Gut microbiota-derived metabolite Trimethylamine-N-oxide (TMAO) and multiple health outcomes

Title: Gut microbiota-derived metabolite Trimethylamine-N-oxide (TMAO) and multiple health outcomes: an umbrella review and updated meta-analysis

Running title: TMAO and health outcomes

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Competing interests

No potential conflicts of interest were disclosed.

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Data share statement

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

Abbreviations used:

BMI, body mass index; CI, confidence interval; CRP, c-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; DM, diabetes mellitus; GDM, gestational diabetes mellitus; GFR, glomerular filtration rate; HDL, high-density lipoprotein cholesterol; HR, hazard ratio; LDL, low -density lipoprotein cholesterol; MACE, major adverse cardiovascular events; MD, mean difference; NOS, Newcastle-Ottawa Scale; OR, odds ratio; PI, prediction interval; RR, risk ratio; SBP, systolic blood pressure; TC, total cholesterol; TMA, trimethylamine; TMAO, Trimethylamine-N-oxide; T2DM, type 2 diabetes mellitus; WMD, weighted mean difference.

Abstract

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

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

Methods: We searched 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 confidence interval (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 levels and health outcomes including all-cause mortality, cardiovascular diseases, diabetes mellitus, cancer, and renal function. We updated these meta-analyses by including a total of 82 individual studies in 18 unique health outcomes. Among them, 14 associations were nominally significant. After evidence credibility assessment, we found six (33%) associations (i.e., all-cause mortality, cardiovascular disease mortality, major adverse cardiovascular events, hypertension, diabetes mellitus, 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, cardiovascular disease, diabetes, cancer and kidney function. Further studies are needed to investigate whether circulating TMAO levels could be an intervention target for chronic disease.

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 has discovered that circulating TMAO levels are linked to a wide range of health outcomes, including cardiovascular and cerebrovascular diseases(4-6), type 2 diabetes mellitus (T2DM)(7), hypertension(8), renal dysfunction(9, 10), cancer and mortality(11, 12). The relationship between elevated plasma TMAO concentrations and health-related traits have also been explored, including glomerular filtration rate (GFR),(9) blood pressure(13, 14), body mass index (BMI)(9, 14), and total cholesterol(15). It has been hypothesized that 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 gut microbiota(17-19).

Most evidence on the health effects of plasma TMAO levels has been generated by observational studies with conflicting results. In addition, some studies were conducted among patients with specific diseases, which questions 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 relationships between increased TMAO levels and risk of obesity(21), stroke(22), diabetes(23), hypertension(24) and all-cause mortality(25) have been conducted. A comprehensive credibility assessment of these association 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 levels.

Methods

Study design

In this umbrella review, all meta-analyses on the associations between plasma TMAO levels 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 (registration number: CRD42021284730).

Literature search

Two investigators (DDL and YL) independently searched 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: "((((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 individuals observational studies to update the identified meta-analyses and reported the results in accordance to the PRISMA checklist(26). All identified publications went through a three-step parallel review of title, abstract, and full text based on pre-defined inclusion and exclusion criteria, and any discrepancies were resolved by consensus.

Eligibility criteria

Meta-analyses performing quantitative analysis of plasma TMAO levels 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 relative risks, odds ratios, hazard ratios or 95% confidence intervals [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 (risk ratio(RR), odds ratio(OR), hazard ratio(HR), or mean difference(MD)) with 95% CIs, the number of participants and cases, and investigated outcomes. For meta-analyses on more than one health outcomes, 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-analysis, we added the newly identified studies and re-estimated 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, relative risk estimates, and 95% CIs. Two investigators (DDL and YL) extracted data independently using a predesigned data extraction form. The quality of individual studies were 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); 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 95% PI is based on the predicted distribution derived from a function of the degree of heterogeneity, number of studies included, and within study standard errors)(29, 30). Egger regression test was used to evaluate the small study effects(31). Excess significance test was conducted to investigate whether the observed number of studies with significant results differs from the expected number of significant studies using the $\chi 2$ test(32-34). The expected number of significant studies for each meta-analysis was calculated by summing 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.

Evaluation of the Evidence Credibility

We used credibility assessment criteria (**Supplementary 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 four categories: convincing, highly suggestive, suggestive, or weak evidence (Class I, II, III, and IV)(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 (**Supplementary Table 2**). There was one meta-analysis published for stroke(22), hypertension(42), diastolic blood pressure (DBP)(24), systolic blood pressure (SBP)(24), diabetes(23), BMI(21), low/high-density lipoprotein cholesterol (LDL/HDL)(24), total cholesterol (TC)(24), triglycerides(24), c-reactive protein (CRP)(41), and glomerular filtration rate (GFR)(47), two meta-analyses for cardiovascular disease(CVD)(39, 46), five meta-analyses for all-cause mortality(25, 38-40, 45) and six 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 association between plasma TMAO levels and 18 unique health outcomes. The quality assessment of included studies is shown in **Supplementary Table 3-5**.

All-cause mortality

The updated meta-analysis included 37 studies from 32 articles(3, 5, 10-12, 48-74) with more than 9,553 cases out of 38,862 participants. All-cause mortality in the highest TMAO category was compared to that in the lowest TMAO category, and it was found that a higher TMAO level was associated with higher mortality (HR: 1.60, 95%CI: 1.43, 1.79; P=8.33×10^{-16}, **Figure 2, Supplementary Figure 1**). A dose-response meta-analysis based on 10 studies (3, 5, 10, 12, 58, 62, 65, 66, 68, 70) showed that a one-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=8.03×10^{-12}, **Figure 3A**). We also conducted a subgroup analysis by disease status and found that the association between TMAO and all-cause mortality was predominant in cardiovascular disease patients (HR: 1.66, 95% CI: 1.46, 1.88; P=1.84×10^{-15},

Supplementary Figure 2), while 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=3.45×10^{-12}, **Supplementary 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 more than 7,070 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=1.13×10^{-22}, **Figure 2, Supplementary 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=1.50×10^{-14}, **Supplementary Figure 5**). Three studies(66, 68, 70) were included in the dose-response analysis, resulting in 11% increased risk of MACE per 1μmol/L increment of TMAO (RR: 1.11, 95% CI: 1.07, 1.14; P=1.04×10^{-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 of 10,293 cases and 18,854 total participants. There was a significant association between TMAO levels and risk of hypertension (RR: 1.39, 95% CI: 1.22, 1.57; P=3.47×10^{-7}, **Figure 2, Supplementary 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=8.58×10^{-5}, **Figure 2**), and the association was still significant when the meta-analysis included only the studies that adjusted for renal function(RR: 1.40, 95%CI: 1.13,1.72; P=1.65×10^{-3}, **Supplementary 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 (1μmol/L) increment of TMAO (RR: 1.07, 95% CI: 1.03, 1.11; P=6.49×10^{-4}, **Figure 3C**).

The updated meta-analysis on CVDs included twelve studies(4, 6, 83, 91-96) with 22,945 participants and showed that high TMAO levels were statistically significantly associated with an increased risk of CVD (OR: 1.50, 95% CI: 1.26, 1.79; P=8.00×10^{-6}, **Figure 2, Supplementary Figure 8**). Eight studies from five articles(11, 14, 60, 72, 83) were used to perform a meta-analysis on CVD mortality. The results revealed that participants with high TMAO levels were more likely to die from CVDs than those with low TMAO levels (HR: 2.02, 95% CI: 1.74, 2.34; P=6.01×10^{-21}, **Figure 2, Supplementary Figure 9**). The association remained significant when the meta-analysis was restricted to cohort studies (HR: 2.00, 95%CI: 1.72 to 2.33, P=3.06×10^{-19}, **Figure 2**)

Result from the updated meta-analysis of stroke showed that higher circulating TMAO levels were associated with a higher risk of stroke (nine studies(66, 69, 83, 90, 97-100) enrolling 9393 participants, OR: 2.88, 95% CI: 1.54, 5.39; P=9.35×10^{-4}, **Figure 2, Supplementary 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 to 11.62, p=0.255, **Figure 2**).

Diabetes mellitus

Our updated meta-analyses, including eighteen studies (from seventeen 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 diabetes mellitus (OR: 1.75, 95% CI: 1.42, 2.16; $P=1.50\times10^{-7}$, **Figure 2, Supplementary Figure 11**). The association was also significant in the confined meta-analysis of cohort studies (OR: 1.81, 95%CI: 1.54, 2.12; $P=2.09\times10^{-8}$, **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; $P=1.12\times10^{-5}$, **Supplementary Figure 12**). In our dose-response meta-analysis, based on data from three articles(87, 88, 102), we found no statistically significant relationship between TMAO levels and DM (P=0.228, **Figure 3D**). Furthermore, our meta-analysis of three studies enrolling 2180 subjects showed that women with high TMAO levels were more likely to have gestational diabetes mellitus (GDM) (OR: 2.24, 95% CI: 1.72, 2.93; $P=3.08\times10^{-9}$, **Figure 2, Supplementary Figure 13**).

Cancer risk

We identified six observational studies that examined the associations of TMAO levels with cancer risk including colorectal cancer(CRC)(106-108), prostate

cancer(109), primary liver cancer(110), and pancreatic cancer(111). Quantitative meta-analysis could only be performed for CRC which included three individual studies and showed a positive association between high TMAO levels and increased risk of CRC (OR: 1.49, 95%CI: 1.19, 1.88; P=5.93×10^{-4}, **Figure 2, Supplementary Figure 14**). Three articles reported positive association 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) (**Supplementary Table 6**).

Blood pressure and cardiometabolic biomarkers

The results of the updated meta-analyses showed no significant association between TMAO and DBP (fourteen studies(9, 13-15, 59, 67, 87-89, 98, 112-114) enrolling 10,085 subjects, weighted mean difference (WMD): -0.25, 95% CI: -0.95, 0.46; P= 0.495, **Figure 4, Supplementary Figure 15**). Higher circulating TMAO was related to higher SBP (sixteen 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 =1.70×10^{-10}, **Figure 4, Supplementary Figure 16**) and BMI (nineteen 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, Supplementary Figure 17**). The association between TMAO levels and SBP remained significant when the meta-analysis included only cohort studies (WMD: 1.91, 95%CI: 1.39, 2.43; P =6.85×10^{-13}, **Figure 4**).

The updated meta-analyses showed that high TMAO levels were associated with

increased CRP levels (WMD: 0.27, 95% CI: 0.06, 0.48; *P*=0.012, **Figure 4, Supplementary Figure 18**) and decreased levels of TC (WMD: -0.57, 95% CI: -1.14, -0.01; *P*= 0.047, **Figure 4, Supplementary Figure 19**) but not of other lipids (HDL, LDL, triglycerides) (**Figure 4, Supplementary Figure 20, 21, 22**). The association between TMAO levels and CRP (WMD: 0.31, 95%CI: 0.09, 0.53; *P*= 0.006, **Figure 4**), HDL (WMD: -1.45, 95%CI: -2.75, -0.16; *P*= 0.028, **Figure 4**), LDL (WMD: -1.74, 95%CI: -3.30, -0.18, *P*= 0.029, **Figure 4**), TC (WMD: -0.79, 95%CI: -1.42, -0.15; *P*= 0.016, **Figure 4**) was significant in the confined meta-analyses of cohort studies.

Renal function

The umbrella review identified one meta-analysis reporting that circulating TMAO was associated with a decrease of GFR (WMD: -12.86, 95%CI: -16.57, -9.15; $P=1.11\times10^{-11}$)(47). Our updated meta-analysis including twenty studies from nineteen 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; $P=3.14\times10^{-14}$, **Figure 4, Supplementary Figure 23**). The association remained significant in the confined meta-analyses of cohort studies (WMD: -15.38, 95%CI: -19.32 to -11.45, $P=1.78\times10^{-14}$, **Figure 4**).

Other health outcomes

We identified 10 original papers(17, 109-111, 117-122) that reported the association

between TMAO levels and other health outcomes (**Figure 1B**, **Supplementary Table 6**). One reported that TMAO was not significantly associated with the risk of preeclampsia(117). Others reported significant association between TMAO levels and other health outcomes (metabolic syndrome(17), diabetic retinopathy(118), hip fracture(119), Parkinson's disease(120), and non-alcoholic fatty liver disease(121, 122)). Quantitative meta-analysis could not be performed due 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 (**Supplementary Table 1**, **Table 1**). Eight (44%) meta-analyses had $P<10^{-6}$, six (33%) had a 95% PI that excluded the null, 12 (67%) had more than 1000 cases (or more than 20000 total participants for continuous outcomes), five (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, six (33 %) health outcomes presented highly suggestive evidence (Class II: CVD mortality, hypertension, MACE, all-cause mortality, DM, GFR), three (17%) presented suggestive evidence (Class III: stroke, CVD and CRC) and five (28%) presented weak evidence (Class IV: SBP, BMI, TC, CRP and GDM) for their associations with circulating TMAO levels.

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 levels. When we restricted meta-analyses to only include cohort studies, 11 outcomes (all-cause mortality, MACE, hypertension, CVD mortality, SBP, CRP, HDL, LDL, TC, DM, GFR) were still significantly associated with TMAO levels. The dose-response analyses reveled that circulating TMAO levels were positively associated with the risk of hypertension and MACE. After assessment of the evidence credibility, we found highly suggestive associations of TMAO levels with six 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 levels 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. Additionally, our study revealed a positive association between TMAO levels 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 formation plaque(123). As atherosclerosis is one of the major causes of CVD, high levels 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 suggested that TMAO might be a novel biomarker indicating the risk of CVD.

Our umbrella review reported a highly suggestive association between TMAO levels and hypertension, and both the former published study(42) and the updated meta-analysis revealed that this association displayed a dose-response relationship. 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 levels 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 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 levels and risk of DM. Previous studies reported supportive evidence on the 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 tolerance and hyperglycemia by blocking the hepatic insulin signaling pathway and causing inflammation in adipose tissue(131), while a decrease of plasma TMAO could reduce plasma glucose and insulin resistance in mice by inhibiting the main TMAO-generating enzyme FMO3(132). Furthermore, we found evidence from two studies(133, 134) reporting a positive association between TMAO levels 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 levels was associated with lower GFR. Previous studies showed that chronic dietary exposures that increased TMAO levels appear to directly contribute to progressive renal fibrosis and dysfunction(10, 135), which is one of the main of end-stage renal diseases (ESRD) 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 levels may play an important role in CKD development and TMAO reduction may be a novel strategy in treating CKD and its cardiovascular disease 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 levels.

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

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 levels 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 levels, our study evaluated the reliability of these associations based on established credibility criteria.

Our study also has limitations. First, since 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 due to limited data. Diet-specific findings could not be obtained due 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 current meta-analyses with the possible reasons being the inclusion of different populations and different study designs. Further, our evidence grading was not sensitive to the use of 95% prediction intervals or excess significance bias since the evidence grading remained the same when we removed them consecutively. In addition, when updating the meta-analyses, we added the newly identified studies and 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 inflated risk of false positive 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 levels. Evidence assessment demonstrated that TMAO levels 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 levels could be an intervention target for chronic diseases.

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Author contributions

XL and YMZ conceived the study and ET contributed to the design. DDL, YL and SY performed the systematic review and data extraction. DDL and YL performed the statistical analysis. DDL, YL and SY wrote the manuscript. Other authors provided significant advice and consultation. All authors critically reviewed the manuscript and contributed important intellectual content. All authors have read and approved the final manuscript as submitted.

Consent to publication

Not applicable.

Data availability statement

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

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Table 1 Association between TMAO levels and health outcomes and evidence class for meta-analyses

Outcomes	Population	Study design	Comparison	No of studies	No of cases	No of participants	Metric	Random effects RR/HR/OR/WMD (95% CI)	P value	95% prediction interval	Egger's P	I^2 (%)	P value for excess significance test	Evidence class
Cardiovascular outcomes														
CVD	CVD/ non-CVD	CC/ CS	high vs low	12	5276	22945	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	10293	18854	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	39314	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	>10510	44480	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	11296	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	17369	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	10085	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	20851	WMD	0.54(0.12, 0.97)	0.012	-1.11, 2.20	0.954	96.13	0.18	IV
HDL	General/DM/CVD/ stroke	CO/ CC/ CS	high vs low	15	NA	21481	WMD	-0.44(-1.59, 0.71)	0.453	-4.42, 3.54	0.151	94.91	NP	NS
LDL	General/DM/CVD/ stroke	CO/ CC/ CS	high vs low	15	NA	20504	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	16523	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	12233	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	16219	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	22999	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	29497	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]NP=not pertinent (because the number of expected significant studies was larger than the number of observed significant studies); NA=not available; NS=not significant.

[2] CO= cohort study; CS= cross-sectional study; CC= case-control study.

[3]CVD: cardiovascular disease; CKD: chronic kidney disease; MACE: major adverse cardiovascular events; DBP: diastolic blood pressure; SBP: systolic blood pressure; BMI: body mass index; LDL: low - density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; TC: total cholesterol; CRP: triglycerides and c-reactive protein; GFR: glomerular filtration rate; DM: diabetes mellitus; GDM: gestational diabetes mellitus; CRC: colorectal cancer.

[4]RR= relative risk; OR= odds ratio; HR: hazard ratio; WMD= weighed mean difference.

[5]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 $\geqslant$50% is considered to indicate substantial heterogeneity. All results are presented as RR/OR/HR/WMD with 95% confidence intervals, using the Mantel-Haenszel method with a random-effects model. Egger regression test was used to evaluate the small study effects. Excess significance test was conducted to investigate whether the observed number of studies with significant results differs from the expected number of significant studies using the $\chi 2$ test.

[6]Evidence class criteria: class I (convincing): statistical significance with P<10^{-6}, more than 1000 cases (or >20 000 participants for continuous outcomes), the largest component study reported statistically significant effect (P<0.05); 95% prediction interval excluded the null; no large heterogeneity (I2 <50%), no evidence of small study effects (P>0.10) and excess significance bias (P>0.10); class II (highly suggestive): statistical significance with P<10^{-6}, more than 1000 cases (or >20 000 participants for continuous outcomes), the largest component study reported statistically significant effect (P<0.05); class III

(suggestive): statistical significance with $P<10^{-3}$, more than 1000 cases (or >20 000 participants for continuous outcomes); class IV (weak): the remaining statistically significant associations with $P<0.05$.

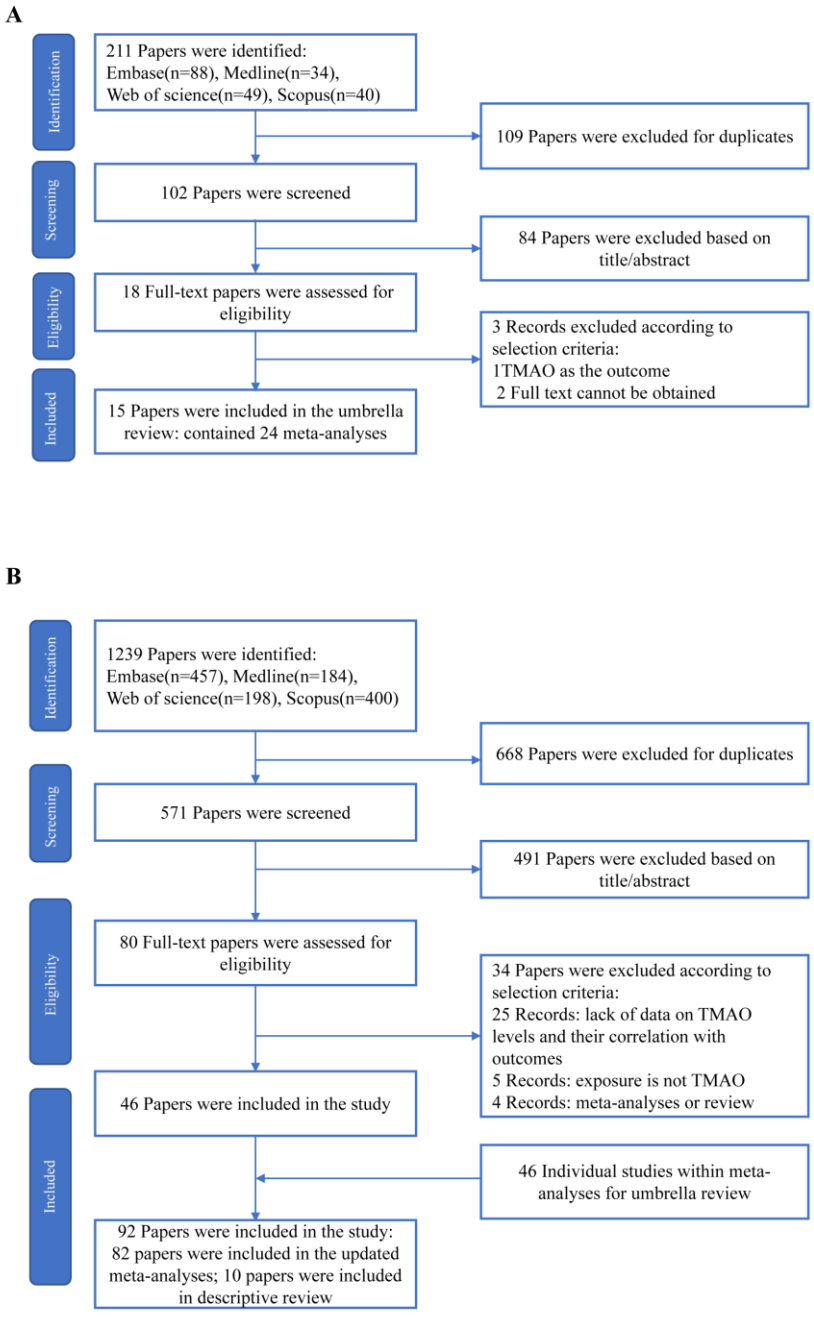

Figure1 Flow diagram of study selection. (A) Study selection for umbrella review; (B) Study selection for the updated meta-analyses.

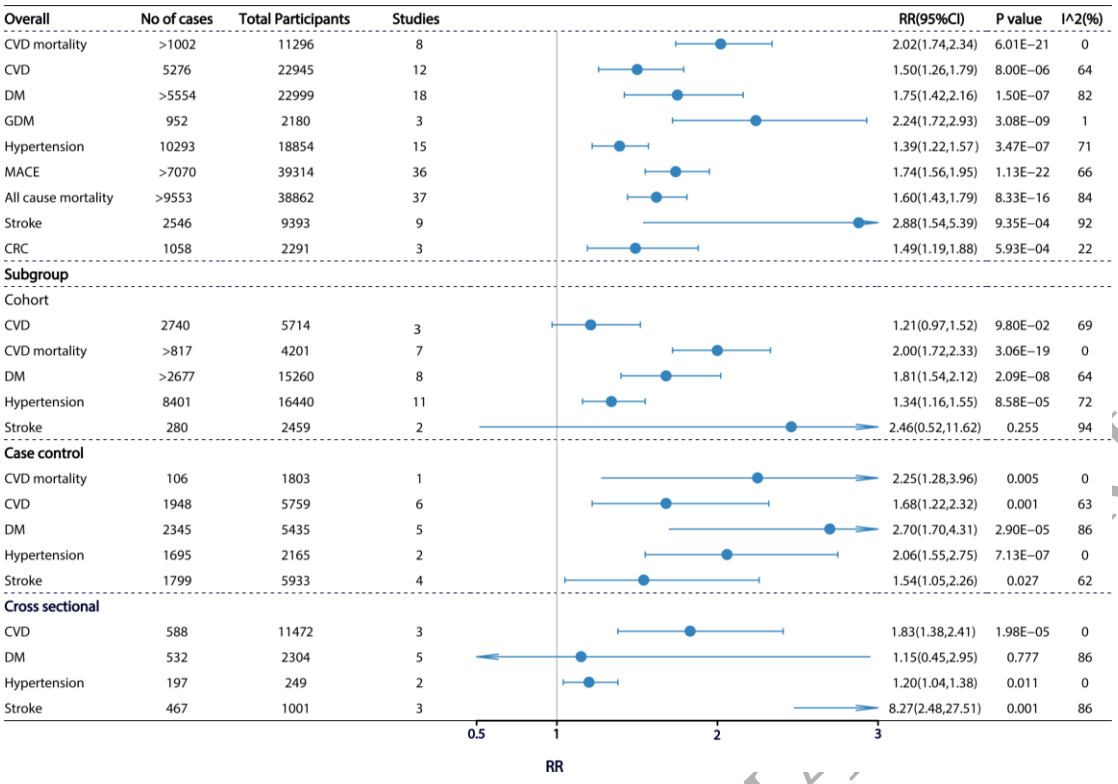

Figure 2 High versus low TMAO concentrations and associations with multiple health outcomes. Estimates are relative risks and meta-analyses are based on random effect models. CVD: cardiovascular disease; DM: diabetes mellitus; GDM: gestational diabetes mellitus; MACE: major adverse cardiovascular events. An I^2 value ≥50% is considered to indicate substantial heterogeneity. All results are presented as hazard ratio with 95% confidence intervals, using the Mantel-Haenszel method with a random-effects model.

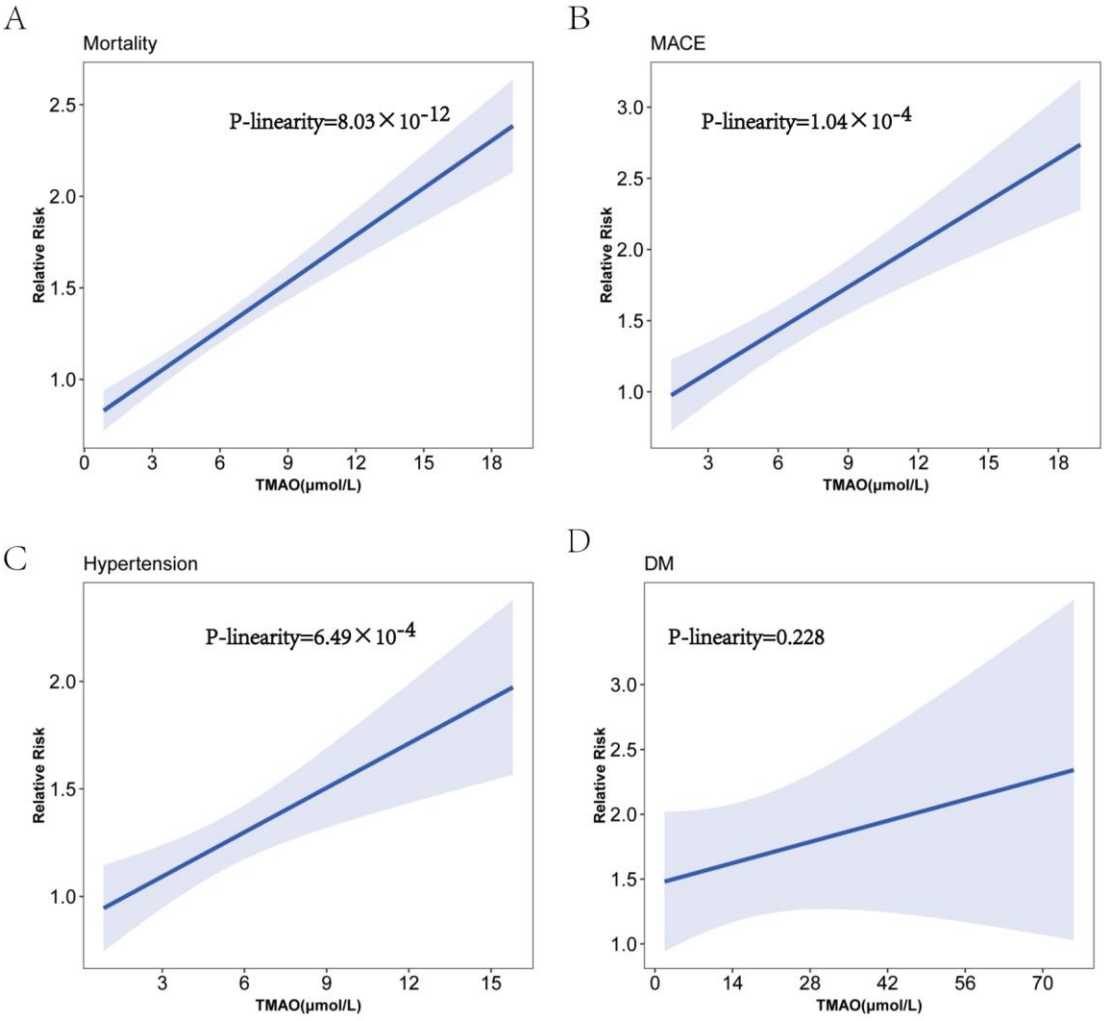

Figure 3 Dose-response association between circulating trimethylamine N-oxide (TMAO) levels and all-cause mortality(A), MACE(B), hypertension(C) and DM(D). Risk Spline (solid line) and 95% confidence intervals (shadow) of pooled relative risk of all-cause mortality, MACE, hypertension, and DM by 1μmol/L of TMAO.

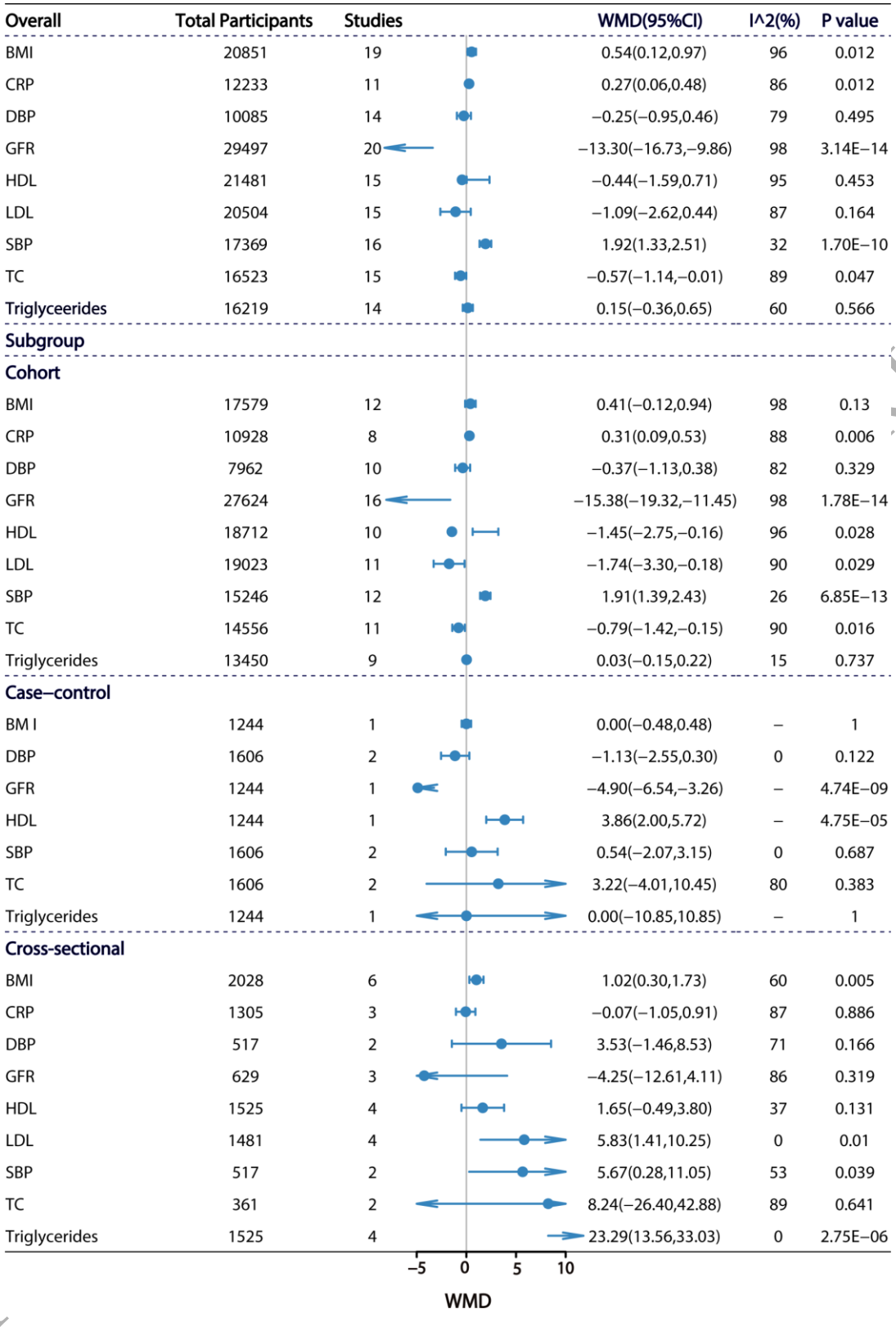

Figure 4 High versus low TMAO concentrations and associations with multiple

health outcomes. Estimates are weighted mean difference and meta-analyses are based on random effect models. DBP: diastolic blood pressure; SBP: systolic blood pressure; BMI: body mass index; LDL: low - density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; TC: total cholesterol; CRP: triglycerides and c-reactive protein; GFR: glomerular filtration rate. An I^2 value ≥50% is considered to indicate substantial heterogeneity. All results are presented as hazard ratio with 95% confidence intervals, using the Mantel-Haenszel method with a random-effects model.