assessed. We quantified associations of blood concentrations of TMAO and its precursors with risks of MACE and mortality.

***Methods and Results*—**PubMed and Embase databases were searched up, and a total of 19 prospective studies from 16 publications (n=19 256, including 3315 incident cases) with quantitative estimates of the associations of TMAO with the development of MACE or death were included in our main analysis. Multivariate-adjusted relative risks (RRs) were used when these were available. Elevated concentrations of TMAO were associated with a pooled RR of 1.62 (95% CI, 1.45, 1.80; $P_{heterogeneity}$=0.2; I^2=23.5%) for MACE compared with low TMAO levels, and 1 study of black participants influenced the heterogeneity of the association. After excluding the data of blacks, the RRs were not different according to body mass index, prevalence of diabetes mellitus, history of cardiovascular diseases, and kidney dysfunction. Furthermore, elevated TMAO concentrations were associated with a pooled RR of 1.63 (1.36, 1.95) for all-cause mortality. Individuals with elevated concentrations of TMAO precursors (L-carnitine, choline, or betaine) had an approximately 1.3 to 1.4 times higher risk for MACE compared to those with low concentrations.

***Conclusions*—**Elevated concentrations of TMAO and its precursors were associated with increased risks of MACE and all-cause mortality independently of traditional risk factors. **(*J Am Heart Assoc*. 2017;6:e004947. DOI: 10.1161/JAHA.116.004947.)**

Key Words: major adverse cardiovascular events • meta-analysis • risk • trimethylamine N-oxide

Gut microbiota has been recently implicated as a novel endocrine organ that plays an important role in regulation of host cardiometabolic function through modulating blood levels of bioactive metabolites.[1,2] Recent studies by Wang and Tang et al reported that circulating concentrations of trimethylamine N-oxide (TMAO), a metabolite derived from the gut microbiota, were predictive of prevalent cardiovascular diseases (CVDs)[1] and future cardiovascular events[3] in clinical cohorts. TMAO is a small organic compound, mainly derived from choline (found in foods such as red meat, fish, poultry, and eggs), which is metabolized to produce trimethylamine (TMA) by microbiota[1,4] and then to TMAO by the hepatic enzyme, flavin monooxygenase 3.[1,5]

An increasing number of studies have investigated the associations of circulating levels of TMAO and its metabolic precursors, such as L-carnitine, choline, and betaine, with the risk of major adverse cardiovascular events (MACE) or all-cause death.[1,3,4,6–26] Whereas these studies largely found that elevated blood concentrations of these TMAO-related metabolites were associated with increased risks of the MACE outcomes and death, inconsistent associations have also been observed.[10,22] Differences in characteristics and disease status of participants across studies might affect TMAO levels[14,27,28] and therefore partly explain such inconsistency. In addition, differential adjustment for potential confounders

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Accompanying Tables S1, S2 and Figure S1 are available at http://jaha.ahajournals.org/content/6/7/e004947/DC1/embed/inline-supplementary-material-1.pdf

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Received October 26, 2016; accepted February 10, 2017.

would add variance to the associations. No study has comprehensively analyzed the strength and consistency of associations of TMAO and its precursors with the risk of MACE.

We therefore performed a systematic review and meta-analysis of prospective studies to quantify the association of blood concentrations of TMAO and its precursors, such as L-carnitine, choline, or betaine, with the risks of MACE and death. We examined whether various clinical factors, including traditional cardiovascular risk factors, might modify the associations.

Methods

Data Sources and Search Strategy

We followed the standard criteria for conducting meta-analyses of observational studies and reporting the results.[29] The protocol of the present study was registered with the PROSPERO database: CRD42016052185. We searched MEDLINE (PubMed) and Embase up to December 6, 2016 for eligible studies with a search strategy that combined text word and Medical Subject Headings identifying reports relating blood concentrations of TMAO and the MACE. The following terms were used for the MEDLINE search: (*trimethylamine n-oxide* [text] OR *TMAO* [text]) AND (*atherosclerosis* [text] OR *death* [text] OR *mortality* [text] OR *stroke* [text] OR *heart failure* [text] OR *coronary* [text], *cardiovascular* [text] OR *Cerebrovascular Disorders* [Mesh] OR *Cardiovascular Diseases* [Mesh]). Similar search terms were used for the Embase search. The reference lists of identified articles were also scanned to identify any other relevant studies. No language restrictions were applied. For TMAO precursors, eligible prospective studies were identified if they reported relative risks (RRs) and 95% CIs of categories of L-carnitine, choline, or betaine for the outcome through the search of TMAO. We contacted authors[4,13,18,19,24] to request additional data on number of incident cases, or RRs across categories of TMAO, L-carnitine, choline, or betaine for the risk of MACE. The term MACE is a composite of clinical events that usually includes endpoints reflecting both "safety" and "effectiveness."[30] The "safety" definition includes death, myocardial infarction (MI), or stroke; and the "effectiveness" definition often includes target vessel revascularization, any repeat revascularization, or rehospitalization.[30] There is no standard definition for MACE, and the definition varies by study, whereas most studies include MI and death to define MACE.[30] In the current study, MACE was indicated by composites of MI, stroke, heart transplant, heart failure (HF), other ischemic cardiovascular events, or death (either cardiovascular or all-cause death) as presented in Tables 1 and 2.

Study Selection, Data Extraction, and Quality Assessment

We included studies in this meta-analysis if they satisfied the following criteria: the study design was prospective, the exposure of interest was blood concentrations of TMAO, the outcome was MACE or death, and the investigators reported RRs with 95% CIs for quantitative categories of TMAO levels. Also, studies with available data on an RR and 95% CIs per 1 μmol/L or per 1 SD log-transformed TMAO for the MACE or death were included to perform the dose-response analysis. We excluded reviews, editorials, comments, and nonhuman studies. Studies of other exposures and other disease outcomes, case-control studies, and cross-sectional studies that examined associations of TMAO and markers of atherosclerosis were also excluded. Two reviewers (Y.H. and W.M.) independently extracted data on study design and population characteristics and compared results to ensure consistency. Inconsistencies in data were resolved by a third reviewer (L.Q.). In detail, we extracted the following data from each study: the first author's last name, publication year, country or region where the study was conducted, ethnicity, number of participants and cases, follow-up time, mean or median TMAO concentrations among total study participants or among the highest category for estimating the risk, ranges or interquartile ranges (IQRs) of TMAO, and other characteristics of the study. The original articles used tertiles, quartiles, or quintiles as categories for TMAO levels. We extracted number of cases/N, exposure levels, RRs and 95% CIs for categories of TMAO, and covariates in adjusted models. Quality assessment was performed using to the Newcastle–Ottawa quality assessment scale.[31] We also extracted RRs and 95% CIs across categories of TMAO precursors (L-carnitine, choline, or betaine) for the outcome.

Main Analysis and Secondary Analyses

We performed a main analysis to estimate a pooled RR of MACE associated with high concentrations of TMAO in comparison with low concentrations. Also, we calculated a pooled RR of elevated TMAO concentrations for all-cause mortality alone. The high TMAO group was indicated by the highest tertile,[13,16,19,23,26] the top 2 tertiles[18] (because a RR of the top 2 tertiles vs the lowest tertile was presented in the original study), the highest quartile,* or the highest quintile.[7,22] Results stratified by disease status at baseline or ethnicity were treated as 2 separate data points.[7,11,22] We performed a sensitivity analysis to check whether use of the publications from the same study group would not alter our results. For studies reporting RRs for multiple outcomes (such

*3, 8, 10, 11, 15, 20, 21, 24, 25.

Table 1. Characteristics of Identified Prospective Studies on TMAO Levels and Risk of MACE and Death

Source	Country (Ethnicity*)	Study Population	Age, Mean, y	TMAO, Mean or Median, μmol/L	TMAO Ranges, μmol/L	Men, %	Total, N	Outcomes Assessed	Events, N	Follow-up Duration, y
Tang et al (2013)[3]	US	Patients undergoing elective coronary angiography	63.0	3.7	2.4 to 6.2 (IQR)	64.0	4007	Death, MI, or stroke	513	Up to 3 years
Koeth et al (2013)[4]	US	Patients undergoing elective cardiac evaluation, GeneBank study	62	4.6	…	70	2595	MACE (composite of death, MI, stroke, and revascularization)	975	3 years
Lever et al (2014)[7]	New Zealand (European ancestry, 83.0%)	Coronary Disease Cohort Study (CDCS), participants without diabetes mellitus	68.0	4.8	3.0 to 29.1 (IQR)	72.7	396	All-cause mortality	62	4.96 years (median)
								Acute MI	65	
								Admission for HF	62	
								Unstable angina	56	
								All cardiovascular disease (CVD) events	227	
		CDCS, participants with diabetes mellitus	74.0	7.5	4.4 to 12.1 (IQR)	73.4	79	All-cause mortality	19	4.82 years (median)
								Acute MI	22	
								Admission for HF	23	
								Unstable angina	16	
								All CVD events	56	
Tang et al (2014)[8]	US	Patients with stable heart failure (HF) undergoing cardiac evaluation	66.0	5.0	3.0 to 8.5 (IQR)	59.0	720	All-cause mortality	207	Up to 5 years
Wang et al (2014)[9]	US	Patients undergoing elective diagnostic coronary angiography	63	3.7	2.4 to 6.2 (IQR)	64	3903	MACE (death, MI, stroke)	495	3 years
Kaysen et al (2015)[10]	US (black, 28% whites, 69% of total)	Comprehensive Dialysis Study	61.8	43.0	28 to 67 (IQR)	55.3	235	All-cause mortality	132	4 years (median)
							152	CVD death or hospitalization	48	2.5 years (median)
Tang et al (2015)[11]	US	Patients with CKD who underwent elective diagnostic coronary angiography for cardiac evaluation	70.0	7.9	5.2 to 12.4 (IQR)	48.0	521	All-cause mortality	174	Up to 5 years

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DOI: 10.1161/JAHA.116.004947

Table 1. Continued

Source	Country (Ethnicity*)	Study Population	Age, Mean, y	TMAO, Mean or Median, μmol/L	TMAO Ranges, μmol/L	Men, %	Total, N	Outcomes Assessed	Events, N	Follow-up Duration, y
		Patients without CKD who underwent elective diagnostic coronary angiography	62.0	3.4	2.3 to 5.3 (IQR)	66.0	3166	All-cause mortality	292	Up to 5 years
Tang et al (2015)[12]	US	Patients with chronic systolic HF	57.0	5.8	3.6 to 12.1 (IQR)	75.0	112	All-cause mortality or cardiac transplantation	40	Up to 5 years
Troseid et al (2015)[13]	Norway	Patients with stable HF	57.0	9.2 in patients with dilated cardiomyopathy; 12.1 in patients with stable CAD	1.2 to 124 (range)	83.0	155	All-cause mortality or heart transplantation	55	5.2 years (median)
Mueller et al (2015)[14]	Austria	Patients who underwent coronary angiography	63.0	1.74	0.382 to 3.48 (IQR)	68.0	339	Cardiac death, MI or stroke	99	Up to 8 years
Zhu et al (2016)[15]	US	GeneBank Study,[3] patients who underwent elective coronary angiography	…	…	…	…	4007	MI or stroke	…	Up to 3 years
Suzuki et al (2016)[16]	UK	Patients with acute HF	78.0	5.6	3.4 to 10.5 (IQR); 0.5 to 151.5 (range)	61.0	972	In-hospital mortality	72	Up to 1 year
								All-cause mortality	268	Up to 1 year
								Death or rehospitalization because of HF	384	Up to 1 year
Skagen et al (2016)[17]	Norway	Patients with carotid artery atherosclerosis	67.6	9.77	0.4 to 161.7 (range)	68.6	259	CVD mortality/non-CVD mortality	24/27	5.3 years (mean)
Missailidis et al (2016)[18]	Sweden	Patients with CKD	55.0	53.4	9.3, 170.0 (10th, 90th)	65.0	179	All-cause mortality	51	Up to 5 years
Stubbs et al (2016)[19]	US (whites, 90.9%; blacks, 7.3%; other, 1.8%)	Patients with CKD, Diabetes Genome Project	69.7	6.9	4.8 to 10.9 (IQR); 0.63 to 163.03 (range)	42.7	220	All-cause mortality	36	Up to 4 years
Kim et al (2016)[20]	Canada (whites, 88.7%)	Patients with CKD, CanPREDDICT	68.2	20.41	12.82 to 32.70 (IQR)	62.5	2529	Ischemic cardiovascular events	264	3 years

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DOI: 10.1161/JAHA.116.004947

Table 1. Continued

Source	Country (Ethnicity*)	Study Population	Age, Mean, y	TMAO, Mean or Median, μmol/L	TMAO Ranges, μmol/L	Men, %	Total, N	Outcomes Assessed	Events, N	Follow-up Duration, y
Senthong et al (2016)[21]	US	Patients with stable coronary artery disease	63.0	3.8	2.5 to 6.5 (IQR)	71.0	2235	All-cause mortality	338	Up to 5 years
Shafi et al (2017)[22]	US (whites, 35%; black 65%)	Hemodialysis patients	57.7	101.9 (whites: 98.4; blacks: 103.8)	62 to 124 (whites: 63–120; blacks: 62–125) (IQR); (whites: 6.42–468; blacks: 2.25–682) (ranges)	43.3	1232 (whites: 431; blacks: 801)	Cardiac death	216 (whites: 96; blacks: 120)	2.3 years
								Sudden cardiac death	124 (whites: 54; blacks: 70)	
							1148 (whites: 388; blacks: 760)	First cardiovascular event or any-cause death	626 (whites: 220; blacks: 406)	
							1232 (whites: 431; blacks: 801)	All-cause mortality	550 (whites: 217; blacks: 333)	
Robinson-Cohen et al (2016)[23]	US (whites, 64.5%; blacks, 25.5%; Asian/Pacific Islander 2.9%; American Indian/Native Alaskan 2.0%; Other 5.2%)	Patients with CKD, Seattle Kidney Study	57.3	23.5	>0 to >133 (ranges)	69.0	339	All-cause mortality	45	3.3 years (median)
Ottiger et al (2016)[24]	Switzerland	Community-acquired pneumonia patients	72.0	3.0	1.7 to 5.4 (IQR)	59.7	317	All-cause mortality	143	6.1 years
Senthong et al (2016)[25]	US	Patients with peripheral artery disease	66	4.8	2.9 to 8 (IQR)	66	821	All-cause mortality	222	5 years
Tang et al (2017)[26]	US	Patients with type 2 diabetes mellitus who underwent elective diagnostic coronary angiography	64.4	4.4	2.8 to 7.7 (IQR)	58	1216	All-cause mortality	227	5 years
								Major adverse cardiac events (death, nonfatal MI, and nonfatal stroke)	209	3 years

*Details on ethnicity were provided if reported. CAD indicates coronary artery disease; CKD, chronic kidney disease; CVD, cardiovascular disease; HF, heart failure; IQR, interquartile range; MACE, major adverse cardiovascular events; MI, myocardial infarction; TMAO, trimethylamine N-oxide.

DOI: 10.1161/JAHA.116.004947

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Table 2. RR of MACE According to TMAO Levels in Prospective Studies

Source	Outcome	Comparison	Model	RR (95% CI)	Adjustment for Covariates
Tang et al (2013)[3]	Death, MI, or stroke	Highest quartile (>6.18 μmol/L) vs lowest	Crude	2.54 (1.96, 3.28)	NA
			Multivariate*	1.43 (1.05, 1.94)	Age, sex, smoking, diabetes mellitus, systolic blood pressure, HDL cholesterol, LDL cholesterol, triglycerides, log-transformed hs-CRP, log-transformed eGFR, myeloperoxidase level, BMI, medication history, and angiographic extent of CAD
		1 SD	Crude	1.40 (1.29, 1.51)	NA
			Multivariate	1.30 (1.20, 1.41)	Traditional risk factors and other baseline covariates
Koeth et al (2013)[4]	MACE	Above median (>4.6 μmol/L) of TMAO and median of carnitine (46.8 μmol/L) vs below median of TMAO and carnitine	Crude	2.5 (1.8, 3.4)	NA
			Multivariate	2.1 (1.5, 2.8)	Age, sex, history of diabetes mellitus, smoking, systolic blood pressure, LDL cholesterol, and HDL cholesterol
Lever et al (2014), participants without diabetes mellitus[7]	All-cause mortality	Highest quintile (>12.0 μmol/L) vs nonhighest quintile	Crude	2.7 (1.6, 4.8)	NA
	Admission for HF	Same as the above	Crude*	1.9 (1.1, 3.4)	NA
Lever et al (2014), participants with diabetes mellitus[7]	All-cause mortality	Highest quintile (>12.0 μmol/L) vs nonhighest quintile	Crude	2.7 (1.1, 7.1)	NA
	Acute MI	Same as the above	Crude	4.0 (1.6, 9.8)	NA
	Admission for HF	Same as the above	Crude	4.6 (2.0, 10.7)	NA
	Unstable angina	Same as the above	Crude	9.1 (2.8, 29.7)	NA
	All CVD events	Same as the above	Crude*	2.0 (1.1, 3.6)	NA
Tang et al (2014)[8]	All-cause mortality	Highest quartile (≥8.51 μmol/L) vs lowest	Crude	3.42 (2.24, 5.23)	NA
			Multivariate*	1.85 (1.14, 3.00)	Age, sex, systolic blood pressure, LDL cholesterol, HDL cholesterol, smoking, diabetes mellitus, log-transformed BNP, log-transformed eGFR, and log-transformed hs-CRP
		log-transformed per 1 SD	Multivariate	1.18 (1.06, 1.31)	Traditional cardiac risk factors
Wang et al (2014)[9]	MACE	Above median (>3.7 μmol/L) of TMAO and median of choline (9.8 μmol/L) vs below median of TMAO and median of choline	Crude	2.1 (1.7, 2.7)	NA
			Multivariate	1.6 (1.2, 2.0)	Age, sex, systolic blood pressure, LDL cholesterol, HDL cholesterol, smoking, diabetes mellitus, and hs-CRP
		Above median (>3.7 μmol/L) of TMAO and median of betaine (41.1 μmol/L) vs below median of TMAO and median of betaine	Crude	1.9 (1.5, 2.4)	NA
			Multivariate	1.6 (1.2, 2.0)	Age, sex, systolic blood pressure, LDL cholesterol, HDL cholesterol, smoking, diabetes mellitus, and hs-CRP

Continued

Table 2. Continued

Source	Outcome	Comparison	Model	RR (95% CI)	Adjustment for Covariates
Kaysen et al (2015)[10]	All-cause mortality	Highest quartile (66.6–184 μmol/L) vs lowest	Crude	0.61 (0.38, 0.97)	NA
			Multivariate	1.14 (0.67, 1.93)	Age, sex, race, BMI, diabetes mellitus, log-transformed CRP, serum prealbumin, and serum albumin
		Per log-transformed	Crude	0.84 (0.65, 1.09)	NA
	Cardiovascular death or hospitalization	Highest quartile (>62 μmol/L) vs lowest	Crude	0.71 (0.32, 1.59)	NA
		Highest quartile (66.6–184 μmol/L) vs lowest	Multivariate*	0.92 (0.40, 2.10)	Race, diabetes mellitus, serum prealbumin
		log-transformed	Crude	0.88 (0.57, 1.35)	NA
Tang et al (2015)[11]	All-cause mortality, CKD cohort	Highest quartile (≥12.4 μmol/L) vs lowest	Crude	2.76 (1.74, 4.37)	NA
			Multivariate*	1.93 (1.13, 3.29)	Age, sex, systolic blood pressure, LDL cholesterol, HDL cholesterol, smoking, diabetes mellitus, log-transformed eGFR, and log-transformed hs-CRP
	All-cause mortality, non-CKD cohort	Highest quartile (≥5.3 μmol/L) vs lowest	Crude	2.21 (1.57, 3.12)	NA
			Multivariate*	1.47 (1.02, 2.12)	Age, sex, systolic blood pressure, LDL cholesterol, HDL cholesterol, smoking, diabetes mellitus, log-transformed eGFR, and log-transformed hs-CRP
Tang et al (2015)[12]	All-cause mortality or cardiac transplantation	log-transformed per 1 SD (0.99 μmol/L)	Crude	1.48 (1.10, 1.96)	NA
			Multivariate	1.46 (1.03, 2.14)	Age, eGFR, mitral E/septal Ea, and NT-proBNP
Troseid et al (2015)[13]	All-cause mortality or heart transplantation	Highest tertile vs remaining groups	Crude	2.24 (1.28, 3.92)	NA
			Multivariate	1.79 (0.90, 1.79)	HF severity, age, hypertension, type 2 diabetes mellitus, HF etiology, eGFR, CRP, and NT-proBNP
		Highest tertile (12.8–124 μmol/L) vs lowest	Crude*	1.76 (0.93, 3.33)†	NA
Mueller et al (2015)[14]	Cardiac death, MI, or stroke	1 μmol/L	Multivariate	1.01 (0.984, 1.04)	Age, sex, smoking, metabolic syndrome, HbA1c, CRP, eGFR
Zhu et al (2016)[15]	MI or stroke	Highest quartile (6.18–312 μmol/L) vs lowest	Multivariate	1.64 (1.12, 2.39)	Age, sex, BMI, systolic blood pressure, LDL cholesterol, HDL cholesterol, triglycerides, estimated creatinine clearance, smoking, diabetes mellitus, medication use, and history of CVD

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DOI: 10.1161/JAHA.116.004947

DOI: 10.1161/JAHA.116.004947

Table 2. Continued

Source	Outcome	Comparison	Model	RR (95% CI)	Adjustment for Covariates
Suzuki et al (2016)[16]	Death or rehospitalization because of HF	Highest tertile (8.2–151.5 μmol/L) vs lowest	Multivariate*	2.12 (1.54, 2.93)	Age, sex, history of HF, IHD, hypertension, diabetes mellitus, HF severity, current smoking, edema, atrial fibrillation, systolic blood pressure, heart rate, Hb, respiratory rate, sodium, urea, eGFR, NT-proBNP
		log-transformed per 1 SD	Crude	1.33 (1.20, 1.46)	NA
			Multivariate	1.18 (1.05, 1.33)	Age, sex, history of HF, IHD, hypertension, diabetes mellitus, HF severity, current smoking, edema, atrial fibrillation, systolic blood pressure, heart rate, Hb, respiratory rate, sodium, urea, NT-proBNP
	All-cause mortality	log-transformed per 1 SD	Crude	1.35 (1.21, 1.51)	NA
			Multivariate	1.16 (1.01, 1.33)	Age, sex, history of HF, IHD, hypertension, diabetes mellitus, HF severity, current smoking, edema, atrial fibrillation, systolic blood pressure, heart rate, Hb, respiratory rate, sodium, urea, and NT-proBNP
Skagen et al (2016)[17]	Cardiovascular (MI or stroke) death	log-transformed per 1 SD	Crude	1.81 (1.29, 2.53)	NA
			Multivariate	1.38 (0.91, 2.08)	Age and eGFR
Missailidis et al (2016)[18]	All-cause mortality	Top 2 tertiles (32.2 or more μmol/L) vs lowest	Crude	6.29 (2.67, 14.8)	NA
			Multivariate	6.68 (2.33, 19.1)	Age, sex, diabetes mellitus, and hs-CRP
			Multivariate*	4.32 (1.32, 14.2)	Age, sex, diabetes mellitus, and hs-CRP and GFR
Stubbs et al (2016)[19]	All-cause mortality	Highest tertile (9.26–163.03 μmol/L) vs lowest	Crude*	1.95 (0.91, 4.17)	NA
		10 μmol/L	Crude	1.19 (1.10, 1.29)	NA
			Multivariate	1.26 (1.13, 1.40)	Age, sex, race, smoking, BMI, hypertension, diabetes mellitus, eGFR, CKD stage, triglycerides, cholesterol, history of percutaneous intervention, histories of coronary artery bypass grafting, MI, cerebrovascular accident, peripheral vascular disease and congestive HF

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Table 2. Continued

Source	Outcome	Comparison	Model	RR (95% CI)	Adjustment for Covariates
Kim et al (2016)[20]	Ischemic cardiovascular events	Highest quartile (>32.67 μmol/L) vs lowest	Crude	2.33 (1.63, 3.33)	NA
			Multivariate*	1.37 (0.91, 2.06)	Age, sex, race, diabetes mellitus, cardiovascular comorbidities at baseline, systolic blood pressure, total cholesterol, HDL cholesterol, smoking, medication use, eGFR, ln-transformed ACR and Hb
		Natural log-transformed per 1 SD	Crude	1.45 (1.28, 1.64)	NA
			Multivariate	1.24 (1.07, 1.43)	Age, sex, race, diabetes mellitus, cardiovascular comorbidities at baseline, systolic blood pressure, total cholesterol, HDL cholesterol, smoking, medication use, eGFR, ln-transformed ACR and Hb
Senthong et al (2016)[21]	All-cause mortality	Highest quartile (median, 9.7 μmol/L) vs lowest	Crude	3.90 (2.78, 5.48)	NA
			Multivariate*	1.71 (1.11, 2.61)	Age, sex, systolic blood pressure, LDL cholesterol, HDL cholesterol, smoking, diabetes mellitus, medication use, number of stenotic vessels, log-transformed hs-CRP, log-transformed myeloperoxidase, log-transformed eGFR, and log-transformed BNP
Shafi et al (2017)[22]	Cardiac death, white	Highest quintile (135–468 μmol/L) vs lowest quintile	Crude	1.82 (1.23, 2.69)	NA
			Multivariate*	1.78 (1.12, 2.82)	Age, sex, index of coexisting disease severity score, cause of end-stage renal disease, BMI, systolic blood pressure, albumin, and relative volume removed on dialysis and residual kidney function
	Sudden cardiac death, white	Same as the above	Crude	2.98 (1.38, 6.44)	NA
			Multivariate	2.76 (1.22, 6.24)	Same
	First cardiovascular event or any-cause death, white	Same as the above	Crude	1.53 (1.01, 2.32)	NA
			Multivariate	1.45 (0.99, 2.15)	Same
	Any-cause mortality, white	Same as the above	Crude	1.40 (0.98, 2.00)	NA
			Multivariate	1.50 (1.03, 2.18)	Same
	Cardiac death, black	Highest quintile (135–682 μmol/L) vs lowest quintile	Crude	0.73 (0.49, 1.09)	NA
			Multivariate*	0.78 (0.51, 1.18)	Same
	Sudden cardiac death, black	Same as the above	Crude	0.73 (0.48, 1.11)	NA
			Multivariate	0.80 (0.51, 1.25)	Same

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DOI: 10.1161/JAHA.116.004947

DOI: 10.1161/JAHA.116.004947

Table 2. Continued

Source	Outcome	Comparison	Model	RR (95% CI)	Adjustment for Covariates
	First cardiovascular event or any-cause death, black	Same as the above	Crude	0.99 (0.70, 1.40)	NA
			Multivariate	1.03 (0.80, 1.31)	Same
	Any-cause mortality, black	Same as the above	Crude	0.85 (0.65, 1.11)	NA
			Multivariate	0.89 (0.66, 1.20)	Same
Robinson-Cohen et al (2016)[23]	All-cause mortality	Highest tertile (1.71 μg/mL) vs lowest tertile	Multivariate*	1.25 (0.48, 3.28)	Age, sex, systolic blood pressure, LDL cholesterol, HDL cholesterol, smoking, log-transformed CRP, log-transformed eGFR
Ottiger et al (2016)[24]	All-cause mortality	Highest quartile (median, 8.9 μmol/L) vs lowest quartile	Crude	3.5 (2.1, 5.8)†	NA
			Multivariate*	1.9 (1.1, 3.3)†	CAD, congestive heart failure, cerebrovascular disease, peripheral artery occlusive disease, diabetes mellitus, CKD, neoplastic disease and chronic obstructive pulmonary disease
		log-transformed	Crude	2.3 (1.7, 3.3)	NA
			Multivariate	1.6 (1.01, 2.4)	CAD, congestive heart failure, cerebrovascular disease, peripheral artery occlusive disease, diabetes mellitus, CKD, neoplastic disease, and chronic obstructive pulmonary disease
Senthong et al (2016)[25]	All-cause mortality	Highest quartile (≥8.01 μmol/L) vs lowest quartile	Crude	2.69 (1.82, 3.97)	NA
			Multivariate*	1.59 (1.03, 2.45)	Age, sex, systolic blood pressure, LDL cholesterol, HDL cholesterol, smoking, diabetes mellitus, log-transformed hs-CRP, and log-transformed eGFR
			Multivariate	1.88 (1.21, 2.92)	Age, sex, systolic blood pressure, LDL cholesterol, HDL cholesterol, smoking, diabetes mellitus, history of CAD, statin use, apolipoprotein A1, apolipoprotein B, log-transformed myeloperoxidase, and log-transformed hs-CRP
		log-transformed per 1 SD	Crude	1.53 (1.35, 1.74)	NA
			Multivariate	1.26 (1.03, 1.53)	Age, sex, systolic blood pressure, LDL cholesterol, HDL cholesterol, smoking, diabetes mellitus, log-transformed hs-CRP, and log-transformed eGFR

Continued

Table 2. Continued

Source	Outcome	Comparison	Model	RR (95% CI)	Adjustment for Covariates
Tang et al (2017)[26]	All-cause mortality	Highest tertile (≥6.3 μg/mL) vs lowest tertile	Crude	3.63 (2.53, 5.21)	NA
			Multivariate	1.85 (1.21, 2.84)	Age, sex, history of CVD, systolic blood pressure, LDL cholesterol, HDL cholesterol, smoking, hs-CRP, log-transformed HbA1c, log-transformed eGFR, log-transformed BMI, and history of HF
	MACE	Highest tertile (≥6.3 μg/mL) vs lowest tertile	Crude	3.03 (2.08, 4.42)	NA
			Multivariate*	1.94 (1.23, 3.05)	Age, sex, history of CVD, systolic blood pressure, LDL cholesterol, HDL cholesterol, smoking, hs-CRP, log-transformed HbA1c, log-transformed eGFR, log-transformed BMI, and history of HF

ACR indicates albumin-to-creatinine ratio; BMI, body mass index; BNP, B-type natriuretic peptide; CAD, coronary artery disease; CKD, chronic kidney disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; HF, heart failure; hs-CRP, high-sensitivity C-reactive protein; IHD, ischemic heart disease; LDL, low-density lipoprotein; MACE, major adverse cardiovascular events; MI, myocardial infarction; NA, not applicable; NT-proBNP, N-terminal pro-B-type natriuretic peptide; RR, relative risk; TMAO, trimethylamine N-oxide.

*Data used for main analysis using 19 data points.

†Data from authors.

as cardiovascular outcomes and all-cause mortality),[7,10,22,26] we used RRs for the vascular outcomes in our main analysis, whereas we performed a sensitivity analysis when we used RRs for all-cause mortality. A pooled RR for MACE without including all-cause mortality was also calculated. We explored heterogeneity of the main results according to study characteristics such as length of follow-up, mean (median) age, proportion of smokers, mean or median TMAO levels among the total participants, mean body mass index (BMI), degree of kidney dysfunction, prevalence of diabetes mellitus, or past cardiovascular histories at a baseline examination.

For the analysis of TMAO precursors and risk of MACE, we calculated a pooled RR of elevated concentrations of betaine (highest in comparison with the lowest)[7,9,13,18,26] and that of elevated concentrations of either L-carnitine or choline.[4,9,13,18,26] Because both choline and L-carnitine are metabolized by intestinal bacteria to induce TMA, and then further metabolized to TMAO,[1,4] therefore L-carnitine and choline were combined in our analysis.

Statistical Analysis

We used unadjusted or multivariable-adjusted RRs and 95% CIs that were reported in the original articles and calculated log-RRs and log-standard error for performing our analysis. If studies reported several multivariate-adjusted RRs, we used the effect estimate that was most fully adjusted for potential confounders, except for 2 studies for which we could not calculate the SE of its logarithm in the final model.[13,20] Heterogeneity was assessed by the Cochran Q test and I^2 statistic; low, moderate, and high I^2 values were considered to be 25%, 50%, and 75%, respectively.[32,33] We used a fixed-effect model, using the method of Mantel and Haenszel when there was no significant heterogeneity, and a random effect model when heterogeneity was significant.[34] The cutpoint of *P* value 0.05 was used for assessing significance of heterogeneity. We assessed publication bias by using Begg's and Egger's tests and visual inspection of the funnel plot if 10 or more studies were available.[35]

For the dose-response analysis of TMAO concentrations and risk of MACE, we used RRs and 95% CIs per 1 μmol/L or per 1 SD log-transformed TMAO for the outcome. We also estimated dose-response associations based on data for categories of TMAO levels on median dose, number of cases and participants, and effect estimates with corresponding SEs using the generalized least-squares for trend estimation method of Greenland and Longnecker.[36] If medians for categories of TMAO levels were not reported, approximate medians were estimated using the midpoint of the lower and upper bounds. For categories without upper limit, median values were defined as 1.5 times the lower limit of the category. An original study reported data separately for whites

and blacks; a nonlinear association of TMAO and cardiovascular events was shown in blacks.[22] Therefore, we only included data in whites in our dose-response analysis. Stratified analysis was performed using meta-regression. All analyses were conducted with Stata software (version 14; StataCorp LP, College Station, TX).

Results

Literature Flow

We identified 24 potentially eligible articles, including 2 cross-sectional studies and 22 prospective studies (Figure 1). We also reviewed details of the cross-sectional studies (Tables S1 and S2), whereas these were not included in the meta-analysis. The 22 publications were enrolled from the United States (14 publications), Canada (1 publication), New Zealand, (1 publication), UK (1 publication), Switzerland (1 publication), Sweden (1 publication), Austria (1 publication), and Norway (2 publications). The mean or median value of TMAO ranged from 1.74 to 103.8 μmol/L across the 22 prospective studies listed in Table 1. One study[14] introduced a newly developed method for the measurement of TMAO showing the lowest value of TMAO. Of the 22 studies, 2 only investigated combinations of TMAO and choline, betaine,[9] or L-carnitine[4]; thus we did not include the 2 studies[4,9] in the analysis for TMAO, although these studies were eligible for the analysis of TMAO precursors and risk of MACE. For publications from the same study group, we treated as different data if the number of study participants and outcome measurements were not completely the same. An exclusion of publications from the same group did not alter our results. Two studies[3,15] included the same number of total study participants from the same cohort so that we only used data from the first publication.[3] We observed fundamentally similar results when we used data from the other study.[15] Subsequently, the remaining 19 articles published from 2013 to 2017 assessed the RRs of TMAO (either as a categorical or continuous variable) and risk of MACE. All of the studies included clinical cohorts, including patients at higher cardiovascular risk, that is, enriched for baseline heart disease,[3,8,12–17,21] kidney disease,[10,11,18–20,22,23] peripheral artery disease,[25] or diabetes mellitus.[7,26] Of the 19 publications, a total of 16 publications, including 19 data points, were eligible for our main analysis to calculate a pooled RR of elevated TMAO levels as compared with low levels[3,7,8,10,11,13,16,18–26] (Table 1 and Figure 1).

The RRs for MACE according to TMAO levels in their original studies are summarized in Table 2. Among the prospective studies, 5 studies[10,13,19,20,23] did not find a significantly elevated RR in the highest category of TMAO levels as compared with the lowest category, in either unadjusted or multivariate-adjusted models among total participants. One study showed a significant association of high TMAO levels and elevated risk of outcomes in whites but not in blacks.[22] Also, another 3 studies did not show a significantly elevated RR when TMAO was analyzed as a continuous variable in model.[10,14,17] In most original studies, RRs were carefully adjusted with various traditional cardiovascular risk factors as shown in Table 2. Of the total 22 publications, multivariate-adjusted RRs were available for 21 (95%) publications; of the 19 data points used for the main analysis, 79% (n=15) were multivariate-adjusted data.

TMAO Levels With the Risk of MACE and All-Cause Mortality

Our meta-analysis of elevated TMAO levels and the risk of MACE enrolled 19 256 participants and 3315 incident cases from the 19 data points. The pooled RR of elevated TMAO levels for the development of MACE as compared to low TMAO levels using the fixed-effect model was 1.62 (95% CI, 1.45, 1.80; $P<0.001$; $P_{heterogeneity}=0.2$; $I^2=23.5\%$; Figure 2A). The pooled RR using the random-effect model was 1.62 (95% CI, 1.43 1.85). The Begg and Egger regression tests showed no substantial publication bias ($P>0.1$ for both tests). Of the 19 data points, the study in blacks by Shafi et al[22] affected the heterogeneity; removal of the data in blacks[22] resulted in a pooled RR of 1.70 (95% CI, 1.52, 1.91; $P<0.001$; $P_{heterogeneity}=0.9$; $I^2=0\%$; fixed-effect model; Figure S1A). In results of a sensitivity analysis omitting 1 study at a time and calculating the pooled RRs for the remainder of 18 studies (the data in blacks were not included), the pooled RRs ranged from 1.65 (95% CI, 1.47, 1.86) to 1.75 (95% CI, 1.55, 1.98), and no other study was identified as an influential outlier across the 18 studies ($P_{heterogeneity}$ ranged 0.8–0.9; I^2 was consistently 0%). When we performed an analysis further omitting data from the same group, the pooled RR of high TMAO levels for the development of MACE was 1.73 (95% CI, 1.50, 2.00; $P_{heterogeneity}=0.3$; $I^2=15.4\%$). Of the 18 data points, 4 were unadjusted and 14 were multivariate adjusted in the original studies; when we only included multivariate-adjusted data, the pooled RR was 1.68 (95% CI, 1.49, 1.89; $P<0.001$; $P_{heterogeneity}=0.7$; $I^2=0\%$). Because we primarily used RRs for the cardiovascular outcomes when there were multiple outcomes presented,[7,10,22,26] we also performed a sensitivity analysis for the 18 data when we replaced RRs for all-cause mortality in these publications[7,10,22,26]; the pooled RR was 1.69 (95% CI, 1.52, 1.89; $P<0.001$; $P_{heterogeneity}=0.6$; $I^2=0\%$). In results of further sensitivity analysis removing 12 studies that included RRs for any death or all-cause mortality, a

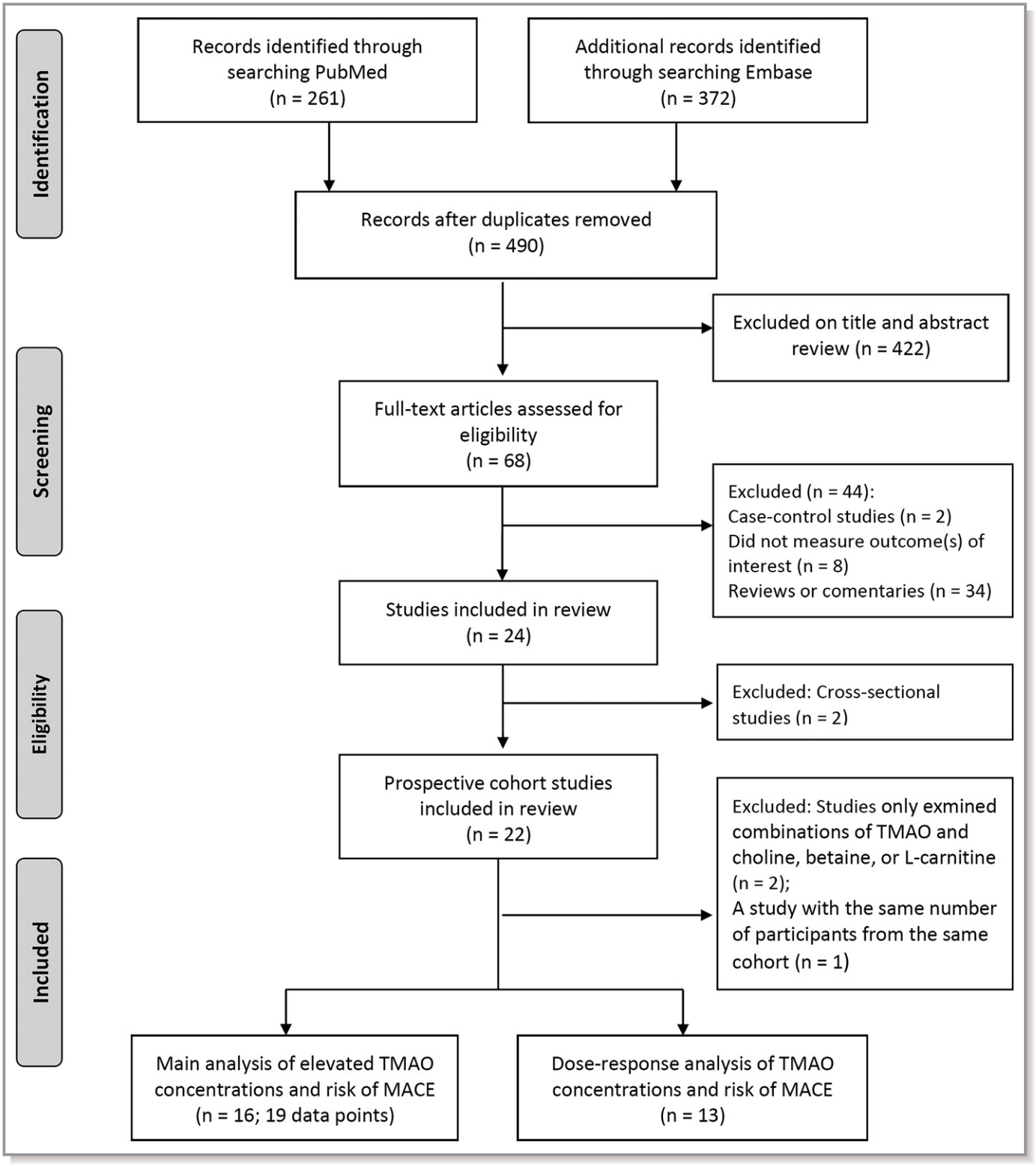

Figure 1. Selection of studies for meta-analysis. MACE indicates major adverse cardiovascular events; TMAO, trimethylamine N-oxide.

pooled RR of elevated levels of TMAO for MACE (MACE: CVD events, admission for HF, cardiovascular death/hospitalization, ischemic cardiovascular events, cardiac death, or MACE) was 1.66 (95% CI, 1.35, 2.05; $P_{heterogeneity}$= 0.6; I^2=0%).

For calculating the risk for only all-cause mortality, 15 data from 12 articles[7,8,10,11,18,19,21–26] were available, including 2498 deaths among 11 676 participants. Overall, elevated concentrations of TMAO were significantly

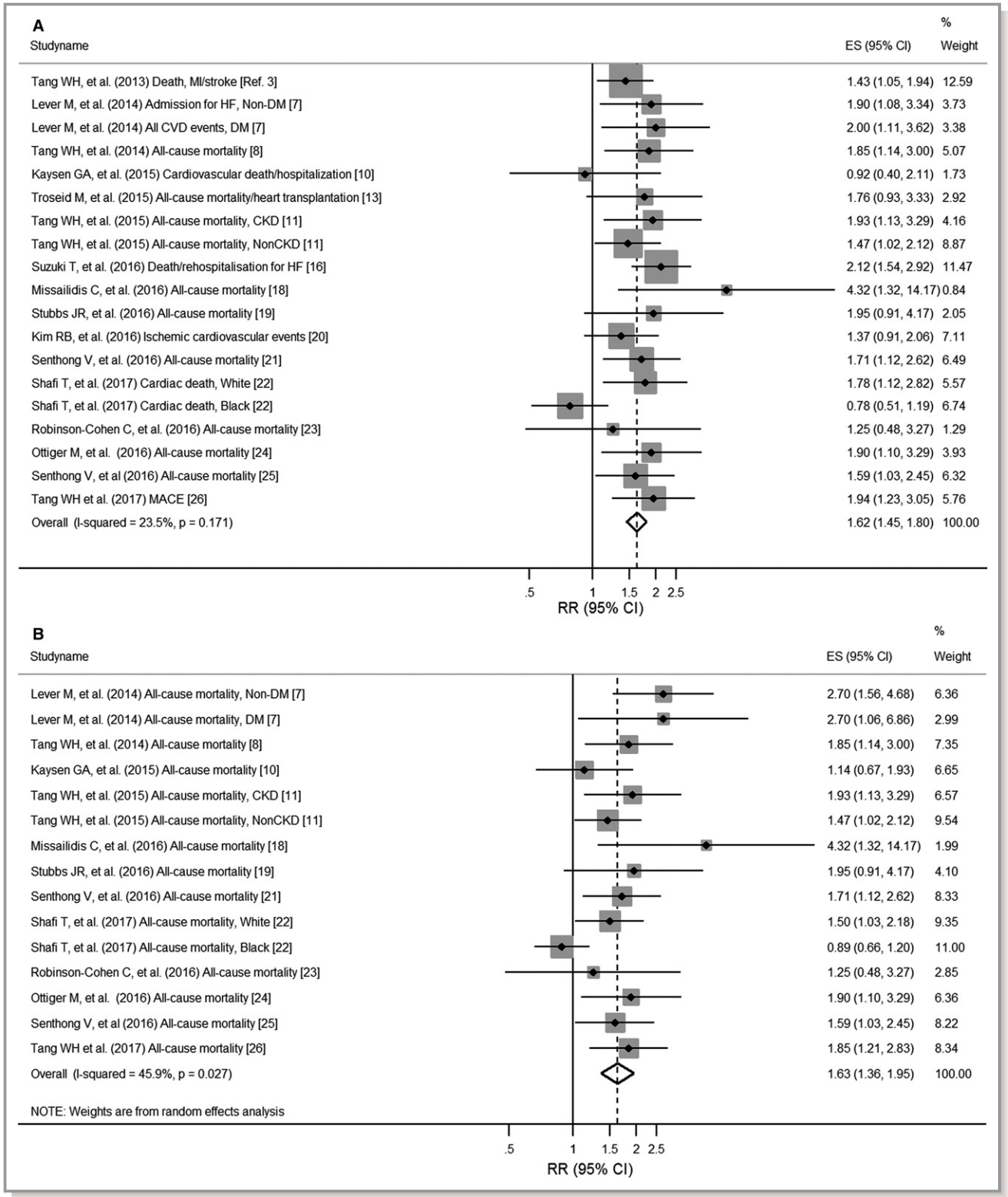

Figure 2. Pooled relative risks of high trimethylamine N-oxide (TMAO) levels for the risk of major adverse clinical events/death (A) and all-cause mortality (B). Dashed lines represent the overall effect, and gray boxes represent weight. ES indicates effect size; RR, relative risk.

associated with an increased risk of all-cause mortality (pooled RR, 1.63; 95% CI, 1.36, 1.95; $P_{heterogeneity}$=0.027; I^2=45.9% using random-effect model; Figure 2B). Again, after removal of the data in blacks, there was no heterogeneity across the studies (pooled RR, 1.72; 95% CI, 1.50, 1.97; $P_{heterogeneity}$=0.7; I^2=0%; Figure S1B).

Table 3. RRs of High TMAO for Major Cardiovascular Events According to Study Characteristics

Characteristics	N of Total Studies [Adjusted data]	RR (95% CI)	*P* for Interaction
Age, y			
<65	9 [8]	1.59 (1.34, 1.90)	0.3
≥65	9 [6]	1.82 (1.53, 2.16)	
Body mass index			
<27.0 kg/m^2	5 [3]	1.91 (1.42, 2.58)	0.2
≥27.0 kg/m^2	7 [5]	1.51 (1.22, 1.87)	
Smoking habit, yes			
<30%	4 [3]	2.12 (1.58, 2.84)	0.1
≥30%	7 [6]	1.61 (1.34, 1.94)	
TMAO levels at baseline (average TMAO)			
(a) <5.0 μmol/L (4.0 μmol/L)	7 [6]	1.62 (1.37, 1.92)	0.4
≥5.0 μmol/L (25.4 μmol/L)	11 [8]	1.79 (1.51, 2.14)	
(b) <8.0 μmol/L (5.3 μmol/L)	13 [9]	1.75 (1.53, 2.00)	0.3
≥8.0 μmol/L (47.7 μmol/L)	5 [5]	1.51 (1.13, 2.02)	
Kidney function			
High (eGFR ≥60 mL/min per 1.73 m^2)	10 [7]	1.67 (1.43, 1.94)	0.6
Low (eGFR <60 mL/min per 1.73 m^2)	8 [7]	1.77 (1.45, 2.17)	
Controlling for eGFR or other renal function markers, or albuminuria in models			
No	5 [1]	1.74 (1.26, 2.38)	0.9
Yes	13 [13]	1.70 (1.49, 1.94)	
Controlling for serum cholesterol levels or use of cholesterol lowering medications in models			
No	9 [5]	1.92 (1.57, 2.34)	0.1
Yes	9 [9]	1.58 (1.36, 1.85)	
Controlling for hs-CRP or CRP in models			
No	9 [5]	1.78 (1.49, 2.14)	0.5
Yes	9 [9]	1.64 (1.39, 1.94)	
Controlling for blood pressure measurements, hypertension, or use of antihypertensive medications in models			
No	8 [4]	1.83 (1.44, 2.32)	0.5
Yes	10 [10]	1.66 (1.44, 1.92)	
Prevalence of diabetes mellitus			
<40%	8 [6]	1.73 (1.47, 2.04)	0.8
≥40%	10 [8]	1.68 (1.40, 2.01)	
Prevalence of individuals with cardiovascular disease histories at baseline			
<40%	6 [4]	1.61 (1.22, 2.13)	0.7
≥40%	9 [9]	1.72 (1.48, 1.99)	
Follow-up time			
<5 years	10 [7]	1.69 (1.44, 1.98)	0.9
≥5 years	8 [7]	1.73 (1.43, 2.09)	

CRP indicates C-reactive protein; eGFR, estimated glomerular filtration rate. RR, relative risks; TMAO, trimethylamine N-oxide.

Stratified Analyses

We then investigated potential sources of heterogeneity in stratified analyses using the metaregression analysis based on the 18 data (4 were unadjusted and 14 were multivariate adjusted) that were used for the main analysis without the data in blacks (Table 3). In the stratified analysis using a cut-off value of median/mean TMAO value of 5.0 μmol/L (lower 39% of the total 18 studies), there was no heterogeneity between <5.0 or ≥5.0 μmol/L of TMAO concentrations at baseline. In addition, there were 5 studies with extremely high mean/median TMAO values (>20 μmol/L). We also performed a stratified analysis using a cut-off value of 8.0 μmol/L. We observed similarly increased risks of MACE regardless of studies with <8.0 (range, 3.0–7.9) or ≥8.0 (range, 20.41–98.4) μmol/L mean/median TMAO levels. Again, there was no heterogeneity between low or high TMAO concentrations at baseline. The association between elevated TMAO levels and the risk of MACE was consistently observed across strata of age, BMI at baseline, smoking, degree of kidney function, prevalence of CVD or diabetes mellitus at a baseline examination, the follow-up duration, and whether kidney function markers, lipids, blood pressure, or C-reactive protein (CRP) levels were controlled for in models. Associations appeared to be stronger among studies with participants who had generally lower BMI, or lower prevalence of smoking, although such differences did not attain statistical significance.

Dose-Response Analysis

We further investigated whether there was a dose-response relationship between TMAO concentrations and the risk for MACE (Table 4). The pooled unadjusted RR was 1.05 (95% CI, 1.03, 1.07) per 1-μmol/L increment in TMAO concentrations. Although the RR was attenuated, it was significantly elevated with a pooled adjusted RR of 1.02 (1.01, 1.03) using data in multivariate-adjusted models. One study[14] introduced a newly developed method for the measurement of TMAO, and their IQRs of TMAO were lower than other studies, so that we calculated a RR without data from the study. Results of the analysis showed a similar pooled adjusted RR per 1-μmol/L increment in TMAO of 1.02 (1.01, 1.04). We also analyzed the dose-response relationship on log-transformed TMAO levels; and the pooled RR per 1-SD increment of log-transformed TMAO was 1.43 (95% CI, 1.34, 1.52) in the unadjusted model or 1.21 (95% CI, 1.14, 1.29) in the adjusted model, respectively.

Associations of TMAO Precursors With the Risk of MACE

Finally, we conducted analyses on the associations between circulating levels of TMAO precursors and the risk and MACE using data from 6 publications[4,7,9,13,18,26] (Figure 3). For the association between elevated L-carnitine or choline concentrations and the risk of MACE, the summary RR was 1.26 (95% CI, 1.10, 1.44; $P_{heterogeneity}$=0.9; I^2=0%; Figure 3A). For the association of elevated betaine concentrations with the risk of MACE, the pooled RR was 1.43 (95% CI, 1.19, 1.73; $P_{heterogeneity}$=0.4; I^2=6.4%; Figure 3B).

Discussion

Our meta-analysis of data from prospective studies provides quantitative pooled estimates of the associations of circulating TMAO level with the incidence of MACE and all-cause death. As compared to participants with low TMAO levels, those with high levels had a 62% increased risk for the development of MACE and a 63% increased risk for all-cause death. In addition, elevated concentrations of TMAO were associated with 1.7-fold increased risks for MACE and all-cause mortality compared with low TMAO levels when we estimated the RRs without using data in blacks. The associations did not significantly differ according to the past histories of CVD, prevalence of diabetes mellitus, kidney dysfunction, follow-up duration, or TMAO levels at baseline. However, no prospective cohort studies were available in the population at general risk. In addition, we found that the association between blood TMAO levels and development of MACE was dose dependent. Moreover, our quantitative

Table 4. Pooled RRs Per 1 μmol/L or 1 SD Log-Transformed Increment of TMAO for Major Adverse Cardiovascular Events

Variables	N	Study ID (Reference)	RR (95% CI)	I^2	Heterogeneity *P* Value
1 μmol/L increment of TMAO, unadjusted	9	11,13,19,20,22,24–26	1.05 (1.03, 1.07)	95.0%	<0.001
1 μmol/L increment of TMAO, adjusted	9	11,14,19,20,22,24–26	1.02 (1.01, 1.03)	81.7%	<0.001
1 μmol/L increment of TMAO, adjusted*	8	11,19,20,22,24–26	1.02 (1.01, 1.04)	83.9%	<0.001
1 SD increment of log-transformed TMAO, unadjusted	5	12,16,17,20,25	1.43 (1.34, 1.52)	22.7%	0.3
1 SD increment of log-transformed TMAO, adjusted	6	8,12,16,17,20,25	1.21 (1.14, 1.29)	0%	0.8

*Without a study of reference.[14] RR indicates relative risks; TMAO, trimethylamine N-oxide.

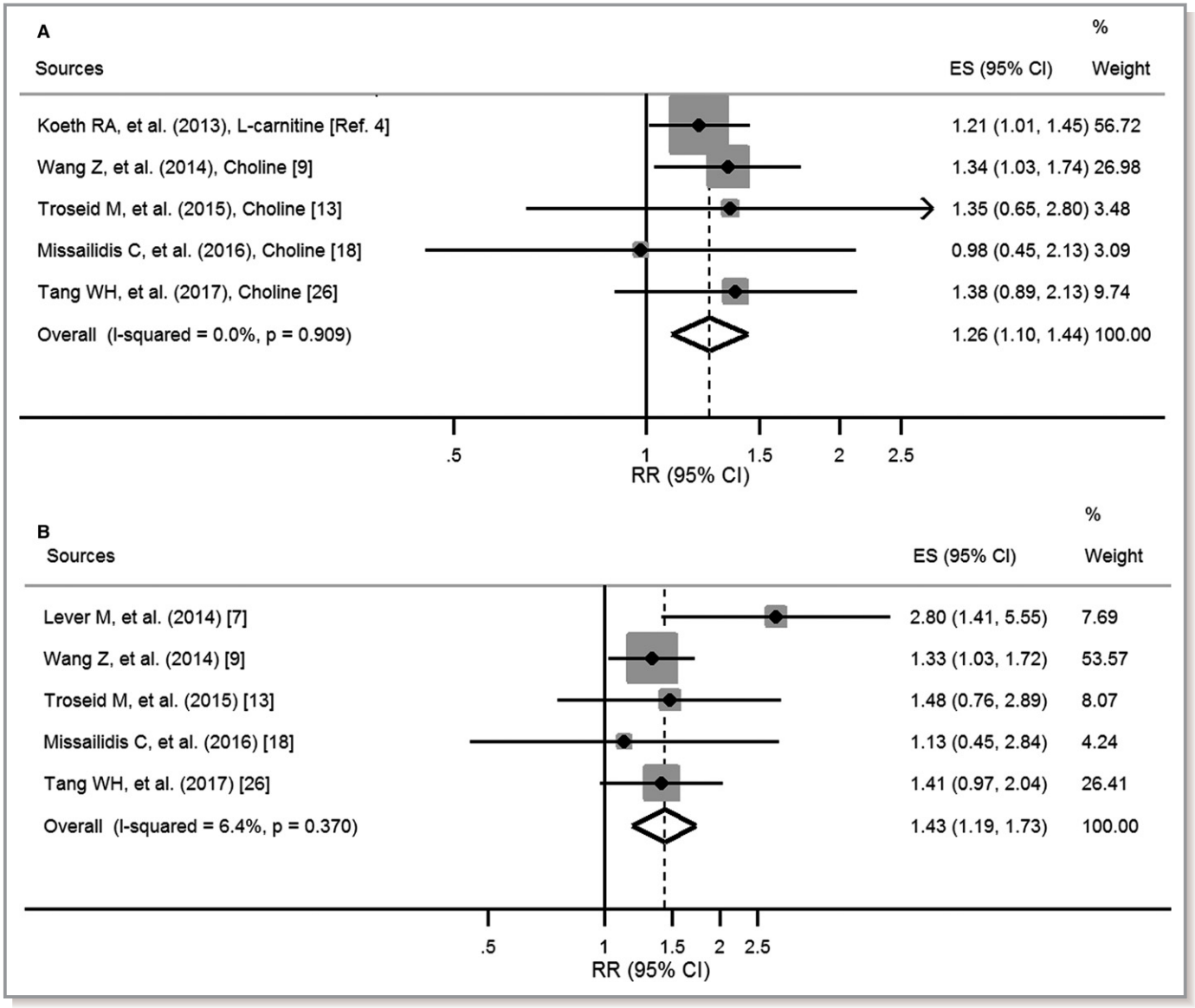

Figure 3. Pooled relative risks of elevated concentrations of L-carnitine or choline (A) and betaine (B) for major adverse cardiovascular events/death. Dashed lines represent the overall effect, and gray boxes represent weight. ES indicates effect size; RR, relative risk.

estimates for the precursors of TMAO and the risk of MACE/death revealed that individuals with elevated concentrations of L-carnitine, choline, or betaine had approximately of 1.3 to 1.4 times higher risk for MACE compared to those with low concentrations. Our results indicated that the relations of elevated TMAO and its precursors with MACE and all-cause death were independent of conventional risk factors, such as kidney dysfunction, diabetes mellitus, and obesity.

Strengths and Limitations

Our study has several strengths. The included original studies were all prospective and therefore our analysis minimized the likelihood of reverse causation. We carefully assessed the influence of several potential confounders and traditional risk factors for CVDs. In addition, our sensitivity analyses indicated that the results were not affected by varying definitions of outcomes or elevated TMAO levels. Several limitations warrant consideration. First, no data were available among general populations for our analysis. The included studies have been conducted in clinical cohorts including patients with pre-existing cardiovascular risk, and thus we cannot determine whether results will be similar in lower-risk populations. Second, the definition of elevated TMAO levels was difference across individual studies. Third, similar to other observational studies, we could not exclude the possibility of residual confounding attributed to unmeasured factors. Fourth, the gut microbiota are affected by environmental factors[37,38] such as dietary intakes,[39–41] which may, in turn, influence blood levels of TMAO and its precursors.[4] However,

detailed assessment of dietary intakes was not available in all the included studies; this limited our abilities to investigate the potential roles of dietary factors in the associations of gut microbiota metabolites and the outcomes. Fifth, most of the study participants were from Europe and the United States and included mainly (approximately of 65–90% of total study participants) whites,[10,19,20,23] so that our results might not be applicable to other race/ethnic groups such as Asians and Africans. Furthermore, TMAO levels were measured at 1 time point, which might not capture the long-term levels of gut microbiota metabolites. Finally, further studies, especially clinical trials to lower TMAO levels, are warranted to inform causality.

Association Between Our Results and Other Studies

The associations of TMAO and the risk of CVDs have been reviewed previously[42–45]; however, these studies did not aim to perform a meta-analysis to quantify the risks of TMAO and its precursors for MACE. We observed the highly consistent positive associations between TMAO levels and the outcomes across studies, even among studies with low (<5.0 μmol/L) concentrations of TMAO at baseline, particularly after excluding the data among blacks. As previously reported that the associations of TMAO and risk of cardiovascular events in hemodialysis patients differed by race,[22] the inclusion of data in black hemodialysis patients influenced the heterogeneity of results in our study. It has also been reported that morality risk may differ between black and white dialysis patients.[46] Nonetheless, median values of TMAO concentrations in healthy individuals were reported as ranges of 3 to 6 μmol/L,[8,17–19] and the study by Shafi et al[22] consisted of hemodialysis patients with the substantially high mean vale of TMAO (101.9 μmol/L among total participants). It may not be generalizable to other groups of blacks from the general population, and further studies are warranted on the ethnic difference in the associations of TMAO and the risk of MACE.

Several studies[10,11,19,28] specifically considered the role of kidney dysfunction in the associations between TMAO and the risk of clinical adverse outcomes. The burden of CVD is high among patients with chronic kidney disease (CKD) or end-stage renal disease,[47] and the circulating TMAO is predominantly excreted by the kidneys.[42] Nonetheless, we did not observe a statistically significant difference in the associations according to kidney dysfunction. Emerging evidence has shown associations of gut microbiota with obesity.[48–51] We found a stronger association in the subgroup with lower BMI and speculated that populations with lower BMI were less likely to be affected by diseases and might have less confounding factors, and effects of dietary differences influencing TMAO levels might be more apparent in the low-BMI group. However, the difference between low- and high-BMI groups was not statistically significant. Whether overweight or obesity accounted for the association of TMAO with the risk of MACE should be further investigated.

Additionally, we found that the pooled risk of elevated TMAO was stronger than those of its precursors, including betaine and choline or L-carnitine, for the risk of MACE or all-cause mortality. In our analyses, the associations of these correlated gut microbiota metabolites could not be mutually adjusted; therefore, we could not determine whether the TMAO or its precursors were independently associated with the outcomes. A study[4] showed that plasma L-carnitine concentrations predicted MACE independent of traditional cardiovascular risk factors, but the significant association disappeared after adjustment for plasma TMAO concentration. In addition, it was noted that elevated concentrations of L-carnitine were only predictive of MACE risk among individuals with higher TMAO levels.[4] In another study, it was found that only TMAO predicted the risk of MACE when choline, betaine, and TMAO were simultaneously included in the multivariate-adjusted model, and choline and betaine predicted risk of MACE only among participants with an above median value of TMAO (>3.7 μmol/L) concentrations.[9]

Potential Mechanisms

In our recent study, we found that high intake of phosphatidylcholine, which could lead to a higher production of TMAO, was significantly associated with an increased risk of all-cause and CVD-specific mortality.[52] Dietary choline and L-carnitine are metabolized by intestinal bacteria to produce TMA, which is, in turn, absorbed into the bloodstream and oxidized to TMAO by enzyme flavin monooxygenase 3 in the liver.[1,4,5] Koeth et al showed that dietary supplementation of mice with choline or L-carnitine[4] increased TMAO levels and enhanced the development of atherosclerosis.[1] Flavin monooxygenase 3 is reported to be a key integrator of hepatic cholesterol and lipid metabolism and inflammation.[53] TMAO was found to modulate cholesterol and sterol metabolism that would, at least partly, contribute to the increasing risk of CVDs.[4] Higher TMAO levels were associated with the presence of increased atherosclerotic burden and complexity among patients with coronary artery disease (CAD).[54] A recent study has shown that TMAO directly interacts with platelets altering calcium signaling, fostering platelet hyper-reactivity and a prothrombotic phenotype in vivo.[15] Similar, TMAO acutely induces aortic endothelial cell inflammatory gene profile, suggesting another potential pathway by which TMAO contributes to CVD.[55] Betaine is a metabolite of choline,[1,56] and dietary betaine administration induced production of TMAO in animals.[9] L-carnitine in red

meat can also be transformed to gamma-butyrobetaine by gut bacteria before being converted to TMA and TMAO.[2,5] Whether betaine, choline, or L-carnitine have independent effects on MACE and all-cause mortality and whether other mechanisms are involved need further investigation.

Conclusion

Our meta-analysis of published prospective studies indicates that higher circulating levels of gut microbiota metabolites, including TMAO and its precursors, are associated with an increased risk of MACE, regardless of conventional risk factors. Further studies are needed to investigate these associations in general-risk populations, as well as the causality of the associations.

Acknowledgments

We thank Drs Stanley L. Hazen, Yuping Wu, Jason Stubbs, Marius Trøseid, Catharina Missailidis, Manuel Ottiger, and Philipp Schuetz, for providing data and clarifying aspects of their studies.

Sources of Funding

The study was supported by grants from the National Heart, Lung, and Blood Institute (HL071981, HL034594, and HL126024), the National Institute of Diabetes and Digestive and Kidney Diseases (DK091718, DK100383, and DK078616), the Boston Obesity Nutrition Research Center (DK46200), and United States–Israel Binational Science Foundation Grant 2011036. Qi was a recipient of the American Heart Association Scientist Development Award (0730094N). Heianza was a recipient of a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

Disclosures

None.

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Supplemental Material

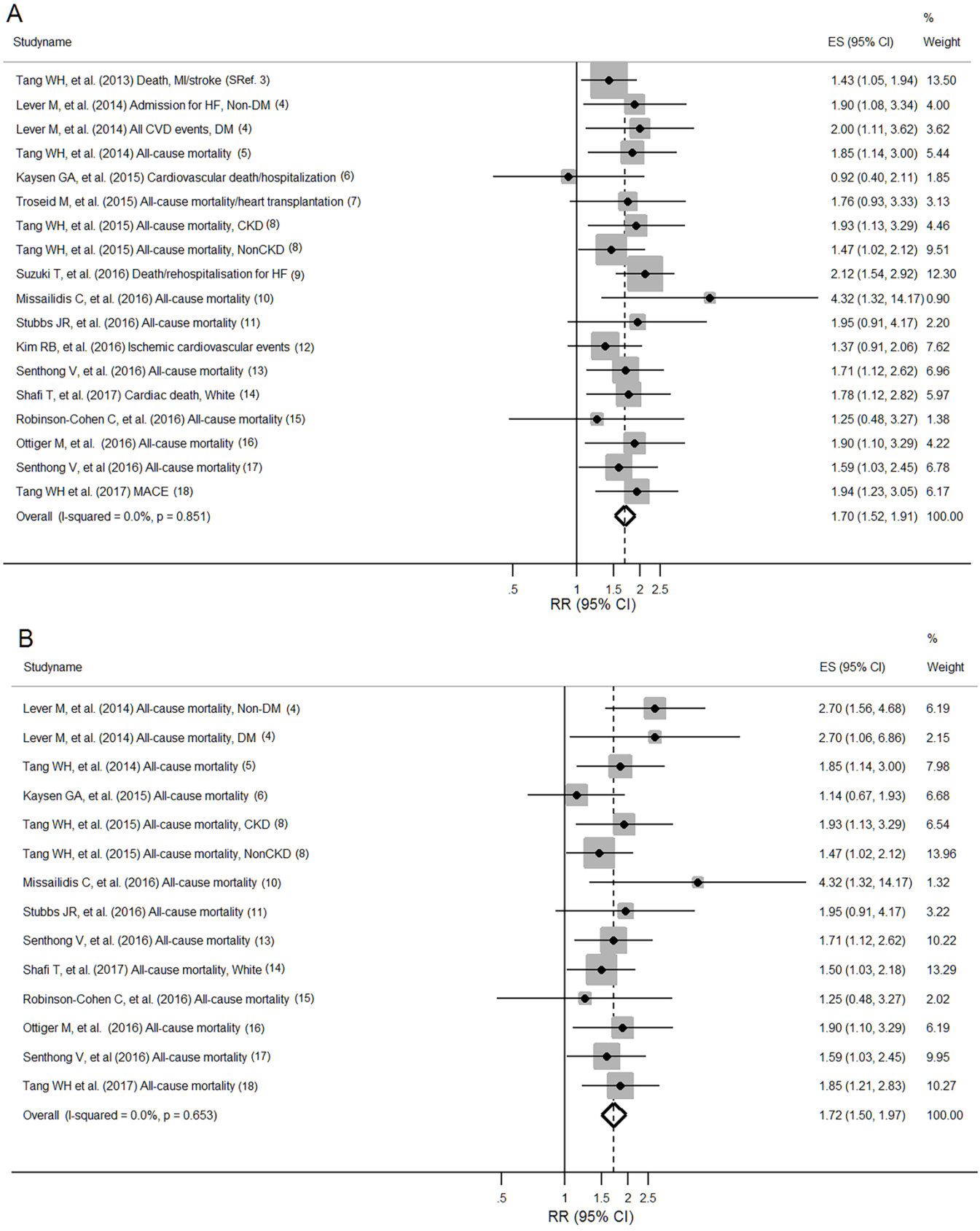
Table S1: Characteristics of the identified studies of trimethylamine N-oxide (TMAO) levels and the prevalent cardiovascular diseases (cross-sectional studies)

Source	Country	Study Population	Age, mean, y	TMAO, mean or median, μM	Men, %	Total N	Outcomes assessed	Events, N
Wang Z, et al. (2011)[1]	US	GeneBank	63.9	NA	49.3	1020	Coronary artery disease (CAD)	As%,41.0
							Peripheral artery disease (PAD)	As%, 24.5
							Cardiovascular disease (CVD) as indicated by either CAD or PAD	As%, 65.4
							Myocardial infarction (MI)	NA
		BioBank	64.5	NA	48.3	856	CAD	As%, 43.7
							PAD	As%, 21.5
							CVD as indicated by either CAD or PAD	As%, 65.2
							MI	NA
Mente A, et al. (2015)[2]	Canada	Study of Health Assessment and Risk in Ethnic Groups [SHARE] and SHARE and Aboriginal Peoples [SHARE-AP]	52.5	1.998	64.9	271	CVD	99

Table S2: Relative risks (RRs) of major cardiovascular events according to trimethylamine N-oxide (TMAO) levels in cross-sectional studies

Source	Outcome	Comparison	Model	Relative risk (95% CI)	Adjustment for Covariates
Wang Z, et al. (2011) GeneBank[1]	Coronary artery disease (CAD)	Highest quartile vs. lowest	Multivariate	2.59 (1.88, 3.55)	Age, sex, smoking, diabetes, medication use, hypertension, lipids, C-reactive protein (CRP), and estimated creatinine clearance
	Peripheral artery disease (PAD)	Same as the above	Multivariate	3.43 (2.26, 5.21)	Same as the above
	CAD + PAD	Same as the above	Multivariate	4.03 (2.54, 6.40)	Same as the above
	Cardiovascular disease (CVD)	Same as the above	Multivariate	2.54 (1.86, 3.47)	Same as the above
	Myocardial infarction (MI)	Same as the above	Multivariate	1.47 (0.90, 2.40)	Same as the above
Wang Z, et al. (2011) BioBank[1]	CAD	Highest quartile vs. lowest	Multivariate	3.08 (1.94, 4.88)	Same as the above
	PAD	Same as the above	Multivariate	3.75 (2.00, 7.03)	Same as the above
	CAD + PAD	Same as the above	Multivariate	4.00 (2.04, 7.82)	Same as the above
	CVD	Same as the above	Multivariate	3.08 (1.96, 4.86)	Same as the above
	MI	Same as the above	Multivariate	2.11 (1.33, 3.34)	Same as the above
Mente A, et al. (2015)[2]	CVD	Highest quintile (≥2.5 μM) vs. lowest	Crude	3.28 (1.37, 7.87)	Crude
			Multivariate	3.17 (1.05, 9.51)	Age, sex, body mass index (BMI), smoking and energy intake
			Multivariate	9.33 (1.88, 46.37)	Age, sex, BMI, smoking, energy intake, diabetes, meat intake, fish intake, and dietary cholesterol

Figure S1: Pooled relative risks of high trimethylamine N-oxide (TMAO) levels for the risk of major adverse clinical events/death (A) and all-cause mortality (B) using 18 data points.[3-18]



Dashed lines represent the overall effect, and gray boxes represent weight. SRef, Supplemental references; DM, diabetes; CKD, chronic kidney disease; HF, heart failure.

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