REVIEW

Modulation of Circulating Trimethylamine *N*-Oxide Concentrations by Dietary Supplements and Pharmacological Agents: A Systematic Review

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ABSTRACT

Discovery of the association of plasma/serum trimethylamine *N*-oxide (TMAO) concentrations with atherosclerosis has sparked immense interest in exploring TMAO as a predictor of cardiovascular disease risk. A spectrum of antibiotics and other therapeutic strategies have been employed to test their potential to modulate TMAO concentrations, assuming the gut microbiome to be the key source of TMAO. The aim of this systematic review was to determine whether dietary supplements or pharmacological agents affect TMAO concentrations in adults. Six databases were searched (Medline, EMBASE, CINAHL, Scopus, ProQuest, and PubMed) for randomized and nonrandomized controlled trials. Searches were limited to the English language and to studies in adults. Thirteen eligible trials were identified, including 6 studies on dietary supplements and 7 on pharmacological agents. Whereas intervention studies involving dietary supplements were mostly randomized controlled trials, those involving pharmacological agents appeared opportunistic and varied greatly in study design and duration. Different interventional products were tested, and the studies lacked the consistency to reliably synthesize any evidence for the modifiability of TMAO concentrations by dietary supplements or pharmacological agents. Choline and L-carnitine are conditionally essential nutrients, and carefully designed placebo-controlled randomized trials specifically aimed at reducing the synthesis of microflora-dependent TMAO production from choline-containing precursors by pro- and/or prebiotics, antibiotics, or other pharmaceutical agents may be the way forward for future research. *Adv Nutr* 2019;10:876–887.

Keywords: trimethylamine *N*-oxide, gut microbiota, dietary supplements, drugs, metabolic disease

Introduction

Trimethylamine *N*-oxide (TMAO) is a low-molecular-weight amine oxide derived by the microbial metabolism of choline-containing compounds (phosphatidylcholine, carnitine, and betaine) to trimethylamine (TMA) in the large bowel, followed by oxidation of TMA to TMAO in the liver by the flavin monooxygenase 3 (FMO3) enzyme (1) (**Figure 1**). TMAO is known to accumulate in the tissues of marine animals, in which it functions to protect against the adverse effects of temperature, salinity, and protein-destabilizing effects of high urea and hydrostatic pressure (2). Although known to marine biologists for a long time, TMAO has generated much research interest in recent years with the discovery of its association with cardiovascular disease (CVD) in humans (3). Foods rich in choline, lecithin, and L-carnitine, such as red meat, eggs, dairy products, and saltwater fish, serve as potential precursors of TMAO in humans. Consumption of diets rich in these foods has been reported to be associated with high TMAO concentrations in the circulation (3–5). A direct association between TMAO and CVD risk was first reported in a large clinical cohort in 2011 (1). The study independently confirmed the prognostic value of elevated concentrations of TMAO, choline, and betaine in the diet in healthy subjects undergoing elective cardiac evaluation, being associated with an increased risk of peripheral artery disease, coronary artery disease, and myocardial infarction. Furthermore, results were consistent with the findings in atherosclerosis-prone mice fed a chow diet enriched with either choline or TMAO, showing a clear dose-dependent relationship between TMAO concentrations

The authors reported no funding received for this study.
Author disclosures: NAK, KA Abbott, KA Alburikan, HAA, ES, and MLG, no conflicts of interest.
Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/advances/.
Address correspondence to MLG (e-mail: manohar.garg@newcastle.edu.au).
Abbreviations used: AXOS, arabinoxylan oligosaccharide; CKD, chronic kidney disease; CVD, cardiovascular disease; FMO3, flavin monooxygenase 3; HD, hemodialysis; LcS, *Lactobacillus casei* Shirota; TMA, trimethylamine; TMAO, trimethylamine *N*-oxide.

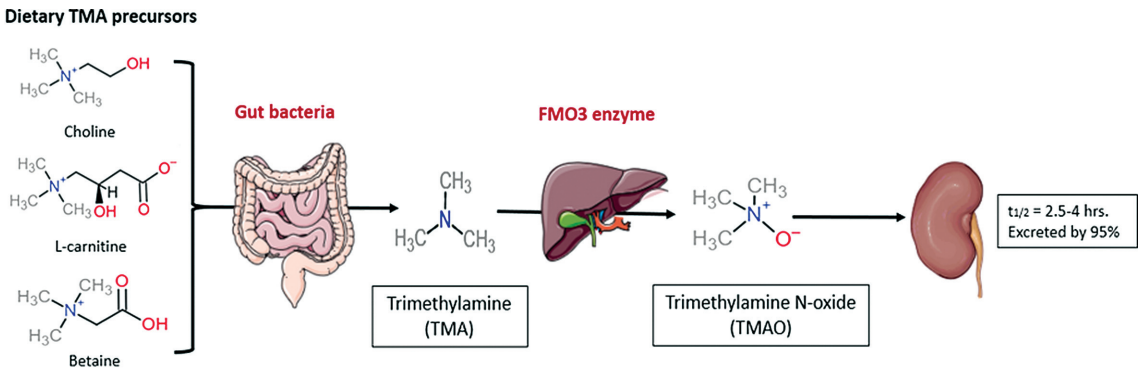

FIGURE 1 TMA–TMAO gut microbiota-dependent production. TMA, trimethylamine; TMAO, trimethylamine *N*-oxide.

and atherosclerotic plaque size (1). Two recently published meta-analyses suggested a strong association between increased TMAO concentrations and elevated risk of heart disease, including heart failure and stroke. An increase in all-cause mortality was also reported in patients with elevated TMAO concentrations (6, 7). Another robust association was found between higher TMAO concentrations (>6.18 μM) and an increased risk of thrombotic events, increased carotid intima media thickness, and atherosclerosis (8). The highest quartile of TMAO concentrations in the range of 6.18–318 μM was associated with a 1.64-fold increase in incident thrombosis risk compared with the lowest quartile (<2.43 μM) (8). Previous studies have proposed an adverse association between plasma TMAO concentrations and diabetes risk in animal models of diabetes (9). However, a direct association between plasma TMAO concentrations and type 2 diabetes mellitus in humans has not been reported. Pharmacokinetics studies in healthy subjects have suggested that TMAO is predominantly eliminated through the kidneys by glomerular filtration; therefore, a strong association of TMAO concentrations with renal function has been postulated (10). Elevated concentrations of TMAO have been associated with kidney damage and dysfunction, and they portend poor long-term survival in patients with chronic kidney disease (CKD) (11). In addition, elevated TMAO concentrations have been demonstrated in certain types of cancers, including colorectal cancer in postmenopausal women (12) and prostate cancer (13, 14). Wide inter- and intra-individual variations in circulating TMAO concentrations exist among human populations (15). Several factors determine TMAO concentrations and can thus confound the association between TMAO and disease etiology, including host gut microbiota, diet, kidney function, liver enzymes, age, and anthropometric measures including BMI (in kg/m^2). However, there appears to be only a marginal role for genetics in determining TMAO concentrations (15, 16). A number of studies have measured fasting plasma concentrations of TMAO in healthy and diseased subjects following dietary interventions. Given the fact that numerous dietary precursors carry a trimethylamine moiety, they have the potential to influence TMAO concentrations and subsequent cardiovascular and metabolic disease risk (3, 17).

Despite reports on the association of TMAO with metabolic diseases, its specific mechanism remains speculative. It is yet to be determined whether TMAO acts as a mediator in causing CVD or whether TMAO concentrations might be influenced by factors affecting clot formation and lipid metabolism. It has been suggested that TMAO exerts its pathological effect through multiple mechanisms, mainly through suppressing the net reverse cholesterol transport in the small intestine and decreasing cholesterol efflux, which will lead to cholesterol accumulation (4, 18, 19). In addition, TMAO may enhance platelet responsiveness (8) and promote foam cell formation by increasing cell surface expression of proatherogenic scavenger receptors on macrophages CD36 and SR-A1, both of which are involved in pathogenesis of atherosclerosis (4, 20). In contrast, a recent study suggested that TMAO may have a protective effect against atherosclerosis by decelerating aortic lesion formation (21). In addition, FMO3 is suggested to play a crucial role in the regulation of circulating TMAO concentrations and the promotion of CVD and atherosclerosis (18, 22, 23). The gastrointestinal microbiome plays an important role in host health and disease (24, 25). It is possible that alterations in the development or composition of gut microbiota (known as dysbiosis) by environmental factors, including host diet and genetics, disturb the partnership between the microbiota and the human immune system, thus generating a disease-prone state (26). The complexity of the interaction of the gut microbiome with such factors has impeded efforts to explore the possibility of modulating the gut microbiota and their metabolites using various therapeutic approaches for health benefits; however, the discovery of TMAO and its association with cardiovascular disease risk has excelled such efforts (15). A spectrum of antibiotics and other therapeutic strategies have been employed to test their potential to modulate TMAO concentrations, assuming the gut microbiome to be the key source of TMAO. In addition, a limited number of oral probiotics have been examined for their ability to exert an influence on TMAO concentrations

TABLE 1 Inclusion and Exclusion Criteria (PICOTSS)[1]

Criterion	Inclusion	Exclusion
Population	Adult humans	In vitro studies, animals, and pediatrics
Intervention	Dietary supplements or pharmacological drugs	Dietary patterns Surgical procedures
Comparison	Placebo, any treatment, or no treatment	None
Outcome	TMAO concentrations in plasma, serum, and urine	Betaine, choline, and other biomarker levels
Timing	Any study duration	None
Study design and article type	RCT or nonrandomized controlled trails Crossover and parallel trials Original articles published in peer review journal or in the gray literature Abstracts Patents	Observational studies Reviews Experts opinions Editorials Protocols
Study setting	Any setting, inpatients or outpatients	None

[1] PICOTSS, population, intervention, comparison, outcome, timing, study design and study setting; RCT, randomized controlled trial; TMAO, trimethylamine *N*-oxide.

and TMAO-producing species (25, 27). Therefore, the aim of our systematic review was to determine whether dietary supplements or pharmacological agents affect TMAO concentrations in adults, as measured in serum, plasma, or urine, in randomized and nonrandomized controlled trials.

Methods

This systematic review followed the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (PRISMA) (28). The protocol was published in the PROSPERO database (http://www.crd.york.ac.uk/PROSPERO) under registration number CRD42018084978.

Literature search and study identification

A preliminary search for existing systematic reviews on this area and related literature was performed. The initial search in Web of Science was performed using TMAO, trimethylamine *N*-oxide, treatment, diet, and drug therapy as keywords. The nails project (Network Analysis Interface for Literature Studies; http://nailsproject.net) was used to identify the highly cited articles and keywords to explore this research scope. A comprehensive literature search was performed in the following electronic databases: *1*) MEDLINE through Ovid, *2*) EMBASE, *3*) ProQuest, *4*) Scopus, *5*) PubMed, and *6*) CINAHL.

We used the following Medical Subject Headings (MeSH) words and their combinations: (TMAO *or* trimethylamine *N*-oxide *or* trimethylamine-*N*-oxide) *and* (gut microbiota *or* gut microbial flora *or* intestinal bacteria *or* Microbiome) *and* (diet *or* (treatment *or* drug therapy *or* agents)) *and* (randomized controlled trial *or* intervention studies *or* clinical trial(s)) *and* (cardiovascular disease *or* CVD *or* atherosclerosis). Full searching in MEDLINE is available as supplementary material (**Supplemental Table 1**). In addition, we conducted a manual search of the reference lists of the eligible studies to identify any further pertinent articles. Authors of primary studies or reviews were contacted for further information when required. We also searched ClinicalTrials.gov to identify any ongoing trials. Google Scholar and ProQuest were used to identify more papers including patents, conference abstracts, dissertations, and theses. The search included all articles published until July 2018.

Eligibility criteria and selection process

Studies were included if they were in adult humans; were randomized or nonrandomized controlled trials; and investigated the effect of specific interventions, either dietary supplements or pharmacological agents, on TMAO concentrations in serum, plasma, or urine. Studies were excluded if they were in animals; in a pediatric population; published in a non-English language; and non-interventional trials including observational studies, reviews, study protocol, and editorials. Also excluded were studies in which TMAO was not a measurable outcome . All inclusion and exclusion criteria are summarized in **Table 1**.

After the initial search, two independent reviewers (NK) and (KA) assessed titles and abstracts to determine whether they met the eligibility criteria for inclusion in the systematic review. Full texts were retrieved if identified as potentially relevant or if the title or abstract did not give sufficient information to make this determination. Discrepancies and conflicts were resolved by a third reviewer (MG). Identified studies were divided into 2 categories: dietary supplements and pharmacological agents.

Assessment of bias

Risk of bias within studies was assessed with the use of the Cochrane Collaboration's tool for randomized controlled trials (29). The Cochrane tool aided in critically appraising the quality constructs of each publication as well as in determining the relevance and validity of the selected publication. Biases were assessed as a judgment (high, low, or unclear) for individual elements from 6 domains (selection, performance, detection, attrition, reporting, and other bias). The studies were scored as positive (high risk of bias), negative (low risk of bias), or neutral (unclear risk of bias).

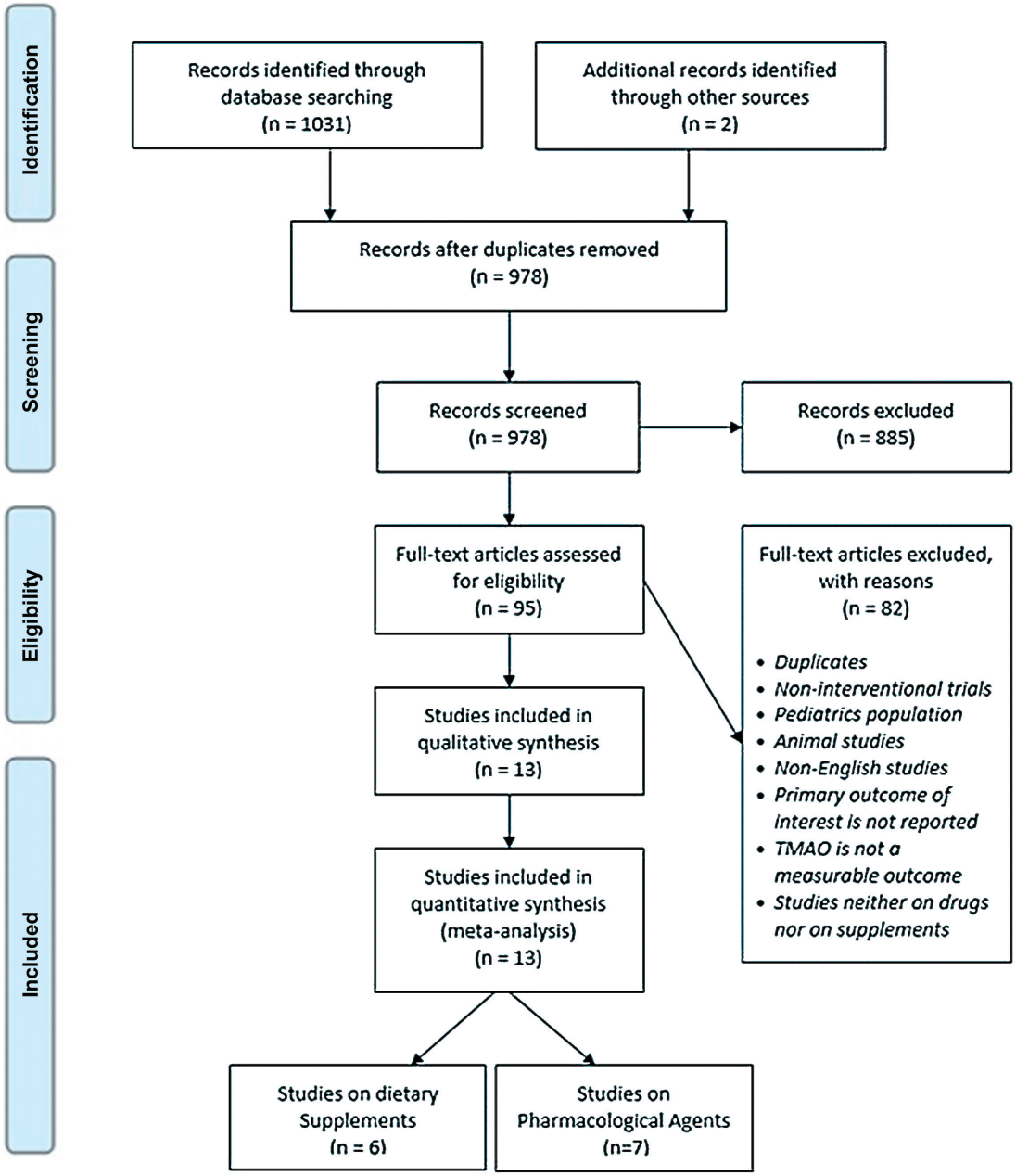

FIGURE 2 PRISMA flow diagram depicting overview of study-selection process for inclusion in the systematic review. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; TMAO, trimethylamine *N*-oxide.

The tool was used by 1 reviewer (NK) and checked by a second reviewer for accuracy and agreement.

Data extraction

Parameters collected during data extraction were author or authors, year of publication, country, sample size of each group, participant characteristics (age, gender, BMI, and health status), characteristics of intervention and comparator, study duration, outcome of interest, and the dietary status among the study participants. The data extraction was entered into an Excel spreadsheet by 1 author (NK) and checked by a second author (KA) for accuracy and completeness. Any disagreements were resolved by discussion. Results are synthesized narratively.

Results

Overview of studies

Our literature searches in 6 databases initially resulted in $n = 1031$ articles. An overview of search results is presented in the PRISMA flow diagram in **Figure 2**. After excluding duplicates, the initial search identified $n = 978$ abstracts for screening. A total of 13 studies, including $n = 6$ studies using

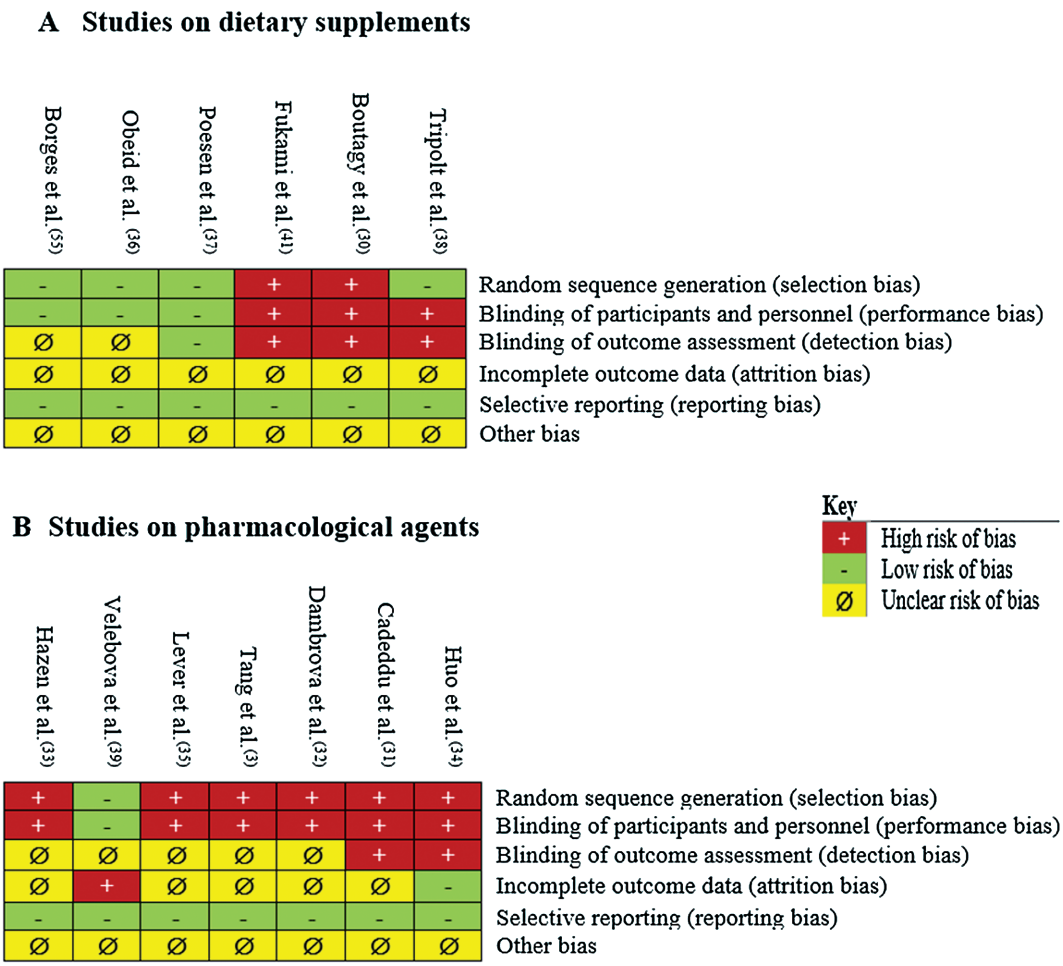

FIGURE 3 Risk of bias assessments for included studies on dietary supplements and pharmacological agents. Graphical display of the results of the methodological analysis of the quality of included studies using the Cochrane Collaboration tool for randomized controlled trials. Studies were scored as positive (high risk of bias), negative (low risk of bias), or neutral (unclear risk of bias).

dietary supplements and $n = 7$ studies using pharmaceutical agents, met the inclusion criteria and were included in this review (3, 30–42). Included studies were published in the United States ($n = 3$), Brazil ($n = 1$), Asia ($n = 2$), and Europe ($n = 7$); no studies were found from the Middle East or Africa. Five studies were conducted in healthy populations, defined as follows: good health; no overt chronic diseases such as heart disease, renal disease, and metabolic diseases; no active infections; and not taking any medications that could influence the study variables. The other 8 studies included subjects with diabetes mellitus ($n = 3$), metabolic syndrome ($n = 1$), or CKD ($n = 1$) and those on hemodialysis ($n = 2$). BMI was reported in 8 studies. The BMI of participants across studies ranged from 21.9 to 35. Three studies reported mean BMI in the healthy category (BMI: 18.5–24.9), 3 studies reported mean BMI in the overweight category (BMI: 25–29.9), and 2 studies reported mean BMI in the obese category (BMI: >30). The mean age of participants among the 9 studies was 52 ± 10 y (range: 22–76 y).

The study designs used in dietary supplements studies were quite homogeneous; all the studies included were randomized controlled trials except 1, which was an open-label interventional study. However, this was not the case with the pharmacological agents, for which different experiential designs were used. Pre-intervention concentrations of TMAO in serum, plasma, and urine were not reported in 3 studies. Changes in TMAO concentrations post-intervention were reported in only 10 studies: serum ($n = 2$), urine ($n = 1$), and plasma ($n = 7$). The risk of biased assessment results is provided in **Figure 3**. Major possible sources of bias among the 13 studies were selection bias and performance bias. Three studies detailed their randomization methods. Two studies mentioned the randomization but did not adequately describe the method of randomization. The remaining 8 studies did not use any randomization technique and hence were classified as having a high risk of bias. A low risk of reporting bias and attrition bias was seen in all studies in

which all expected outcomes were reported, and all studies performed a complete analysis.

Dietary supplements

Six studies on dietary supplements involving probiotics, prebiotics, L-carnitine, and vitamin combinations met the inclusion criteria (30, 36–38, 41, 42). A summary of study characteristics is shown in **Table 2**. The following three different types of probiotic supplementation were included in the review: *Lactobacillus casei* strain Shirota (LcS); VSL#3; and the strain-specific probiotic formulation known as Renadyl™, which includes *Streptococcus thermophiles* (KB19), *Lactobacillus acidophilus* (KB27), and *Bifidobacteria longum* (KB31). The prebiotic supplement included arabinoxylan oligosaccharide (AXOS); vitamin combinations included cholecalciferol (vitamin D-3) and B vitamins such as folic acid, pyridoxine (B-6), and cyanocobalamin (B-12). L-carnitine was provided as an oral dietary supplement in 1 study. Four studies on dietary supplements used a parallel design, 1 was a crossover study, and 1 was an open-label interventional trial. Four studies provided the interventions in a random order, whereas 1 study provided them in sequential order. Comparators included cornstarch ($n = 1$), maltodextrin ($n = 1$), wheat germ ($n = 1$), and standard therapy ($n = 2$). Trials on pre- and probiotics ranged from 4 to 12 wk in duration, whereas the study on vitamins lasted 12 mo. Subjects with L-carnitine deficiency were followed up after 6 mo of L-carnitine supplementation. In the crossover design study, the washout period was 4 wk. Studies included $n = 219$ participants, both males and females, except for 1 study, which included males only. Pre-intervention concentrations of TMAO were reported in all studies, but not with VSL#3 probiotic supplement. Dietary consumption of foods rich in seafood, fish, choline, L-carnitine, and betaine by the study participants was not controlled among the studies, except in 1 study, in which subjects were on a eucaloric control diet (55% carbohydrate, 30% fat, and 15% protein) for two weeks before the intervention and continued on a hypercaloric (+1000 kcal d^{-1}) and high-fat diet (55% fat, 30% carbohydrate, and 15% protein) simultaneously with the interventional dietary supplement. The change in serum and plasma concentrations of TMAO after the administration of dietary supplements was considered the study endpoint. Among the 3 oral probiotics used (30, 38, 42), there was no significant change in the TMAO concentrations in the LcS, VSL#3, and Renadyl intervention groups compared with the control groups. AXOS prebiotic treatment resulted in a minor reduction in TMAO concentration compared with placebo. After 12 mo of treatment with vitamin D-3 and B vitamins, serum TMAO concentrations did not differ between the treatment groups and control groups. However, the amount of reduction in TMAO concentrations from baseline was highly significant in the treatment groups compared with the control groups (36). Plasma TMAO concentrations in hemodialysis (HD) patients were markedly higher at baseline compared with those in healthy subjects. After oral administration of L-carnitine in HD patients, plasma TMAO concentrations were significantly increased.

Pharmacological agents

A total of 7 interventional studies involving 5 different pharmacological agents met the inclusion criteria (3, 31–35, 39). Pharmacological agents included metformin ($n = 3$), meldonium ($n = 1$), broad-spectrum antibiotic ($n = 1$), fenofibrate ($n = 1$), and aspirin ($n = 1$). Studies included $n = 185$ participants, both males and females, healthy ($n = 3$), with DM ($n = 3$), and unspecified ($n = 1$), and with a mean age of 50 ± 10 y. A summary of study characteristics is shown in **Table 3**. Study design included 1 open-label nonrandomized metabolomic study; 1 prospective nonrandomized clinical trial; 3 open-label, nonrandomized, non-placebo-controlled studies; 1 nonrandomized, non-placebo-controlled, crossover study; and 1 randomized, placebo-controlled, double-blind, crossover study. Study duration ranged from 3 wk to 12 mo. Concentrations of TMAO were quantified at baseline in serum ($n = 3$), plasma ($n = 3$), and urine ($n = 1$). Dietary status among the study participants was not controlled in 4 studies; however, 3 studies specified the type and the amount of food consumed during the intervention.

Metformin

Three studies assessed the effects of metformin on TMAO concentrations in patients with DM (31, 34, 39). TMAO serum concentrations were measured after 3 mo of metformin treatment in 3 studies; however, only 1 study reported baseline concentrations (39). Serum TMAO concentrations were markedly increased in the metabolomic study and the nonrandomized trial (31, 34), whereas the crossover trial did not provide any evidence of an effect of metformin on TMAO serum concentrations (39).

Meldonium

One human study assessed the impact of meldonium as a metabolic modulator on TMAO plasma concentrations in 8 healthy participants (32), with an equal number of males and females. Plasma TMAO concentrations were increased up to 16-fold following administration of a TMA-rich diet, which included approximately 150 g of fish and sea products such as salmon, cod, herring, or shrimps. After the addition of meldonium to the TMA-rich diet, TMAO plasma concentrations increased 9-fold, a significant reduction in TMAO plasma concentrations from that seen in participants on the TMA-rich diet alone.

Antibiotics/antimicrobials

One study assessed the impact of broad-spectrum antibiotics, including metronidazole and ciprofloxacin, on TMAO concentrations (3). Forty healthy participants were assigned to a phosphatidylcholine challenge for 4 wk. Dietary phosphatidylcholine challenge consisted of the ingestion of 2 hard-boiled eggs including yolk (~250 mg of total choline in each) by study participant during their first visit.

TABLE 2 Characteristics and outcomes from studies that reported the effect of dietary supplements on TMAO concentrations[1]

Reference and country	Participant characteristics	Study design	Intervention compared with control[2]	Duration and follow-up	Baseline TMAO concentration (μM)[3]	Outcome[4]	Dietary status[5]
Tripolt et al. (38) Austria	$n = 13{:}15$[6] Female: 4% Age: 51 ± 11[2] y BMI: 35 ± 5 Subjects with metabolic syndrome	Open-label, randomized controlled, parallel study	LcS milk probiotic (3 bottles/d, 65 mL of 10^8/mL LcS) Compared with no milk	12 wk	Plasma I: 4.7 ± 2.7 C: 4.6 ± 2.8	NC*	Uncontrolled[7]
Boutagy et al. (30) United States	$n = 9{:}10$[6] Female: 0% Age: 22.4 ± 1.1 y BMI: 24.5 ± 1.1 Healthy subjects on hypercaloric HFD	Randomized, placebo-controlled, double-blind, parallel study	VSL#3 probiotic (2 packets, 900 billion live bacteria) Compared with placebo (2 packets, cornstarch)	4 wk	Plasma NR	NC*	Controlled
Fukami et al. (41) Japan	$n = 31{:}47$[6] Female: 56% Age: 55.9 ± 5.2 y 64.6 ± 10.8 y BMI: 21.2 ± 1.9 Healthy subjects compared to subjects on HD	Open label, nonrandomized, interventional study	Oral L-carnitine supplementation (900 mg OD) to both groups	6 mo	Plasma I: 222.5 ± 111.7 C: 174.3 ± 99.7	↑***	Uncontrolled
Poesen et al. (37) Belgium	$n = 40$ Female: 30% Age: 70 ± 6.0 y BMI: 28.7 ± 5.0 Subjects with CKD	Randomized, placebo-controlled, double-blind, crossover study	AXOS prebiotic (10 g bid) Compared with placebo (maltodextrin 6.7 g bid)	12 wk	Serum I: 8.9 IQR: (7.1–12.3)[8]	↓**	Uncontrolled
Obeid et al. (36) Germany	$n = 25{:}27$[6] Female: 16% Age: 68 ± 9.2 y BMI: 26.4 ± 3.1 Healthy subjects	Randomized, single-blind, non-placebo-controlled, parallel study	B vitamins (0.5 mg folic acid + 50 mg B-6 + 0.5 mg B-12) + cholecalciferol (1200 IU OD) + calcium carbonate (800 mg OD) Compared with cholecalciferol (1200 IU OD) + calcium carbonate (800 mg OD)	12 mo	Plasma I: 4.2 (5.2) C: 4.3 (2.3)	↓**	Uncontrolled
Borges et al. (42) Brazil	$n = 11{:}10$[6] Female: 72% Age = 54.0 ± 22 y BMI: 24.6 ± 7 Subjects with CKD on HD	Randomized, double-blind, parallel study	Strain-specific probiotic formulation, 3 capsules (9×10^{13} CFU/d) Compared with placebo	3 mo	Plasma I: 6.2 (2.6–14.9) C: 7.5 (3.1–17.7)	NC*	Uncontrolled

[1] $n = 6$. AXOS, arabinoxylan oligosaccharide; bid, twice daily; C, control; CKD, chronic kidney disease; HD, hemodialysis; HFD, high-fat diet; I, intervention; IU, international unit; LcS, *Lactobacillus casei* Shirota; NC, no change; NR, not reported; OD, once daily; TMAO, trimethylamine *N*-oxide.
[2] Values are means ± SDs unless otherwise indicated.
[3] Baseline TMAO concentration in micromolar (μM) = μmol/L unless otherwise indicated.
[4] Outcome denotes effect of intervention on TMAO levels (increase or decrease).
[5] Dietary intake of trimethylamine dietary precursors including seafood, fish, choline, L-carnitine, and betaine.
[6] n = intervention: control groups.
[7] "Controlled" indicates dietary intake of TMAO precursors was controlled; "uncontrolled" indicates dietary intake of TMAO precursors was not controlled.
[8] Median (interquartile range).
*$P > 0.05$.
**$P < 0.05$.
***$P < 0.001$.

TABLE 3 Characteristics and outcomes from studies that reported the effect of pharmacological agents on TMAO concentrations[1]

Reference and country	Participant characteristics	Study design	Intervention compared with control	Duration and follow-up	Baseline TMAO concentration (μM)[2]	Outcome[3]	Dietary intake[4]
Huo et al. (34) China	$n = 15{:}20$[5] Female: 53% Age: 57.2 ± 10.7[6] y BMI: 28.8 ± 2.39 Subjects with DM	Open-label, nonrandomized metabonomic study	Metformin Compared with no treatment	3 mo	Serum NR	↑**	Uncontrolled[7]
Cadeddu et al. (31) Italy	$n = 20$ Female: 45% Age: 47 ± 13 y BMI: 30.8 ± 5.2 Subjects with DM	Open-label, nonrandomized, non-placebo-controlled study	Metformin (850 mg bid)	3 mo	Serum NR	↑**	Uncontrolled
Dambrova et al. (32) Latvia	$n = 8$ Female: 50% Healthy subjects	Open-label, nonrandomized interventional study	Meldonium (500 mg bid) + TMA-rich diet (seafood)	3 wk	Plasma 4.9 ± 1.3	↓**	Controlled
Tang et al. (3) United States	$n = 40$ Healthy subjects	Prospective, nonrandomized clinical trial	Metronidazole (500 mg bid) plus ciprofloxacin (500 mg OD) + phosphatidylcholine diet	4 wk	Plasma 4.2 ± 2.0	↓↓***	Controlled
Lever et al. (35) United Kingdom	$n = 26$ Female: 42% Age: 50 ± 15 y Healthy subjects	Open-label, nonrandomized, non-placebo-controlled interventional study	Fenofibrate tablet (145 mg OD)	6 wk	Urine[8] 38.9 (21.4–49.0)	NC*	Uncontrolled
Velebova et al. (39) Prague, Czech Republic	$n = 40$ Subjects with DM	Randomized, placebo-controlled, double-blind, crossover study	Metformin (2 g OD) Compared with placebo (not specified)	6 mo	Serum 1.03 ± 1.29	NC*	Uncontrolled
Hazen (33) United States	$n = 7{:}\ 9$[5]	Nonrandomized, non-placebo-controlled, crossover study	Aspirin (81 mg OD) + choline diet Compared with choline diet only	12 mo	Plasma I: 2.6 ± 0.3 C: 2.8 ± 0.4	↓↓***	Controlled

[1] $n = 7$. bid, twice daily; C, control; DM, diabetes mellitus; HD, hemodialysis; I, intervention; NC, no change; NR, not reported; OD, once daily; tab, tablet; TMA, trimethylamine; TMAO, trimethylamine *N*-oxide.
[2] Baseline TMAO concentration in micromolar (μM) = μmol/L unless otherwise indicated.
[3] Outcome denotes effect of intervention on TMAO levels (increase or decrease).
[4] Dietary intake of trimethylamine dietary precursors including seafood, fish, choline, L-carnitine, and betaine.
[5] n = intervention: control groups.
[6] Values are means ± SDs unless otherwise indicated.
[7] "Controlled" indicates dietary intake of TMAO precursors was controlled; "uncontrolled" indicates dietary intake of TMAO precursors was not controlled.
[8] Median (interquartile range) urine excretions (mmol/mol creatinine).
*$P > 0.05$.
**$P < 0.05$.
***$P < 0.001$.

Participants were assigned to receive broad-spectrum antibiotics, including metronidazole and ciprofloxacin, for 1 wk, followed by a second phosphatidylcholine challenge during their second visit. After 1 mo of antibiotic discontinuation, study participants were again challenged during their third visit. Plasma TMAO concentrations were found to be high after egg ingestion; however, after treatment with antibiotics for 1 wk, TMAO concentrations were rendered undetectable. In addition, TMAO was detected in the intervention group 4 wk after ceasing the antibiotic intervention (3).

Fenofibrate

Fenofibrate is one of the pharmacological agents that have been investigated for their effect on TMAO renal excretion (35, 43). Urine TMAO samples were quantified following administration of fenofibrate for 6 wk. This study showed that urinary TMAO concentrations were not affected by fenofibrate treatment.

Aspirin

A crossover design study, with a washout period of 4 mo, was used to examine the effects of aspirin on plasma TMAO concentrations and platelet aggregation in volunteers on a diet rich in choline equivalent to the intake of 2 hard-boiled eggs on a daily basis (33). TMAO was measured at baseline, 1 mo, and 2 mo. Aspirin therapy significantly reduced TMAO concentrations as well as the prothrombotic effect associated with a high-choline diet.

Discussion

The aim of this systematic review was to determine whether dietary supplements or pharmacological interventions can modulate TMAO concentrations. It is important to map the evidence regarding different therapeutic approaches that could potentially have an influence on TMAO production and excretion or a role in pathogenesis. Modulation of the gut microbiota, which can alter their TMA-producing capacity, is potentially a logical intervention strategy in the prevention or treatment of TMAO-induced metabolic diseases (1, 6, 8, 44). Rogers and Aronoff (45) found differences in the relative abundance of specific bacteria between individuals, depending on the number and type of medications used. In addition, FMO3 plays a major role in the regulation of TMAO circulating concentrations; however, targeting FMO3 to modulate TMAO concentrations remains unfavorable because of the accumulation of TMA, resulting in trimethylaminuria (19). Oral ingestion of prebiotics and probiotics can modulate the human gut microbiota composition by selectively stimulating the growth and/or activity of one or more bacteria in the colon (microbiota) or by suppressing the growth of harmful microorganisms. Pre- and probiotics are well known to improve host well-being and health by transforming the gut flora to its normal composition after it has been affected by dietary and environmental stresses (25, 27, 46, 47). Three different types of oral probiotics were studied in this review, exerting their effects through several unique mechanisms. First, LcS is a typical probiotic strain that has been reported in randomized controlled trials to significantly influence the gut microbiota composition by increasing the total bacterial count and maintaining the anaerobic environment in the large intestine (48). Second, VSL#3 has been reported to be a probiotic cocktail supplement of different strains of lactic acid–producing bacteria, including *Streptococcus thermophiles*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*. Third, a strain-specific probiotic formulation (Renadyl) has been reported to improve CKD signs and symptoms by utilizing nitrogenous uremic toxins as nutrients to maintain the gut ecosystem and prevent their accumulation to high toxic levels (49). Although their efficacy in directly and indirectly modulating the gut microbiota in human hosts has long been established (30, 50), none of these probiotics altered TMAO concentrations.

Notably, none of the 3 probiotics studied was specifically focused on TMAO-producing bacteria or their contribution to TMA–TMAO production. Studies in humans and animals suggested that several families of bacteria are engaged in TMA–TMAO production, such as Deferribacteraceae, Enterobacteriaceae, Anaeroplasmataceae, and Prevotellaceae (4, 15, 51). An in vitro study identified 9 strains of bacteria capable of producing TMA from choline using isolates of bacteria in the human intestine. These encompassed the 2 major phyla (Bacteroidetes and Firmicutes) found in the human gut and included *Anaerococcus hydrogenalis*, *Clostridium asparagiforme*, *Clostridium hathewayi*, *Clostridium sporogenes*, *Escherichia fergusonii*, *Proteus penneri*, *Providencia rettgeri*, and *Edwardsiella tarda* (52, 53). Therefore, future studies involving manipulation of one or more of the TMA–TMAO-producing microorganisms are warranted. AXOS are prebiotic carbohydrates that result from the enzymatic hydrolysis of cereal arabinoxylans in the colon, and they act by promoting the activity of specific beneficial colonic bacteria, particularly *Bifidobacteria* (37, 54). AXOS showed a small but significant effect on serum TMAO concentrations in CKD patients. However, this finding cannot be generalized, taking into consideration the study participants' health status, origin, and the study power. Activities of both probiotics and prebiotics may depend on the strain, dose, duration, and components used to exert their effects (25, 46). In addition, the studies involving probiotics supplementation for 4–12 wk found this period of time to be sufficient to alter the gut microbiota (30, 55). However, it is yet undetermined whether this duration was adequate to influence TMAO concentrations. In the AXOS study, participants with dietary changes were excluded because the diet has the potential to interfere with prebiotic effects. This was not the case with the LcS and Renadyl probiotics because the participants' diet was not controlled, and this was considered to be a study limitation.

The only study on dietary supplements involving L-carnitine found a significant increase in plasma TMA and TMAO concentrations after its consumption in HD patients. L-Carnitine has been suggested for use in multiple diseases, including atherosclerosis and CKD, to protect against

vascular injury; however, the benefit of carnitine supplementation remains questionable and has not been confirmed in clinics.

A study involving fat-soluble vitamins (vitamin D-3) and water-soluble vitamins (B vitamins) showed a significant effect on lowering plasma TMAO concentrations; however, the mechanism by which they exerted their beneficial effect was not suggested, and B vitamin status in the study participants at baseline was not taken into consideration (36). To date, studies suggest that only choline is involved in functions that overlap with those of folate and other B vitamins, and in the case of folate (B vitamin) deficiency in diet, the need for dietary choline arises because it is the primary methyl donor (56). Therefore, the status of B vitamins should be taken into consideration in all study participants because their concentrations might confound the results and mask the true association between choline and the observed outcome.

The only study on broad-spectrum antibiotics, including metronidazole and ciprofloxacin, showed a remarkable suppression of TMAO concentrations when administered following a phosphatidylcholine diet. However, TMAO concentrations subsequently increased 1 mo after antibiotic cessation. It is not clear whether this finding is due to a phenomenon called "antibiotic-resistant intestinal microbiota" or whether it may be due to TMAO's ability to identify a different pathway within intestinal microbiota amenable to therapeutic modulation (3). This effect needs to be investigated further in randomized control trials to determine the long- and short-term impact of systemic and local antibiotics on TMAO concentrations.

The majority of the pharmacological agents studied involving metformin, meldonium, and fenofibrate did not suppress TMAO-producing bacteria or inhibit the TMA–lyase enzyme. Although there was no rationale for investigating its effects, meldonium showed a beneficial effect on TMAO concentrations through altering TMA–TMAO gut microbiota-dependent production (32). On the other hand, metformin was found to significantly increase TMAO concentrations in 2 nonrandomized, non-placebo-controlled studies (31, 34). However, no association was reported in the placebo-controlled, crossover studies. Therefore, carefully designed randomized controlled trials to identify the specific mechanism of these pharmacological agents in modulating TMAO concentrations are warranted. The nonsteroidal anti-inflammatory drug aspirin has been reported to suppress TMAO concentrations via inhibiting microbial TMA–lyase activity and reducing the proatherogenic effects associated with a high-choline diet (33). This finding highlights the need to initiate aspirin as a prophylaxis therapy to prevent platelet hyperactivity and clot formation risk or to treat other diseases associated with elevated TMAO concentrations.

The current review has several limitations. Although a comprehensive search strategy was used with no time restriction, only articles published in English were included. The significant heterogeneity within the studies included in this review may have arisen from the methodology used in the individual studies. The discrepancies found regarding study duration, drug dosing, and the small number of participants included in the studies make it challenging to generalize these findings to other populations. In addition, these studies were conducted on different populations with different dietary patterns; however, dietary status was not controlled among most of study subjects, which could potentially confound study outcomes. None of the pharmacological agents studied, except aspirin, were focusing on the two main proposed mechanisms for targeting TMAO concentrations. All but 5 studies were non-placebo-controlled studies; thus, their findings might be confounded by other factors. Finally, not all studies included in this review reported TMAO concentrations at baseline; therefore, the change from preintervention concentrations could not be described, and thus the effect of some pharmacological agents is difficult to interpret.

Conclusion

To date, studies involving dietary supplements and pharmacological agents have been opportunistic and are missing a sound rationale for intervention with the test products. In addition, a controlled dietary status among the study subjects should be taken into consideration when conducting future intervention trials to prevent any confounding on TMAO concentrations. Investigators should aim at either suppressing TMAO–gut microbiota-dependent production or blocking TMAO formation through TMA–lyase enzyme inhibition. Despite the clinical benefit of TMAO precursors such as choline and L-carnitine, their consumption was associated with an increased concentration of TMAO. Therefore, their use should be minimized, and either blocking their conversion to TMAO or suppressing their concentrations by pro- and/or prebiotics, antibiotics, or other pharmaceutical agents may be the way forward for future research.

Acknowledgments

We thank the University of Newcastle Medical Librarian, Ms. Debbie Booth, for her assistance with database searches. The authors' responsibilities were as follows—NAK, KA Abbott, and MLG: designed the study; NAK and KA Abbott: conducted the research; NAK: prepared the manuscript with contributions from KA Abbott, KA Alburikan, HAA, ES, and MLG; and all authors: read and approved the final manuscript.

References

1. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, DuGar B, Feldstein AE, Britt EB, Fu X, Chung YM, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472(7341):57.
2. Lidbury I, Murrell JC, Chen Y. Trimethylamine *N*-oxide metabolism by abundant marine heterotrophic bacteria. Proc Natl Acad Sci USA 2014;111(7):2710–5.
3. Tang WW, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 2013;368(17):1575–84.
4. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, et al. Intestinal microbiota metabolism of L-carnitine,

a nutrient in red meat, promotes atherosclerosis Nat Med 2013;19(5): 576.
5. Li Y, Wang DD, Chiuve SE, Manson JE, Willett WC, Hu FB, Qi L. Dietary phosphatidylcholine intake and type 2 diabetes in men and women. Diabetes Care 2015;38(2):e13–e4.
6. Heianza Y, Ma W, Manson JE, Rexrode KM, Qi L. Gut microbiota metabolites and risk of major adverse cardiovascular disease events and death: a systematic review and meta-analysis of prospective studies. J Am Heart Assoc 2017;6(7):e004947.
7. Qi J, You T, Li J, Pan T, Xiang L, Han Y, Zhu L. Circulating trimethylamine *N*-oxide and the risk of cardiovascular diseases: a systematic review and meta-analysis of 11 prospective cohort studies. J Cell Mol Med 2018;22(1):185–94.
8. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, Li L, Fu X, Wu Y, Mehrabian M, et al. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. Cell 2016;165(1):111–24.
9. Dambrova M, Latkovskis G, Kuka J, Strele I, Konrade I, Grinberga S, Hartmane D, Pugovics O, Erglis A, Liepinsh E. Diabetes is associated with higher trimethylamine *N*-oxide plasma levels. Exp Clin Endocrinol Diabetes 2016;124(04):251–6.
10. Missailidis C, Hällqvist J, Qureshi AR, Barany P, Heimbürger O, Lindholm B, Stenvinkel P, Bergman P. Serum trimethylamine-*N*-oxide is strongly related to renal function and predicts outcome in chronic kidney disease. PLoS One 2016;11(1):e0141738.
11. Gruppen EG, Garcia E, Connelly MA, Jeyarajah EJ, Otvos JD, Bakker SJ, Dullaart RPF. TMAO is associated with mortality: impact of modestly impaired renal function. Sci Rep 2017;7(1):13781.
12. Bae S, Ulrich CM, Neuhouser ML, Malysheva O, Bailey LB, Xiao L, Brown EC, Cushing-Haugen KL, Zheng Y, Cheng TY, et al. Plasma choline metabolites and colorectal cancer risk in the Women's Health Initiative Observational Study. Cancer Res 2014;74(24):7442–52.
13. Cho CE, Caudill MA. Trimethylamine-*N*-oxide friend, foe, or simply caught in the cross-fire? Trends Endocrinol Metab 2017;28(2):121–30.
14. Mondul AM, Moore SC, Weinstein SJ, Karoly ED, Sampson JN, Albanes D. Metabolomic analysis of prostate cancer risk in a prospective cohort: the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study. Int J Cancer 2015;137(9):2124–32.
15. Velasquez MT, Ramezani A, Manal A, Raj DS. Trimethylamine *N*-oxide: the good, the bad and the unknown. Toxins 2016;8(11):326.
16. Hartiala J, Bennett BJ, Tang WW, Wang Z, Stewart AF, Roberts R, McPherson R, Lusis AJ, Hazen SL, Allayee H, et al. Comparative genome-wide association studies in mice and humans for trimethylamine *N*-oxide, a proatherogenic metabolite of choline and L-carnitine. Arterioscler Thromb Vasc Biol 2014;34(6):1307–13.
17. Rajaie S, Esmaillzadeh A. Dietary choline and betaine intakes and risk of cardiovascular diseases: review of epidemiological evidence. ARYA Atherosclerosis 2011;7(2):78–86.
18. Wang Z, Tang WW, Buffa JA, Fu X, Britt EB, Koeth RA, Levison BS, Fan Y, Wu Y, Hazen SL. Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-*N*-oxide. Eur Heart J 2014;35(14):904–10.
19. Zhang LS, Davies SS. Microbial metabolism of dietary components to bioactive metabolites: opportunities for new therapeutic interventions. Genome Med 2016;8(1):46.
20. Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, Gu X, Huang Y, Zamanian-Daryoush M, Culley MK, et al. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. Cell 2015;163(7):1585–95.
21. Collins HL, Drazul-Schrader D, Sulpizio AC, Koster PD, Williamson Y, Adelman SJ, Owen K, Sanli T, Bellamine A. L-Carnitine intake and high trimethylamine *N*-oxide plasma levels correlate with low aortic lesions in ApoE$^{-/-}$ transgenic mice expressing CETP. Atherosclerosis 2016;244:29–37.
22. Veeravalli S, Karu K, Scott F, Fennema D, Phillips IR, Shephard EA. Effect of flavin-containing monooxygenase genotype, mouse strain, and gender on trimethylamine *N*-oxide production, plasma cholesterol concentration, and an index of atherosclerosis. Drug Metab Dispos 2018;46(1):20–5.
23. Fennema D, Phillips IR, Shephard EA. Trimethylamine and trimethylamine-*N*-oxide, a flavin-containing monooxygenase 3 (FMO3)-mediated host–microbiome metabolic axis implicated in health and disease. Drug Metab Dispos 2016;44(11):1839–50.
24. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell 2012;148(6):1258–70.
25. Gareau MG, Sherman PM, Walker WA. Probiotics and the gut microbiota in intestinal health and disease. Nat Rev Gastroenterol Hepatol 2010;7(9):503.
26. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 2009;9(5):313.
27. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 1995;125(6):1401–12.
28. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA; PRISMA-P Group. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev 2015;4(1):1.
29. Cochrane Collaboration. Cochrane handbook for systematic reviews of interventions. Chichester (UK): Wiley-Blackwell; 2008.
30. Boutagy NE, Neilson AP, Osterberg KL, Smithson AT, Englund TR, Davy BM, Hulver MW, Davy KP. Probiotic supplementation and trimethylamine-*N*-oxide production following a high-fat diet. Obesity 2015;23(12):2357–63.
31. Cadeddu C, Deidda M, Nocco S, Locci E, Cossu E, Baroni M, Atzori L, Mercuro G. Effects of metformin treatment on myocardial and endothelial functioning in insulin resistance patients: a metabolomic study. J Diabetes Metab 2013;4:302–4.
32. Dambrova M, Skapare-Makarova E, Konrade I, Pugovics O, Grinberga S, Tirzite D, Petrovska R, Kalvins I, Liepins E. Meldonium decreases the diet-increased plasma levels of trimethylamine *N*-oxide, a metabolite associated with atherosclerosis. J Clin Pharmacol 2013;53(10):1095–8.
33. Hazen SL, inventor; Cleveland Clinic Foundation, assignee. Treating and preventing disease with TMA and TMAO lowering agents. United States patent application US 14/866, 375. 2016.
34. Huo T, Cai S, Lu X, Sha Y, Yu M, Li F. Metabonomic study of biochemical changes in the serum of type 2 diabetes mellitus patients after the treatment of metformin hydrochloride. J Pharm Biomed Anal 2009;49(4):976–82.
35. Lever M, McEntyre CJ, George PM, Slow S, Chambers ST, Foucher C. Fenofibrate causes elevation of betaine excretion but not excretion of other osmolytes by healthy adults. J Clin Lipidol 2014;8(4):433–40.
36. Obeid R, Awwad HM, Kirsch SH, Waldura C, Herrmann W, Graeber S, Geisel J. Plasma trimethylamine-*N*-oxide following supplementation with vitamin D or D plus B vitamins. Mol Nutr Food Res 2017;61(2).
37. Poesen R, Evenepoel P, de Loor H, Delcour JA, Courtin CM, Kuypers D, Augustijns P, Verbeke K, Meijers B. The influence of prebiotic arabinoxylan oligosaccharides on microbiota derived uremic retention solutes in patients with chronic kidney disease: a randomized controlled trial. PLoS One 2016;11(4):e0153893.
38. Tripolt NJ, Leber B, Triebl A, Köfeler H, Stadlbauer V, Sourij H. Effect of *Lactobacillus casei* Shirota supplementation on trimethylamine-*N*-oxide levels in patients with metabolic syndrome: an open-label, randomized study. Atherosclerosis 2015;242(1):141–4.
39. Velebova K, Hoang T, Veleba J, Belinova L, Kopecky J, Jr, Kuda O, Segetova M, Kopecky Sr J, Melenovsky V, Pelikanova T. The effect of metformin on serum levels of trimethylamine-*N*-oxide in patients with type 2 diabetes/prediabetes and chronic heart failure. In Diabetologia, 2016, 59, SS533–SS533.
40. Wong NC, Kulikowski E, Calosing C, Tsujikawa L, Wasiak S, Gilham D, Halliday C, Johansson J, Sweeney M. Apabetalone (RVX-208) lowers cardiovascular disease (CVD) in diabetes mellitus by a mechanism involving microbiome mediated activity on the complement pathway. J Am Coll Cardiol 2017;69(11 Suppl):2009.
41. Fukami K, Yamagishi S-I, Sakai K, Kaida Y, Yokoro M, Ueda S, Wada Y, Takeuchi M, Shimizu M, Yamazaki H, et al. Oral

L-carnitine supplementation increases trimethylamine-*N*-oxide but reduces markers of vascular injury in hemodialysis patients. J Cardiovasc Pharmacol 2015;65(3):289–95.
42. Borges NA, Stenvinkel P, Bergman P, Qureshi AR, Lindholm B, Moraes C, Stockler-Pinto MB, Mafra D. Effects of probiotic supplementation on trimethylamine-*N*-oxide plasma levels in hemodialysis patients: a pilot study. Probiotics Antimicrob Proteins 2018; doi: 10.1007/s12602-018-9411-1.
43. Latkovskis G, Makarova E, Mazule M, Bondare L, Hartmane D, Cirule H, Grinberga S, Erglis A, Liepinsh E, Dambrova M. Loop diuretics decrease the renal elimination rate and increase the plasma levels of trimethylamine-*N*-oxide. Br J Clin Pharmacol 2018;84(11):2634–44.
44. Tang WHW, Hazen SL. The contributory role of gut microbiota in cardiovascular disease. J Clin Invest 2014;124(10):4204–11.
45. Rogers MAM, Aronoff DM. The influence of nonsteroidal anti-inflammatory drugs on the gut microbiome. Clin Microbiol Infect 2016;22(2):178.e1–e9.
46. Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 2017;9(9):1021.
47. Levin R. Probiotics—the road map. Int J Probiotics Prebiotics 2011;6(3/4):133–40.
48. Matsumoto K, Takada T, Shimizu K, Moriyama K, Kawakami K, Hirano K, Kajimoto O, Nomoto K. Effects of a probiotic fermented milk beverage containing *Lactobacillus casei* strain Shirota on defecation frequency, intestinal microbiota, and the intestinal environment of healthy individuals with soft stools. J Biosci Bioeng 2010;110(5):547–52.
49. Natarajan R, Pechenyak B, Vyas U, Ranganathan P, Weinberg A, Liang P, Mallappallil MC, Norin AJ, Friedman EA, Saggi SJ. Randomized controlled trial of strain-specific probiotic formulation (Renadyl) in dialysis patients. BioMed Res Int 2014;2014:568571.
50. Arthur JC, Gharaibeh RZ, Uronis JM, Perez-Chanona E, Sha W, Tomkovich S, Mühlbauer M, Fodor AA, Jobin C. VSL#3 probiotic modifies mucosal microbial composition but does not reduce colitis-associated colorectal cancer. Sci Rep 2013;3:2868.
51. Craciun S, Balskus EP. Microbial conversion of choline to trimethylamine requires a glycyl radical enzyme. Proc Natl Acad Sci USA 2012;109(52):21307.
52. Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-*N*-oxide. MBio 2015;6(2):e02481–14.
53. Oellgaard J, Winther SA, Hansen TS, Rossing P, von Scholten BJ. Trimethylamine *N*-oxide (TMAO) as a new potential therapeutic target for insulin resistance and cancer. Curr Pharm Des 2017;23(25):3699–712.
54. Rivière A, Moens F, Selak M, Maes D, Weckx S, De Vuyst L. The ability of bifidobacteria to degrade arabinoxylan oligosaccharide constituents and derived oligosaccharides is strain dependent. Appl Environ Microbiol 2014;80(1):204–17.
55. Spanhaak S, Havenaar R, Schaafsma G. The effect of consumption of milk fermented by *Lactobacillus casei* strain Shirota on the intestinal microflora and immune parameters in humans. Eur J Clin Nutr 1998;52(12):899.
56. Institute of Medicine (US) Standing Commitee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. National Academies Press; 1998.