Gut microbiota–derived metabolite trimethylamine-N-oxide and multiple health outcomes: an umbrella review and updated meta-analysis

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ABSTRACT

Background: Trimethylamine-N-oxide (TMAO) is a gut microbiota–derived metabolite produced from dietary nutrients. Many studies have discovered that circulating TMAO concentrations are linked to a wide range of health outcomes.

Objectives: This study aimed to summarize health outcomes related to circulating TMAO concentrations.

Methods: We searched the Embase, Medline, Web of Science, and Scopus databases from inception to 15 February, 2022 to identify and update meta-analyses examining the associations between TMAO and multiple health outcomes. For each health outcome, we estimated the summary effect size, 95% prediction CI, between-study heterogeneity, evidence of small-study effects, and evidence of excess-significance bias. These metrics were used to evaluate the evidence credibility of the identified associations.

Results: This umbrella review identified 24 meta-analyses that investigated the association between circulating TMAO concentrations and health outcomes including all-cause mortality, cardiovascular diseases (CVDs), diabetes mellitus (DM), cancer, and renal function. We updated these meta-analyses by including a total of 82 individual studies on 18 unique health outcomes. Among them, 14 associations were nominally significant. After evidence credibility assessment, we found 6 (33%) associations (i.e., all-cause mortality, CVD mortality, major adverse cardiovascular events, hypertension, DM, and glomerular filtration rate) to present highly suggestive evidence.

Conclusions: TMAO might be a novel biomarker related to human health conditions including all-cause mortality, hypertension, CVD, DM, cancer, and kidney function. Further studies are needed to investigate whether circulating TMAO concentrations could be an intervention target for chronic disease. This review was registered at www.crd.york.ac.uk/prospero/ as CRD42021284730. *Am J Clin Nutr* 2022;116:230–243.

Keywords: umbrella review, updated meta-analyses, trimethylamine-N-oxide, TMAO, all-cause mortality, cardiovascular disease, hypertension, diabetes mellitus

Introduction

Trimethylamine-N-oxide (TMAO) is a gut microbiota metabolite derived from phosphatidylcholine, choline, betaine, and L-carnitine, which are abundant in seafoods, dairy products, egg yolks, muscle, and organ meats (1, 2). These nutrients can be hydrolyzed by trimethylamine (TMA) lyase from gut flora to form the TMAO precursor TMA, which is further oxidized by hepatic flavin monooxygenases to form TMAO (2, 3). A multitude of studies have discovered that circulating TMAO concentrations are linked to a wide range of health outcomes, including cardiovascular and cerebrovascular diseases (4–6), type 2 diabetes mellitus (DM) (7), hypertension (8), renal dysfunction (9, 10), cancer, and mortality (11, 12). The relations between

elevated plasma TMAO concentrations and health-related traits have also been explored, including glomerular filtration rate (GFR) (9), blood pressure (13, 14), BMI (9, 14), and total cholesterol (15). It has been hypothesized that the intestinal microbiota may alter the risk of disease by inducing TMAO changes in the metabolome profile (16), and therefore TMAO might be a novel biomarker representing human health conditions related to the gut microbiota (17–19).

Most evidence on the health effects of plasma TMAO concentrations has been generated by observational studies with conflicting results. In addition, some studies were conducted among patients with specific diseases, which calls into question whether such associations can be generalized to a healthy population. Hence, it is necessary to synthesize the current evidence to provide a comprehensive overview of the claimed associations of TMAO concentrations with health outcomes.

Umbrella review is designed to provide a comprehensive overview of evidence from systematic review with or without meta-analysis (20). Several meta-analyses on the relations between increased TMAO concentrations and risks of obesity (21), stroke (22), diabetes (23), hypertension (24), and all-cause mortality (25) have been conducted. A comprehensive credibility assessment of these associations will help elucidate the role of TMAO in human health. Using a standardized approach, we performed an umbrella review to evaluate the validity and credibility of the evidence from updated meta-analyses of observational studies. In detail, we summarized the range of related health outcomes; presented the magnitude, direction, and significance of the reported associations; assessed the potential biases; and identified the most convincing evidence in relation to the health impact of TMAO concentrations.

Methods

Study design

In this umbrella review, all meta-analyses on the associations between plasma TMAO concentrations and health outcomes were identified. Original studies that evaluated the associations between TMAO and health outcomes were also identified to update the identified meta-analyses. The protocol of the present study was registered in PROSPERO (CRD42021284730).

Literature search

Two investigators (DL and YL) independently searched the Embase, Medline, Web of Science, and Scopus databases from inception to 15 February, 2022 using a search strategy to identify meta-analyses of observational studies. The literature search algorithm was as follows: "((((meta-analysis) OR (meta)) OR (systematic overview)) OR (systematic review)) AND ((((trimethylamine oxide) OR (trimethylamine N-oxide)) OR (trimethylammonium oxide)) OR (TMAO))." We also searched for individual observational studies to update the identified meta-analyses and reported the results in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (26). All identified publications went through a 3-step parallel review of title, abstract, and full text based on predefined inclusion and exclusion criteria, and any discrepancies were resolved by consensus.

Eligibility criteria

Meta-analyses performing quantitative analysis of plasma TMAO concentrations and health outcomes were included in the umbrella review. All relevant population-based observational studies including prospective cohort, nested case–control, case cohort, case–control, or analytical cross-sectional studies were combined in the updated meta-analysis, and we conducted subgroup analysis by study design. Guidelines, narrative reviews, literature reviews, and genetic studies were excluded. We further excluded studies in which TMAO was not the primary exposure. Meta-analyses or original studies that had inadequate data (e.g., lack of information on RRs, ORs, HRs, or 95% CIs) were also excluded.

Data extraction and quality assessment

From each eligible meta-analysis, we extracted information on the lead author's name, study design, publication year, study sample, number of studies included, the reported summary risk estimates [RR, OR, HR, or weighted mean difference (WMD)] with 95% CIs, the number of participants and cases, and the investigated outcomes. For meta-analyses on >1 health outcome, each outcome was recorded separately. Furthermore, we searched for recently published original articles on TMAO and combined them with studies identified from the previous meta-analyses to update the meta-analyses. When updating the meta-analyses, we added the newly identified studies and re-estimated the summary effect estimates using random-effects models. To account for potential confounding and reverse causality, we performed subgroup analyses by confining the meta-analyses to include only cohort studies with adjustment for renal function and diet (if possible). Data extraction at this stage covered information on study design, number of cases, total number of participants, RR estimates, and 95% CIs. Two investigators (DL and YL) extracted data independently using a predesigned data extraction form.

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Supplemental Tables 1–6 and Supplemental Figures 1–23 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: CKD, chronic kidney diseases; CRC, colorectal cancer; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; DM, diabetes mellitus; GDM, gestational diabetes mellitus; GFR, glomerular filtration rate; MACE, major adverse cardiovascular events; PI, prediction interval; SBP, systolic blood pressure; TC, total cholesterol; TMA, trimethylamine; TMAO, trimethylamine-N-oxide; WMD, weighted mean difference.

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The quality of individual studies was assessed by the Newcastle-Ottawa Scale (NOS) for observational studies (27).

Statistical analysis

For each unique meta-analysis of observational studies, several metrics were estimated, including the summary effect and corresponding 95% CI using the random-effects model; the heterogeneity among studies (Q statistic and I^2 metric); and the 95% prediction interval (95% PI) to predict the range of effect size that would be expected in a new original study after accounting for both the heterogeneity among individual studies and the uncertainty of the summary effect estimated in the random-effects model (28) (the calculation of the 95% PI is based on the predicted distribution derived from a function of the degree of heterogeneity, number of studies included, and within-study SEs) (29, 30). Egger's regression test was used to evaluate the small-study effects (31). The excess significance test was conducted to investigate whether the observed number of studies with significant results differed from the expected number of significant studies using the χ^2 test (32–34). The expected number of significant studies for each meta-analysis was calculated by summing the statistical power estimates for each component study. We estimated the power of each study for an effect equal to the effect of the largest study (the study with the smallest variance), as previously described (35). All statistical analyses were performed using the "metafor" and "forestplot" R packages, R software version 4.0.2 (The R Foundation, Boston, MA).

Evaluation of evidence credibility

We used credibility assessment criteria (**Supplemental Table 1**), as described in previously published umbrella reviews (35–37). Evidence from meta-analyses of observational studies with nominally significant summary results ($P < 0.05$) was classified into 4 categories: convincing, highly suggestive, suggestive, or weak evidence (class I, II, III, and IV, respectively) (35–37). For meta-analyses performed on the same outcome, we examined the consistency between studies and the largest meta-analysis was retained for further analyses.

Results

Figure 1A shows the process of literature searching and screening for the umbrella review. The literature search retrieved 211 unique articles. After literature screening, 15 articles (21–25, 38–47) were eligible, which contained 24 meta-analyses for 15 unique outcomes (**Supplemental Table 2**). There was 1 meta-analysis published for stroke (22), hypertension (42), diastolic blood pressure (DBP) (24), systolic blood pressure (SBP) (24), diabetes (23), BMI (21), LDL/HDL cholesterol (24), total cholesterol (TC) (24), triglycerides (24), C-reactive protein (CRP) (41), and GFR (47); there were 2 meta-analyses for cardiovascular disease (CVD) (39, 46); 5 meta-analyses for all-cause mortality (25, 38–40, 45); and 6 meta-analyses for major adverse cardiovascular events (MACE) (25, 38, 43–45).

Figure 1B shows the process of selection of original studies in conducting the updated meta-analyses. The initial search yielded 1239 publications. After literature screening, we retrieved 46 new articles; and together with 46 individual studies from previous meta-analyses, a total of 92 individual studies were included in the study. Among them, 82 individual studies were included in the meta-analyses. The updated meta-analyses evaluated the associations between plasma TMAO concentrations and 18 unique health outcomes. **Supplemental Tables 3–5** show the quality assessment of the included studies.

All-cause mortality

The updated meta-analysis included 37 studies from 32 articles (3, 5, 10–12, 48–74) with >9553 cases out of 38,862 participants. All-cause mortality in the highest TMAO category was compared with that in the lowest TMAO category, and it was found that a higher TMAO concentration was associated with higher mortality (HR: 1.60; 95% CI: 1.43, 1.79; $P = 8.33 \times 10^{-16}$) (**Figure 2**, **Supplemental Figure 1**). A dose-response meta-analysis based on 10 studies (3, 5, 10, 12, 58, 62, 65, 66, 68, 70) showed that a 1-unit increment of TMAO (1 μmol/L) was associated with a 9% increased risk of all-cause mortality (HR: 1.09; 95% CI: 1.07, 1.11; $P = 8.03 \times 10^{-12}$) (**Figure 3**A). We also conducted a subgroup analysis by disease status and found that the association between TMAO and all-cause mortality was predominant in CVD patients (HR: 1.66; 95% CI: 1.46, 1.88; $P = 1.84 \times 10^{-15}$) (**Supplemental Figure 2**), whereas no significant association was reported in other populations. The association with all-cause mortality remained significant when including only the studies that adjusted for renal function (HR: 1.56; 95% CI: 1.38, 1.77; $P = 3.45 \times 10^{-12}$) (**Supplemental Figure 3**).

Cardiovascular outcomes

Regarding MACE, 36 studies from 32 articles (2, 5, 10, 48–53, 55–61, 63, 65, 66, 68, 70, 75–85) were included in the updated meta-analysis, contributing >7070 cases in 39,314 participants. In the random-effects model, circulating TMAO was positively associated with an increased risk of MACE (HR: 1.74; 95% CI: 1.56, 1.95; $P = 1.13 \times 10^{-22}$) (Figure 2, **Supplemental Figure 4**). The association remained significant in the confined meta-analysis of cohort studies that adjusted for renal function (HR: 1.65; 95% CI: 1.45, 1.88; $P = 1.50 \times 10^{-14}$) (**Supplemental Figure 5**). Three studies (66, 68, 70) were included in the dose-response analysis, resulting in 11% increased risk of MACE per 1-μmol/L increment of TMAO (RR: 1.11; 95% CI: 1.07, 1.14; $P = 1.04 \times 10^{-4}$) (Figure 3B).

Fifteen studies (3, 15, 53, 55, 58, 65, 66, 77, 83, 84, 86–90) were included in the updated meta-analysis of hypertension, comprising 10,293 cases and 18,854 total participants. There was a significant association between TMAO concentrations and risk of hypertension (RR: 1.39; 95% CI: 1.22, 1.57; $P = 3.47 \times 10^{-7}$) (Figure 2, **Supplemental Figure 6**), which was consistent with a former published meta-analysis (42). The association remained significant in the confined meta-analysis of cohort studies only (RR: 1.34; 95% CI: 1.16, 1.55; $P = 8.58 \times 10^{-5}$) (Figure 2), and the association was still significant when the meta-analysis

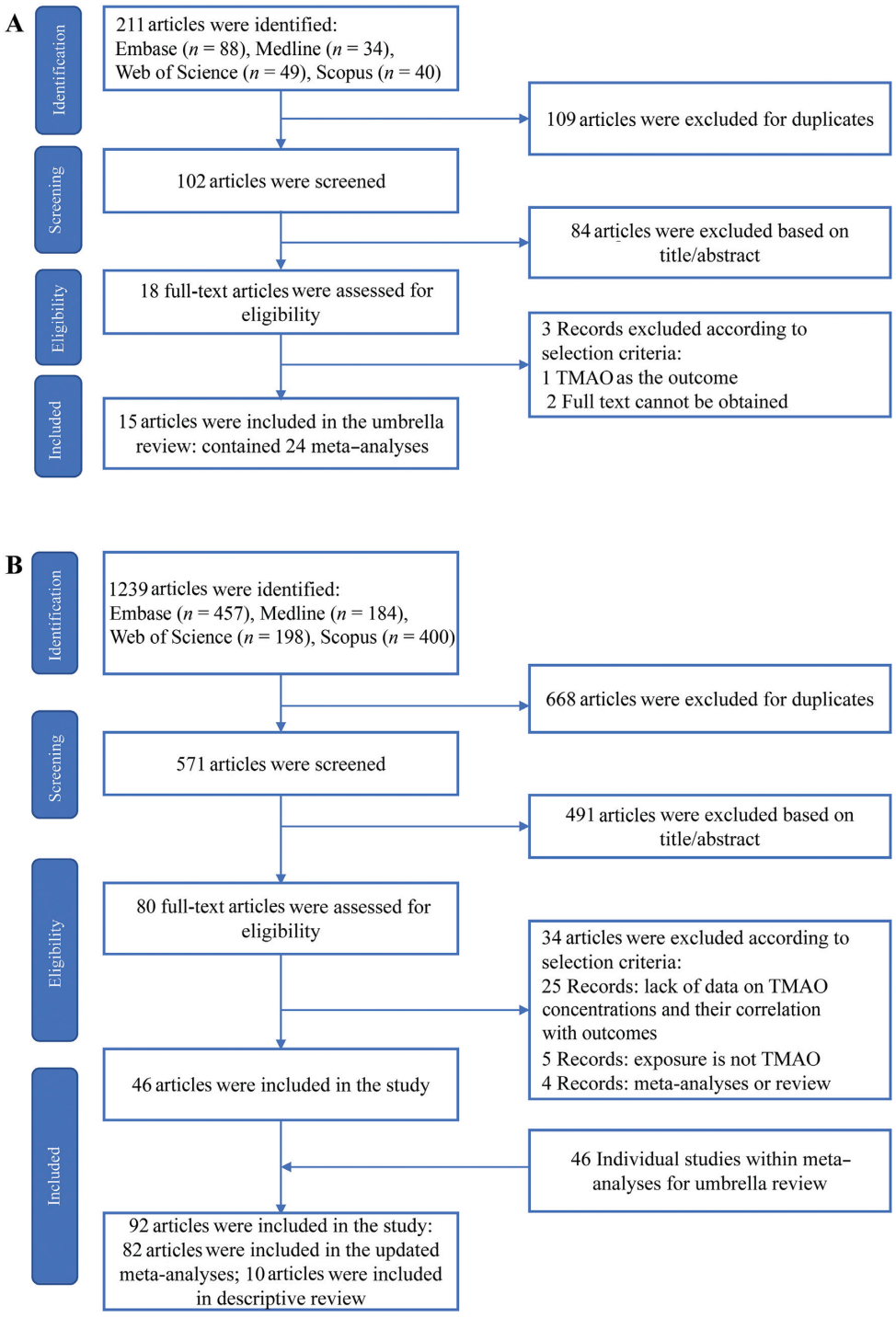

FIGURE 1 Flow diagram of study selection. (A) Study selection for umbrella review; (B) study selection for the updated meta-analyses. TMAO, trimethylamine N-oxide.

included only the studies that adjusted for renal function (RR: 1.40; 95% CI: 1.13, 1.72; $P = 1.65 \times 10^{-3}$) (**Supplemental Figure 7**). Eight studies (3, 53, 55, 58, 66, 87–89) were eligible for dose-response analysis, which showed that the risk of hypertension increased by 7% per (1-μmol/L) increment of TMAO (RR: 1.07; 95% CI: 1.03, 1.11; $P = 6.49 \times 10^{-4}$) (Figure 3C).

The updated meta-analysis on CVDs included 12 studies (4, 6, 83, 91–96) with 22,945 participants and showed that high TMAO concentrations were statistically significantly associated with an increased risk of CVD (OR: 1.50; 95% CI: 1.26, 1.79; $P = 8.00 \times 10^{-6}$) (Figure 2, **Supplemental Figure 8**). Eight studies from 5 articles (11, 14, 60, 72, 83) were used to perform a meta-analysis on CVD mortality. The results

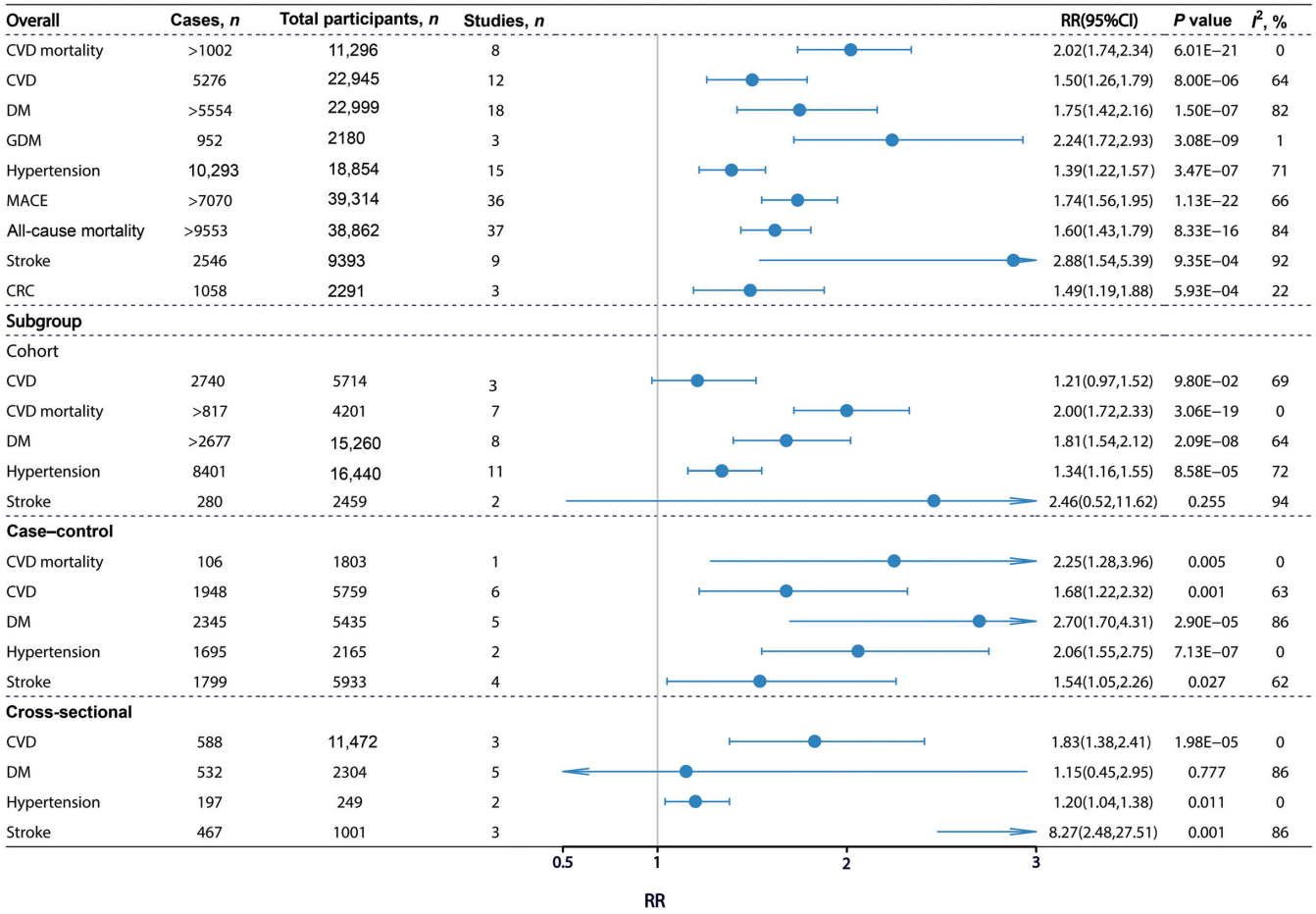

FIGURE 2 High compared with low TMAO concentrations and associations with multiple health outcomes. Estimates are RRs and meta-analyses are based on random-effect models. An I^2 value ≥50% is considered to indicate substantial heterogeneity. All results are presented as HR with 95% CIs, using the Mantel–Haenszel method with a random-effects model. CRC, colorectal cancer; CVD, cardiovascular disease; DM, diabetes mellitus; GDM, gestational diabetes mellitus; MACE, major adverse cardiovascular events.

revealed that participants with high TMAO concentrations were more likely to die from CVDs than those with low TMAO concentrations (HR: 2.02; 95% CI: 1.74, 2.34; $P = 6.01 \times 10^{-21}$) (Figure 2, **Supplemental Figure 9**). The association remained significant when the meta-analysis was restricted to cohort studies (HR: 2.00; 95% CI: 1.72, 2.33; $P = 3.06 \times 10^{-19}$) (Figure 2).

Results from the updated meta-analysis of stroke showed that higher circulating TMAO concentrations were associated with a higher risk of stroke [9 studies (66, 69, 83, 90, 97–100) enrolling 9393 participants, OR: 2.88; 95% CI: 1.54, 5.39; $P = 9.35 \times 10^{-4}$] (Figure 2, **Supplemental Figure 10**). However, this association was attenuated and not significant when the meta-analysis was restricted to cohort studies (RR: 2.46; 95% CI: 0.52, 11.62; $P = 0.255$) (Figure 2).

DM

Our updated meta-analyses, including 18 studies [from 17 articles (3, 7, 15, 55, 65, 77, 83, 84, 86, 87, 90, 93, 101–105) enrolling 22,999 subjects], found a significant association between TMAO and DM (OR: 1.75; 95% CI: 1.42, 2.16; $P = 1.50 \times 10^{-7}$) (Figure 2, **Supplemental Figure 11**). The association was also significant in the confined meta-analysis of cohort studies (OR: 1.81; 95% CI: 1.54, 2.12; $P = 2.09 \times 10^{-8}$) (Figure 2), and the association remained significant when the meta-analysis was restricted to cohort studies that adjusted for renal function (OR: 1.71; 95% CI: 1.35, 2.18; $P = 1.12 \times 10^{-5}$) (**Supplemental Figure 12**). In our dose-response meta-analysis, based on data from 3 articles (87, 88, 102), we found no statistically significant relation between TMAO concentrations and DM ($P = 0.228$) (Figure 3D). Furthermore, our meta-analysis of 3 studies enrolling 2180 subjects showed that women with high TMAO concentrations were more likely to have gestational diabetes mellitus (GDM) (OR: 2.24; 95% CI: 1.72, 2.93; $P = 3.08 \times 10^{-9}$) (Figure 2, **Supplemental Figure 13**).

Cancer risk

We identified 6 observational studies that examined the associations of TMAO concentrations with cancer risk including colorectal cancer (CRC) (106–108), prostate cancer (109), primary liver cancer (110), and pancreatic cancer (111). Quantitative

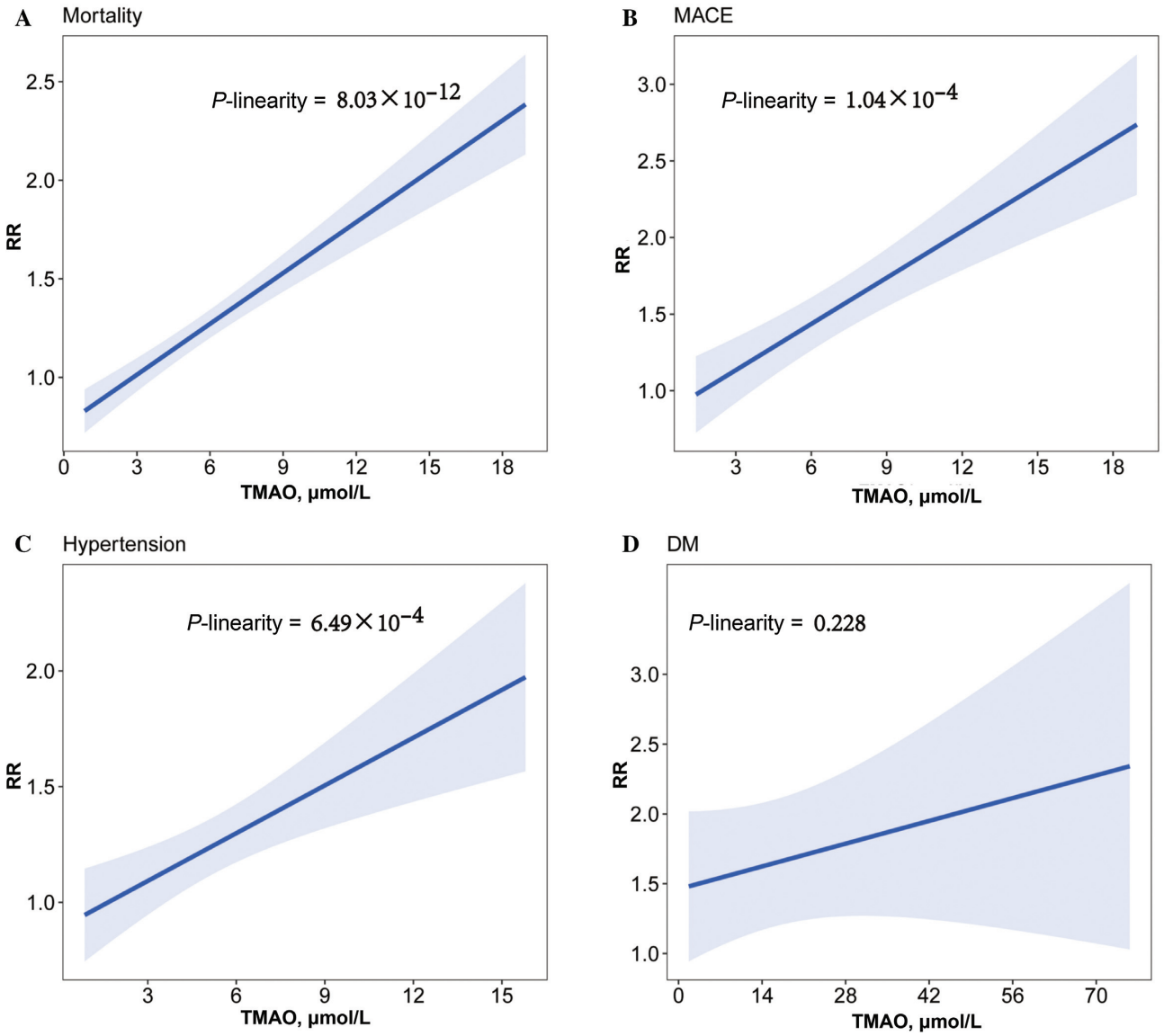

FIGURE 3 Dose–response association between circulating TMAO concentrations and all-cause mortality (A), MACE (B), hypertension (C), and DM (D). Risk spline (solid line) and 95% CIs (shadow) of pooled RR of all-cause mortality, MACE, hypertension, and DM by 1 μmol/L of TMAO. DM, diabetes mellitus; MACE, major adverse cardiovascular events; TMAO, trimethylamine N-oxide.

meta-analysis could only be performed for CRC, which included 3 individual studies and showed a positive association between high TMAO concentrations and increased risk of CRC (OR: 1.49; 95% CI: 1.19, 1.88; $P = 5.93 \times 10^{-4}$) (Figure 2, **Supplemental Figure 14**). Three articles reported positive associations of TMAO with prostate cancer (OR: 1.36; 95% CI: 1.02, 1.81; $P = 0.039$) (109), primary liver cancer (OR: 2.85; 95% CI: 1.59, 5.11; $P = 0.003$) (110), and pancreatic cancer (OR: 2.36; 95% CI: 1.30, 4.26; $P = 0.02$) (111) (**Supplemental Table 6**).

Blood pressure and cardiometabolic biomarkers

The results of the updated meta-analyses showed no significant association between TMAO and DBP [14 studies (9, 13–15, 59, 67, 87–89, 98, 112–114) enrolling 10,085 subjects, WMD: −0.25; 95% CI: −0.95, 0.46; $P = 0.495$] (**Figure 4**, **Supplemental Figure 15**). Higher circulating TMAO was related to higher SBP [16 studies (3, 9, 13–15, 59, 67, 87–89, 98, 112–115) enrolling 17,369 subjects, WMD: 1.92; 95% CI: 1.33, 2.51; $P = 1.70 \times 10^{-10}$] (Figure 4, **Supplemental Figure 16**) and BMI [19 studies (3, 9, 13, 14, 53, 65, 67, 84, 87–90, 98, 103, 113–116) enrolling 20,851 subjects, WMD: 0.54; 95% CI: 0.12, 0.97; $P = 0.012$] (Figure 4, **Supplemental Figure 17**). The association between TMAO concentrations and SBP remained significant when the meta-analysis included only cohort studies (WMD: 1.91; 95% CI: 1.39, 2.43; $P = 6.85 \times 10^{-13}$) (Figure 4).

The updated meta-analyses showed that high TMAO concentrations were associated with increased CRP concentrations (WMD: 0.27; 95% CI: 0.06, 0.48; $P = 0.012$) (Figure 4, **Supplemental Figure 18**) and decreased concentrations of TC

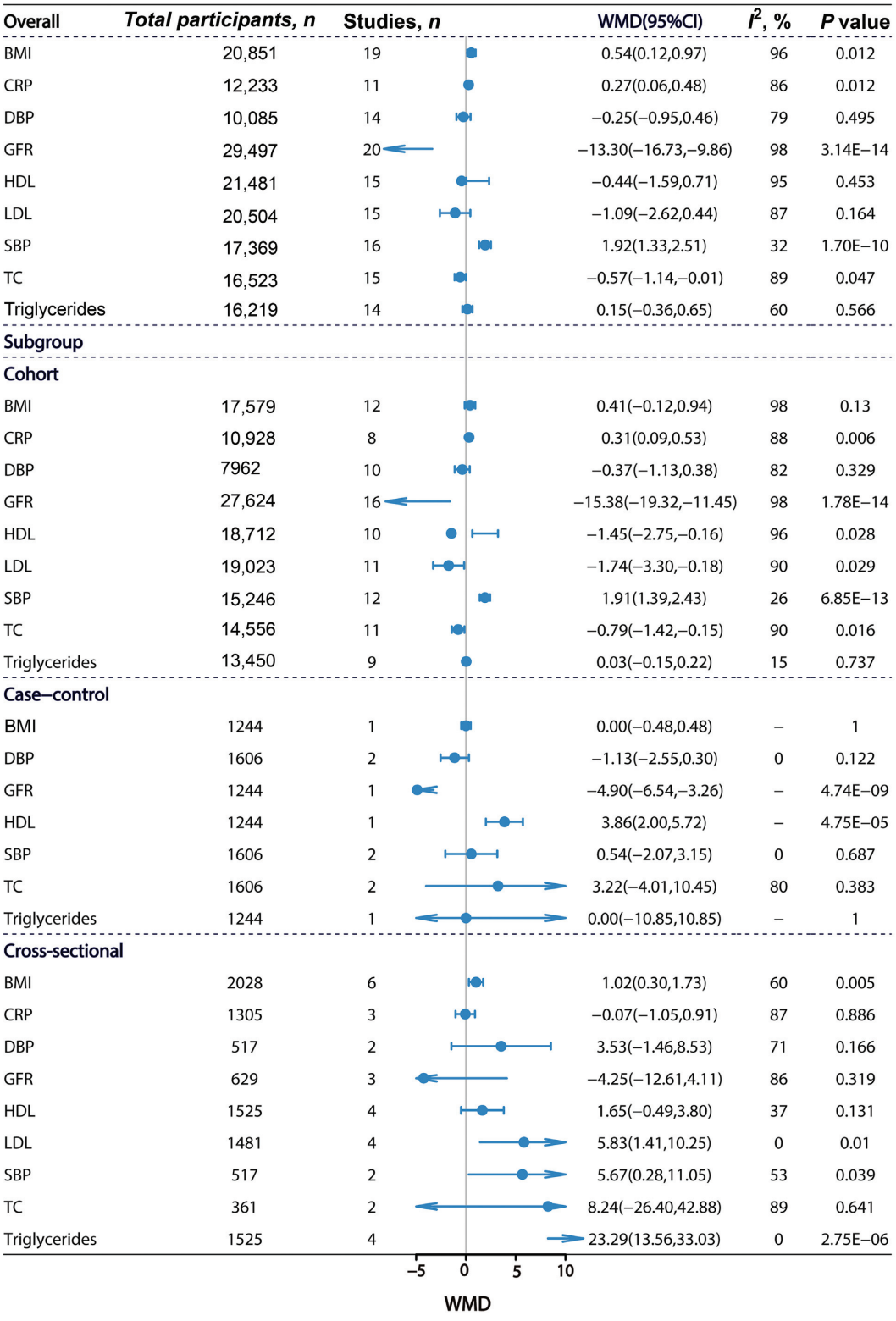

FIGURE 4 High compared with low TMAO concentrations and associations with multiple health outcomes. Estimates are WMD and meta-analyses are based on random-effect models. An I^2 value ≥50% is considered to indicate substantial heterogeneity. All results are presented as HR with 95% CIs, using the Mantel–Haenszel method with a random-effects model. CRP, triglycerides and C-reactive protein; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HDL, HDL cholesterol; LDL, LDL cholesterol; SBP, systolic blood pressure; TC, total cholesterol; WMD, weighted mean difference.

(WMD: −0.57; 95% CI: −1.14, −0.01; $P = 0.047$) (Figure 4, **Supplemental Figure 19**) but not of other lipids (HDL cholesterol, LDL cholesterol, triglycerides) (Figure 4, **Supplemental Figures 20–22**). The associations between TMAO concentrations and CRP (WMD: 0.31; 95% CI: 0.09, 0.53; $P = 0.006$) (Figure 4), HDL cholesterol (WMD: −1.45; 95% CI: −2.75, −0.16; $P = 0.028$) (Figure 4), LDL cholesterol (WMD: −1.74; 95% CI: −3.30, −0.18; $P = 0.029$) (Figure 4), and TC (WMD: −0.79; 95% CI: −1.42, −0.15; $P = 0.016$) (Figure 4) were significant in the confined meta-analyses of cohort studies.

Renal function

The umbrella review identified 1 meta-analysis reporting that circulating TMAO was associated with a decrease of GFR (WMD: −12.86; 95% CI: −16.57, −9.15; $P = 1.11 \times 10^{-11}$) (47). Our updated meta-analysis including 20 studies from 19 articles (3, 9, 13, 14, 48, 53, 55, 59, 65–67, 70, 77, 80, 89, 98, 113–115) enrolling 29,497 subjects found a consistently significant association (WMD: −13.30; 95% CI: −16.73, −9.86; $P = 3.14 \times 10^{-14}$) (Figure 4, **Supplemental Figure 23**). The association remained significant in the confined meta-analysis of cohort studies (WMD: −15.38; 95% CI: −19.32, −11.45; $P = 1.78 \times 10^{-14}$) (Figure 4).

Other health outcomes

We identified 10 original articles (17, 109–111, 117–122) that reported associations between TMAO concentrations and other health outcomes (Figure 1B, Supplemental Table 6). One reported that TMAO was not significantly associated with the risk of pre-eclampsia (117). Others reported significant associations between TMAO concentrations and other health outcomes [metabolic syndrome (17), diabetic retinopathy (118), hip fracture (119), Parkinson disease (120), and nonalcoholic fatty liver disease (121, 122)]. Quantitative meta-analysis could not be performed owing to the limited number of studies identified for these health outcomes.

Evidence assessment of included studies

Evidence assessment of the identified associations was performed according to our credibility assessment criteria (Supplemental Table 1, **Table 1**). Eight (44%) meta-analyses had $P < 10^{-6}$, 6 (33%) had a 95% PI that excluded the null, 12 (67%) had >1000 cases (or >20,000 total participants for continuous outcomes), 5 (28%) had no large heterogeneity ($I^2 < 50\%$), and 11 (61%) had neither small-study effects nor excess significance bias. After credibility assessment, no outcome presented convincing evidence; 6 (33%) health outcomes presented highly suggestive evidence (class II: CVD mortality, hypertension, MACE, all-cause mortality, DM, GFR); 3 (17%) presented suggestive evidence (class III: stroke, CVD, and CRC); and 5 (28%) presented weak evidence (class IV: SBP, BMI, TC, CRP, and GDM) for their associations with circulating TMAO concentrations.

Discussion

Our updated meta-analyses included a total of 82 individual studies and examined the associations of TMAO with 18 unique health outcomes. Among them, 14 outcomes (all-cause mortality, CVD, MACE, stroke, hypertension, CVD mortality, SBP, BMI, CRP, TC, DM, GDM, GFR, CRC) were found to be significantly associated with TMAO concentrations. When we restricted meta-analyses to only include cohort studies, 11 outcomes (all-cause mortality, MACE, hypertension, CVD mortality, SBP, CRP, HDL cholesterol, LDL cholesterol, TC, DM, GFR) were still significantly associated with TMAO concentrations. The dose-response analyses revealed that circulating TMAO concentrations were positively associated with the risk of hypertension and MACE. After assessment of the evidence credibility, we found highly suggestive associations of TMAO concentrations with 6 health outcomes, including all-cause mortality, CVD mortality, MACE, hypertension, DM, and GFR.

Former published meta-analyses (25, 38–40, 45) demonstrated that high TMAO concentrations were related to an increased risk of all-cause mortality and the updated meta-analysis showed consistent results. When conducting subgroup analysis by disease status, TMAO showed a significant association with all-cause mortality only in patients with CVD. In addition, our study revealed a positive association between TMAO concentrations and CVD risk. Given that the majority of evidence was from case–control studies, we cannot rule out reverse causality. It has been reported that TMAO may affect platelet reactivity, lipid metabolism, and endothelial dysfunction, which could result in the acceleration of atherosclerotic plaque formation (123). Because atherosclerosis is one of the major causes of CVD, high concentrations of TMAO could be related to high incidence of CVD, due to TMAO's contribution in the development of atherosclerosis. However, no causal association between TMAO and CVD was identified in a recent bidirectional Mendelian randomization study (124). Taken together, current evidence suggests that TMAO might be a novel biomarker indicating the risk of CVD.

Our umbrella review reported a highly suggestive association between TMAO concentrations and hypertension, and both the former published study (42) and the updated meta-analysis revealed that this association displayed a dose–response relation. Previous studies have found that hypertensive patients had more gut microbial enzymes involved in TMA production than those without hypertension (125). Animal studies have also found that elevated plasma concentrations of TMAO can prolong the duration of elevated blood pressure (126–128). TMAO could also promote Ang II–induced vasoconstriction via the PERK/ROS/CaMKII/PLCβ3 (protein kinase r-like endoplasmic reticulum kinase (PERK), reactive oxygen species (ROS), calmodulin-dependent protein kinase (CaMK), phospholipase c β3 (PLCβ3) axis, thereby facilitating Ang II–induced hypertension (126).

Both the former published study (23) and the updated meta-analysis revealed a positive association between TMAO concentrations and risk of DM. Previous studies reported supportive evidence on associations between TMAO and diabetes-related traits, including insulin resistance, impaired glucose metabolism, and metabolic syndrome (17, 129, 130). Animal studies also found that TMAO may exacerbate impaired glucose

TABLE 1 Association between TMAO concentrations and health outcomes and evidence class for meta-analyses[1]

Outcomes	Population	Study design	Comparison	Studies, *n*	Cases, *n*	Participants, *n*	Metric	Random-effects RR/HR/OR/WMD (95% CI)	*P* value	95% PI	Egger's *P*[2]	I^2,[3] %	*P* value for excess significance test[4]	Evidence class[5]
Cardiovascular outcomes														
CVD	CVD/non-CVD	CC/CS	High vs. low	12	5276	22,945	OR	1.50 (1.26, 1.79)	8.00E−06	0.92, 2.44	0.000	63.97	0.16	III
Hypertension	Healthy/hypertension	CO/CC/CS	High vs. low	15	10,293	18,854	RR	1.39 (1.22, 1.57)	3.47E−07	0.97, 1.99	0.201	70.99	NP	II
MACE	CKD/CVD/DM	CO/CC/CS	High vs. low	36	>7070	39,314	HR	1.74 (1.55, 1.95)	1.13E−22	1.07, 2.82	0.011	65.59	0.00	II
Stroke	Stroke/CVD/DM	CO/CC/CS	High vs. low	9	2546	9393	OR	2.88 (1.54, 5.39)	9.35E−04	0.44, 18.81	0.439	91.54	NP	III
Mortality														
All-cause mortality	General/CVD/CKD/DM	CO	High vs. low	37	>10,510	44,480	HR	1.60 (1.43, 1.79)	8.33E−16	0.91, 2.82	0.000	83.63	0.10	II
CVD mortality	CVD/non-CVD	CO/CC	High vs. low	8	>1002	11,296	HR	2.02 (1.74, 2.34)	6.01E−21	1.74, 2.34	0.480	0.00	0.24	II
Blood pressure and cardiometabolic biomarkers														
SBP	General/DM/CVD/stroke	CO/CC/CS	High vs. low	16	NA	17,369	WMD	1.92 (1.33, 2.51)	1.70E−10	0.74, 3.10	0.530	32.42	0.45	IV
DBP	General/DM/CVD/stroke	CO/CC/CS	High vs. low	14	NA	10,085	WMD	− 0.25 (−0.95, 0.46)	0.495	−2.07, 1.57	0.338	79.15	0.85	NS
BMI	General/DM/CVD/stroke	CO/CC/CS	High vs. low	19	NA	20,851	WMD	0.54 (0.12, 0.97)	0.012	−1.11, 2.20	0.954	96.13	0.18	IV
HDL cholesterol	General/DM/CVD/stroke	CO/CC/CS	High vs. low	15	NA	21,481	WMD	− 0.44 (−1.59, 0.71)	0.453	−4.42, 3.54	0.151	94.91	NP	NS
LDL cholesterol	General/DM/CVD/stroke	CO/CC/CS	High vs. low	15	NA	20,504	WMD	− 1.09 (−2.62, 0.44)	0.164	−5.31, 3.14	0.286	87.44	0.70	NS
TC	General/DM/CVD/stroke	CO/CC/CS	High vs. low	15	NA	16,523	WMD	− 0.57 (−1.14, −0.01)	0.047	−2.16, 1.02	0.513	88.65	0.65	IV
CRP	General/DM/CVD/stroke	CO/CS	High vs. low	11	NA	12,233	WMD	0.27 (0.06, 0.48)	0.012	−0.27, 0.81	0.112	86.11	NP	IV
Triglycerides	General/DM/CVD	CO/CC/CS	High vs. low	14	NA	16,219	WMD	0.15 (−0.36, 0.65)	0.566	−0.83, 1.13	0.000	60.02	0.76	NS
Diabetes mellitus														
Diabetes	CVD/diabetes/renal disease	CO/CC/CS	High vs. low	18	>5554	22,999	OR	1.75 (1.42, 2.16)	1.50E−07	0.83, 3.70	0.886	82.05	0.10	II
GDM	GDM/non-GDM	CC	High vs. low	3	952	2180	OR	2.24 (1.72, 2.93)	3.08E−09	1.71, 2.94	0.240	0.94	0.78	IV
Renal outcomes														
GFR	CKD/general	CO/CC/CS	High vs. low	20	NA	29,497	WMD	− 13.30 (−16.73, −9.86)	3.14E−14	−28.65, 2.05	0.724	97.92	0.76	II
Cancer														
CRC	CRC/non-CRC	CC	High vs. low	3	1058	2291	OR	1.49 (1.19, 1.88)	5.93E−04	1.11, 2.00	0.194	21.72	0.65	III

[1]CC, case–control study; CKD, chronic kidney disease; CO, cohort study; CRC, colorectal cancer; CRP, C-reactive protein; CS, cross-sectional study; CVD, cardiovascular disease; DBP, diastolic blood pressure; DM, diabetes mellitus; GDM, gestational diabetes mellitus; GFR, glomerular filtration rate; MACE, major adverse cardiovascular events; NA, not available; NP, not pertinent (because the number of expected significant studies was larger than the number of observed significant studies); NS, not significant; PI, prediction interval; SBP, systolic blood pressure; TC, total cholesterol; WMD, weighted mean difference.

[2]Egger's regression test was used to evaluate the small-study effects.

[3]Interstudy heterogeneity was tested using the Cochran *Q* statistic (t^2) at a significance level of $P < 0.10$ and quantified by the I^2 statistic. An I^2 value ≥50% is considered to indicate substantial heterogeneity. All results are presented as RR/OR/HR/WMD with 95% CIs, using the Mantel–Haenszel method with a random-effects model.

[4]Excess significance test was conducted to investigate whether the observed number of studies with significant results differed from the expected number of significant studies using the χ^2 test.

[5]Evidence class criteria: class I (convincing): statistical significance with $P < 10^{-6}$, >1000 cases (or >20,000 participants for continuous outcomes), the largest component study reported a statistically significant effect ($P < 0.05$), 95% PI excluded the null, no large heterogeneity ($I^2 < 50\%$), no evidence of small-study effects ($P > 0.10$) or excess significance bias ($P > 0.10$); class II (highly suggestive): statistical significance with $P < 10^{-6}$, >1000 cases (or >20,000 participants for continuous outcomes), the largest component study reported a statistically significant effect ($P < 0.05$); class III (suggestive): statistical significance with $P < 10^{-3}$, >1000 cases (or >20,000 participants for continuous outcomes); class IV (weak): the remaining statistically significant associations with $P < 0.05$.

tolerance and hyperglycemia by blocking the hepatic insulin signaling pathway and causing inflammation in adipose tissue (131), whereas a decrease of plasma TMAO could reduce plasma glucose and insulin resistance in mice by inhibiting the main TMAO-generating enzyme FMO3 (flavin-containing monooxygenase-3) (132). Furthermore, we found evidence from 2 studies (133, 134) reporting a positive association between TMAO concentrations and GDM, but the involvement of TMAO in any causal or compensatory pathway has not been proven. Therefore, further studies should be conducted to understand the mechanism of TMAO influencing GDM.

The former published study (47) and updated meta-analysis showed that an increase of TMAO concentrations was associated with lower GFR. Previous studies showed that chronic dietary exposures that increased TMAO concentrations appeared to directly contribute to progressive renal fibrosis and dysfunction (10, 135), which is one of the main end-stage renal diseases and a common outcome of almost all progressive chronic kidney diseases (CKDs) (136). Animal studies demonstrated that inhibition of TMAO production attenuated CKD development and cardiac hypertrophy in mice, suggesting that TMAO concentrations may play an important role in CKD development and TMAO reduction may be a novel strategy in treating CKD and its CVD complications (137). However, in this umbrella review, we only assessed the observational association of TMAO with GFR as an intermediate surrogate trait of CKD. Future studies focusing on CKD as an endpoint need to be performed to examine the association with TMAO concentrations.

It is widely known that TMAO is produced from the fermentation of dietary nutrients (choline, betaine, and carnitine) by the gut microbiota. Considering high concentrations of TMAO being associated with gut microbiota balance and several diseases, nonpharmacologic strategies, including foods and dietary supplements rich in bioactive compounds or nutrients, have the potential to modulate the gut microbiota to reduce TMAO concentrations, and therefore decrease the risk of several diseases. There is evidence showing that TMAO concentrations can be reduced by some bioactive compounds, such as resveratrol, allicin, capsanthin, and dietary components present in the apple, oolong tea, natural wheat bran, and low-fat diet, whereas strategies such as the paleolithic diet, high-fat diet, and high-protein diet promote increased TMAO concentrations (138). Because TMAO is a metabolite produced by the gut microbiota, targeting the gut microbiota and the metabolic pathway of TMAO might provide new strategies for the prevention of these related diseases (139). Further studies should be conducted to evaluate these dietary components' effectiveness, dose, and intervention time on TMAO concentrations and whether their health effects could be mediated through regulating TMAO concentrations.

Study strengths and limitations

Although previous meta-analyses of TMAO and the risk of disease outcomes have been conducted, our study is the first to summarize and present the evidence for the associations between TMAO concentrations and a wide spectrum of health outcomes systematically and thoroughly by incorporating information from meta-analyses of observational studies. In addition, our dose-response analyses revealed that there were no critical concentrations of TMAO in terms of varying degrees of risk in patients with all-cause mortality, diabetes, hypertension, and MACE disease. Subgroup analyses further evaluated the associations by only including prospective studies or studies adjusted for certain confounding factors. Although previous studies reported multiple health outcomes associated with TMAO concentrations, our study evaluated the reliability of these associations based on established credibility criteria.

Our study also has limitations. First, because all the included studies were observational, causal associations between circulating TMAO and related outcomes cannot be inferred. Second, sex- and ethnicity-specific findings could not be obtained owing to limited data. Diet-specific findings could not be obtained owing to limited data, and therefore we were not able to perform subgroup analyses to further explore the associations by minimizing the potential confounding of dietary patterns. Third, there was high heterogeneity in the current meta-analyses, possible reasons being the inclusion of different populations and different study designs. Further, our evidence grading was not sensitive to the use of 95% PIs or excess significance bias because the evidence grading remained the same when we removed them consecutively. In addition, when updating the meta-analyses, we added the newly identified studies, re-estimated summary effect estimates using random-effects models, and applied a set of well-established criteria to properly classify the evidence according to the reported *P* values, heterogeneity, and excess significance bias, with consideration of the inflated risk of false positives inherited by the updated meta-analyses (140). Finally, the underlying mechanisms between TMAO and the development of various diseases have not been explored in depth.

Conclusions

In conclusion, our umbrella review and updated meta-analyses identified multiple health outcomes associated with TMAO concentrations. Evidence assessment demonstrated that TMAO concentrations are associated with several health conditions, including all-cause mortality, CVD, hypertension, diabetes, and CKD. Our dose-response meta-analyses indicated that there were no critical concentrations of TMAO in terms of its health impact. Further studies are needed to investigate whether circulating TMAO concentrations could be an intervention target for chronic disease.

The authors' responsibilities were as follows—XL and YZ: conceived the study; ET: contributed to the study design; DL, YL, and SY: performed the systematic review and data extraction and wrote the manuscript; DL and YL: performed the statistical analysis; other authors (XC, YH, JC, QW, DH, AF, YB, PS, DB, KT, SCL, HY, and HZ): provided significant advice and consultation; and all authors: critically reviewed the manuscript, contributed important intellectual content, and read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

All data relevant to the study are included in the article or as supplementary information.

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