Trimethylamine N-oxide in cardiovascular disease

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Abstract

Although traditional cardiovascular risk factors are well established and understood, mortality and morbidity in patients with cardiovascular disease (CVD) remains high. Exploring new pathophysiological pathways enables a better understanding of CVD at both the molecular and clinical levels. Gut microbiota as a potential modulator of CVD are the subject of extensive research. In recent years, trimethylamine N-oxide (TMAO), a biologically active molecule generated by the gut microbiota, has been widely tested in studies on various populations of patients. The ultimate TMAO levels depend on individual features and gut microbiota composition. Most of the research on TMAO has focused on atherosclerotic CVD and heart failure (HF). Studies conducted so far support the use of TMAO as a prognostic marker in CVD. Several studies describe diverse interventions aimed at reducing the concentration of TMAO and its harmful effects. This article summarizes the findings from research, discusses the major insights into TMAO metabolism and related pathophysiological processes, as well as indicates the directions for future research.

Key words: gut microbiota, coronary artery disease, heart failure, TMAO, trimethylamine oxide

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Introduction

According to the World Health Organization (WHO), cardiovascular disease (CVD) remains the major cause of death and disability worldwide.[1] Exploring new pathophysiological pathways enables a better understanding of CVD at both the molecular and clinical levels. Owing to the development of metabolomics and metagenomics, the intestinal microbiota have been indicated as a potential modulator of the course of CVD. Trimethylamine N-oxide (TMAO) is a gut-derived metabolite whose usefulness has been evaluated in numerous studies. Despite promising results, TMAO is an example of a deeply researched gut microbiome biomarker which is still not used in everyday clinical practice. In order to determine the utility of a new biomarker, it is first necessary to assess its metabolism and related pathophysiological processes, followed by clinical trials.[2] This review summarizes extensive literature on TMAO and indicates gaps in knowledge existing after more than 10 years of research.

Objectives

The purpose of this article was to provide an overview of the metabolism of TMAO and associated pathophysiological processes, and the results of major studies. An attempt was also made to indicate the direction of further research.

Methodology

Literature search was carried out in the PubMed database on November 3, 2021 using the queries: "(TMAO) AND (atherosclerosis)", "(TMAO) AND (coronary artery disease)", "(TMAO) AND (atrial fibrillation)", "(TMAO) AND (heart failure)", and "(TMAO) AND (gut microbiota)". Selected key studies concern diverse groups of patients with CVD. Their results are discussed in the text and presented in tables below.

Metabolism of TMAO

The TMAO is an organic compound with the chemical formula of $(CH_3)_3NO$. It is commonly found in the tissues of marine organisms, where it mitigates the adverse effects of temperature, salinity, as well as high urea and hydrostatic pressure.[3] In humans, TMAO is produced by the oxidation of trimethylamine (TMA) and is absorbed directly from food. The TMAO is most abundant in fish and seafood.[4] Gut microbiota produce TMA from the dietary precursors: choline, L-carnitine, and betaine. These TMA precursors are most abundant in red meat and eggs.[5] The most recent research indicates that the intake of foods rich in TMA precursors does not translate directly into an increase in plasma TMAO level because it depends on individual metabolic features, such as hepatic enzymes activity and gut microbiota composition.[6,7]

Carnitine metabolism is key to human TMAO production and 3 major bacterial metabolic pathways leading to TMA synthesis from dietary precursors were described[8]:

a) anaerobic choline degradation by choline TMA-lyase;

b) hydroxylation of L-carnitine to TMA by carnitine oxidoreductase; and

c) conversion of L-carnitine to γ-butyrobetaine, which is then converted to TMA.

Trimethylamine produced by gut microbiota is excreted from the gut via 3 mechanisms: it can be absorbed to the circulation, excreted with stool or used by other bacteria in the process of syntrophy. Trimethylamine that has been absorbed to the circulation is oxidized to TMAO by hepatic flavin-containing monooxygenase (FMO).[5] Trimethylamine absorption occurs in the small intestine (Fig. 1).[9] After absorption, TMA is almost immediately oxidized to TMAO. Following the oral intake of phosphatidylcholine, the highest plasma and urinary TMAO levels are observed after 12 h and 24 h, respectively. After 48 h from intake, the plasma TMAO level returns to baseline.[9] The TMAO taken with food is absorbed through the intestinal barrier and is detectable in blood after 15 min. The maximum blood concentration is reached after 1 h and is maintained for approx. 6 h. After 24 h, 96% of the TMAO dose taken with food is excreted with urine, mostly in an unchanged form.[10]

To determine the concentration of TMAO, liquid chromatography coupled with tandem mass spectrometry and automated nuclear magnetic resonance spectrometry are most often used.[4] Due to the need of specialized equipment, reliable TMAO determination is possible in research or academic facilities; however, mentioned methods become more available.

The effect of TMAO on pathophysiological processes

The first studies reporting the negative effects of TMAO were conducted on an animal model and focused on atherogenesis. Initially, it was determined that TMAO accelerates the production of foam cells from macrophages. The TMAO was shown to promote the upregulation of the scavenger receptors CD36 and SR-A1[11] as well as induce inflammation[12] via the MAPK/JNP pathway, which regulates the synthesis of pro-inflammatory cytokines – tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and intercellular adhesion molecule 1 (ICAM-1). This leads to cholesterol overload in macrophage foam cells and their faster migration and adhesion to endothelial cells. A study on human umbilical vein endothelial cells (HUVECs) confirmed a link between high plasma TMAO levels and the development of atherosclerosis. Moreover, it indicated that TMAO impaired endothelial self-repair

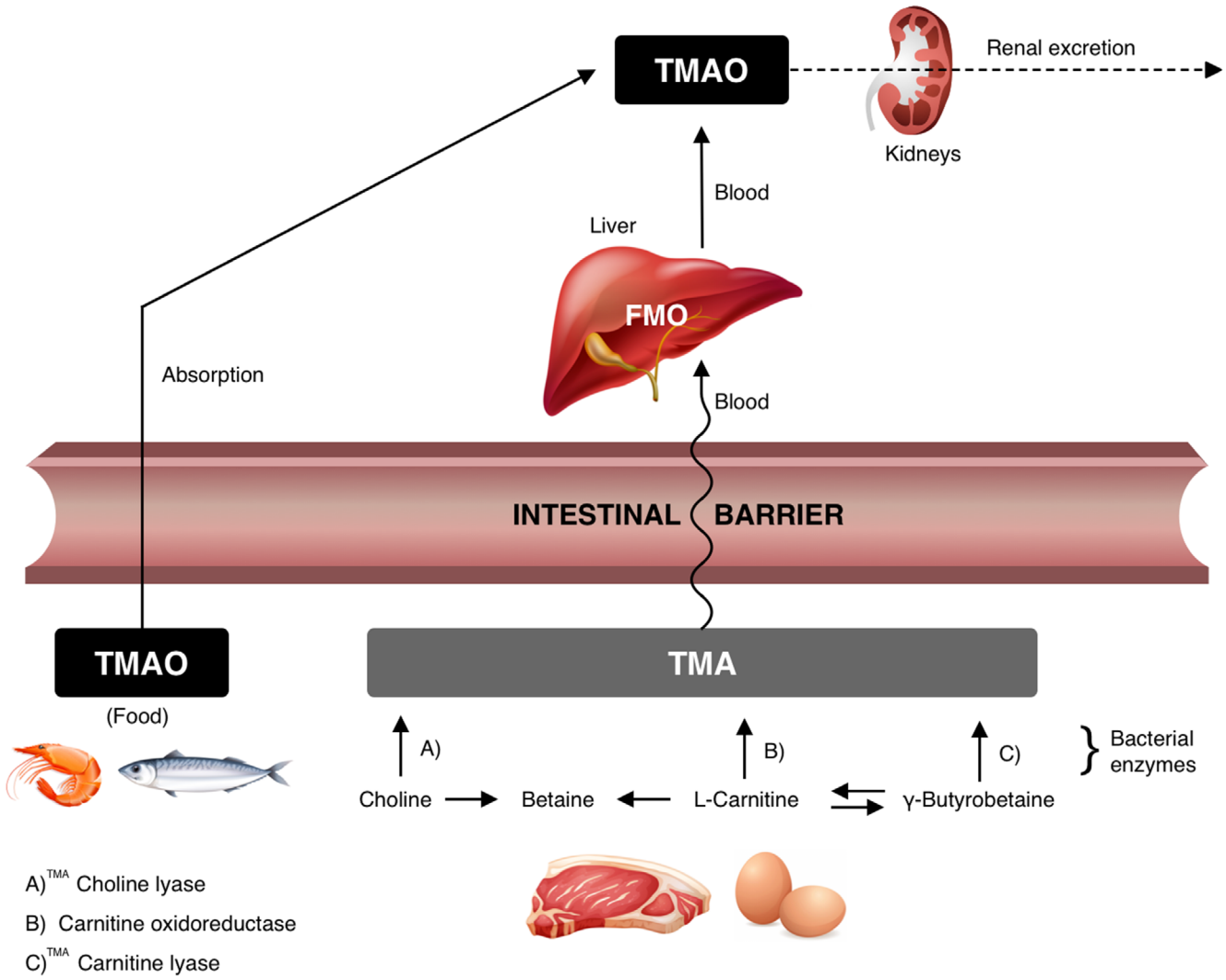

Fig. 1. Schematic presentation of the intestinal absorption of TMA and TMAO

TMA – trimethylamine; TMAO – trimethylamine N-oxide; FMO – flavin-containing monooxygenase.

already in the early stages of atherogenesis.[13] This is due to the inhibitory effect of TMAO on endothelial cell proliferation during the G0/G1 phase of the cell cycle[14] as well as its cytotoxic effect on circulating endothelial progenitor cells.[15] This cascade of events is accompanied by increased oxidative stress, another postulated effect of chronically elevated TMAO levels, observed also in healthy individuals.[16] The effect of TMAO on pathophysiological processes is depicted in Fig. 2.

At a systemic level, TMAO promotes atherogenesis by altering lipid metabolism. In a study on a mice model, Koeth et al. demonstrated that TMAO inhibited the expression of the Cyp7a1 enzyme and bile acid transport proteins.[17] The Cyp7a1 is responsible for bile acid synthesis and inhibition of cholesterol catabolism. The lack of Cyp7a1 leads to the reduced bile acid synthesis and secretion, resulting in atherosclerosis progression. At the same time, a reduction in the expression of bile acid transporter proteins in the liver negatively affects the major pathway of elimination of cholesterol from the body.[17]

Along with research on atherosclerosis, there have been studies investigating the prothrombotic effect of TMAO. Impaired intracellular calcium ion transport in platelets, heightened platelet reactivity and increased platelet adhesion to collagen fibers were reported.[18] In endothelial cells, an increased tissue factor synthesis and downregulation of thrombomodulin were described.[19]

Direct cardiotoxic and proarrhythmic effects of TMAO

The cardiotoxicity of TMAO was confirmed in morphological and functional studies, mainly in animal models. By activating the inflammatory pathways, TMAO promotes cardiac fibrosis, heart weight gain and cardiac remodeling.[20] At a cellular level, TMAO impairs the intracellular microtubule network and alters calcium concentration control in cardiac muscle cells. This leads to a decrease in contraction amplitude, longer time of peak and reduced

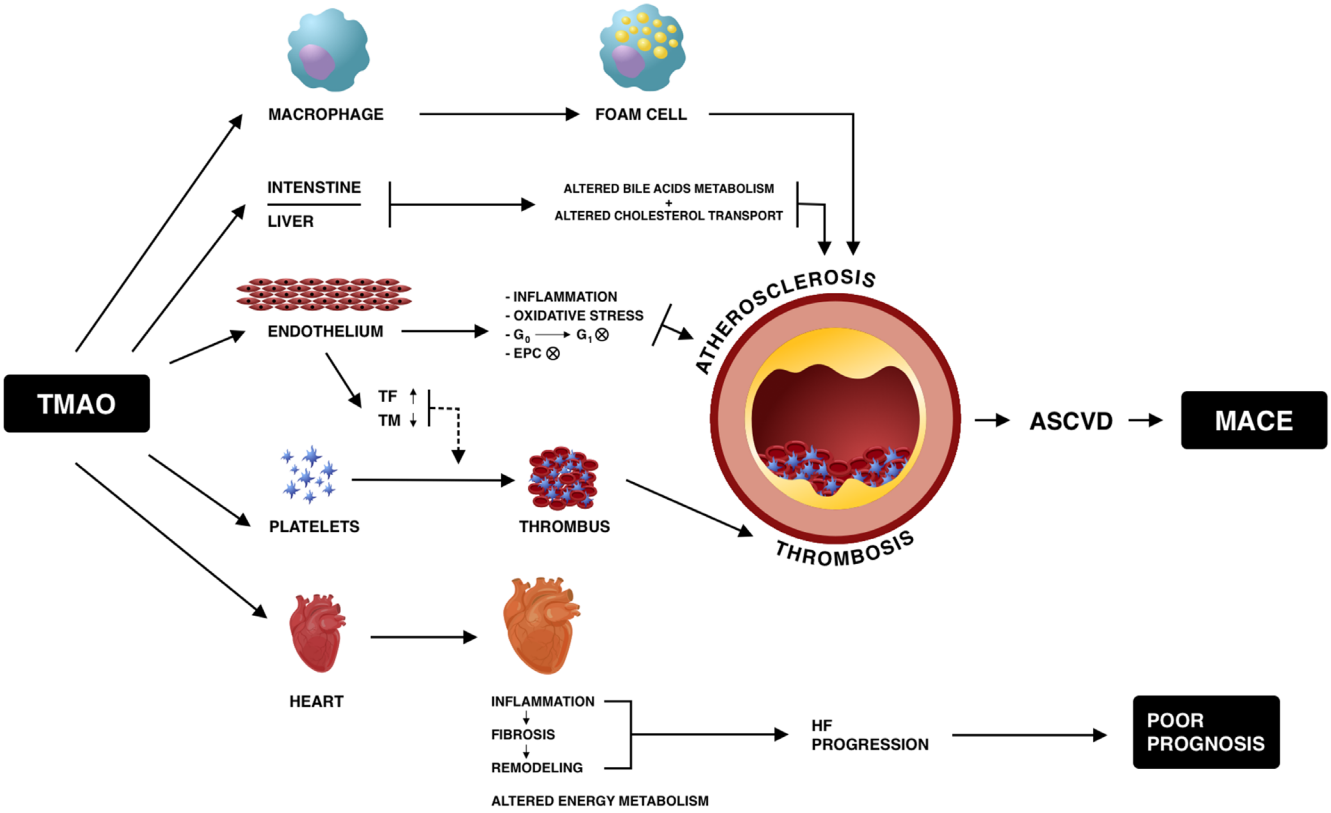

Fig. 2. Effect of TMAO on pathophysiological processes

TMAO – trimethylamine N-oxide; ASCVD – atherosclerotic cardiovascular disease; EPC – endothelial progenitor cells; HF – heart failure; MACE – major cardiovascular events; TF – tissue factor; TM – thrombomodulin.

DRUGS	SURGERY	DIET	GUT MICROBIOTA MODIFICATION
• STATIN [56] • ASPIRIN [57] • RIFAXIMIN [60] • RESVERATROL [64] • MELDONIUM [65]	• GASTRIC BYPASS [63] • DUODENAL SWITCH [63]	• DISCONTINUATION OF RED MEAT [5]	• Probiotics: -Saccharomyces Boulardi [60] -VSL#3 [61] • Fecal microbiota transplant [62]

Fig. 3. Therapeutic strategies affecting TMAO levels and metabolism in humans. Detailed information included in Table 3, with consistent reference numbers

TMAO – trimethylamine N-oxide.

synchronization.[21] Similar TMAO-induced abnormalities in contractility were also reported in ex-vivo human cardiac tissue.[22]

Results from studies on TMAO

Over the past 10 years, numerous studies investigating the prognostic value of TMAO have been conducted. The research included various populations of patients, both with acute and chronic illness. Most of those studies enrolled patients with coronary artery disease (CAD) and heart failure (HF). Selected studies are discussed below and summarized in Table 1.

The first meaningful study on the effect of TMAO in CAD was published in 2013.[23] Tang et al. demonstrated that higher plasma TMAO levels correlated with an increased risk of major adverse cardiovascular events (MACEs) during a 3-year follow-up in 4007 patients referred for elective coronary angiography.[23] The correlation was revealed even after adjustment for traditional risk factors. Similar findings were reported by Senthong et al.

in a study of 2235 patients with significant coronary artery stenosis receiving optimal treatment. Higher TMAO levels predicted mortality independent of traditional risk factors during a 5-year follow-up.[24] In a longitudinal study by Lee et al.,[25] a significant association between higher levels of TMAO and increased risk of incident and recurrent atherosclerotic CVD was shown.[25] Another large community-based study of middle-aged participants revealed that

Table 1. Results of studies on the association of TMAO with cardiovascular risk

Author	Type of study	Population characteristics	Follow-up	Study endpoint	Results HR/OR (95% CI)	TMAO [μmol/L], median (IQR)
Coronary artery disease						
Tang et al.[23]	prospective study	n = 4007; patients undergoing elective coronary angiography USA age: 63 ±11 male sex: 64%	3 years	MACE (death, myocardial infarction, stroke)	TMAO Q4 compared to Q1 MACE n = 513, HR 2.54 [1.96; 3.28]; p < 0.001 multivariate HR 1.43 [1.05; 1.94] death HR 3.37 [2.39; 4.75]; p < 0.001 nonfatal myocardial infarction or stroke HR 2.13 [1.48; 3.05]; p < 0.001	3.7 (2.4–6.20) MACE 5.0 (3.0–8.8) no MACE 3.5 μM (2.4–5.9); p < 0.001
Senthong et al.[24]	prospective study	n = 2235; patients with stable coronary artery disease who underwent elective coronary angiography USA age: 63 ±11 years male sex: 71%	5 years	death (all-cause)	TMAO Q4 compared to Q1 death n = 338 HR 3.90 [2.78; 5.48]; p < 0.0001	3.8 (2.5–6.5) Q4 > 6.5; 9.7 (7.7–14.9)
Lee et al.[25]	prospective multicenter community-based cohort study	n = 5580 USA age: 72 ±5.3 years male sex: 36% a) participants free of prevalent cardiovascular disease (n = 4131) age: 72.2 ±5.3 years male sex: 36% b) participants with prevalent cardiovascular disease (n = 1449) age: 73.6 ±5.8 years male sex = 53%	15 years	ASCVD defined as MI (fatal and nonfatal), fatal coronary heart disease, stroke (fatal and nonfatal), sudden cardiac death, and other atherosclerotic death	quintile 5 compared to quintile 1 multivariable HR 1.23 [1.04; 1.45]; p = 0.028 multivariable, diet and renal function adjusted HR 1.08 [0.91; 1.29]; p = 0.579 a) multivariable, diet and continuous eGFR, adjusted HR 1.07 [0.90; 1.27]; p = 0.516 b) multivariable HR 1.25 [1.01; 1.56]; p = 0.009 multivariable, diet and continuous eGFR, HR 1.10 [0.87; 1.39]; p = 0.179	4.7 (3.2–7.7) quintile 1 = 2.29 (1.84–2.61) quintile 5 = 13.2 (10.4–19.9) a) 4.72 (3.19–7.69) eGFR 70.1 (16.2) b) 5.43 (3.57–8.74) eGFR 63.8 (17.9)
Tang et al.[26]	nested case-control study	n = 2181; healthy individuals Europe age: 65 ±8 years male sex: 65%	8 years	CAD – hospital admission and/or death with CAD as underlying cause (ICD9 Code 410–414)	Q4 compared to Q1 n = 908 OR 1.86 [1.46; 2.37]; p < 0.001 adjusted for traditional risk factors OR 1.58 [1.21; 2.06]; p < 0.001	3.4 (2.3–5.7)
Suzuki et al.[27]	retrospective study	n = 1079; acute MI patients UK age: 67 (57–77) years male sex: 72%	2 years	all-cause mortality death/MI	TMAO T3 compared to T1 n = 292 events all-cause mortality n = 119 HR 1.21 [0.98; 1.48] p = 0.074 death/MI n = 232 HR 1.40 [1.26; 1.55] p < 0.0005 multivariate HR 1.21 [1.03; 1.4] p = 0.023	3.7 (4.6–6.4) T3 > 5.1; 8.5 (6.2–15.0)
Matsuzawa et al.[28]	observational study	n = 112; STEMI patients who underwent primary PCI Japan age: 63 (56–71) years male sex: 88%	median: 5.4 years	cardiovascular events	death n = 5 nonfatal myocardial infarction n = 5, unstable angina requiring revascularization n = 2 nonfatal stroke n = 5 TMAO >6.76 compared to <6.76 adjusted HR 6.21 [1.69; 30.285]; p = 0.005 adjusted HR for 0.1 increase in log TMAO 1.343 [1.122; 1.636]; p = 0.001	6.76 (3.82–12.53)

Table 1. Results of studies on the association of TMAO with cardiovascular risk – cont.

Author	Type of study	Population characteristics	Follow-up	Study endpoint	Results HR/OR (95% CI)	TMAO [μmol/L], median (IQR)
Li et al.[29]	prospective study	n = 530; patients presenting to the emergency department with chest pain of suspected cardiac origin (112 troponin T-positive) USA age: 62.4 ±13.9 years male sex: 57.5% n = 1683 who underwent coronary angiography for ACS Switzerland age: 63.9 ±12.4 years male sex: 77.8%	1 month, 6 months, 7 years, 1 year	MACE defined as a composite of MI, stroke, revascularization, or all-cause mortality (1 month, 6 months), all-cause mortality (7 years)	TMAO Q4 compared to Q1 MACE 1 month OR 6.30 [1.89; 21.0]; p < 0.01 MACE 6 months n = 220 (death n = 29) OR 5.65 [1.91; 16.7]; p < 0.01 mortality 7 years HR 1.81 [1.04; 3.15]; p < 0.05 MACE n = 119 (death n = 79) (1 year Q4 HR 1.57 [1.03; 2.41]; p < 0.05	4.28 (2.55–7.91) 2.87 (1.94–4.85)
Sheng et al.[30]	prospective observational study	n = 335; patients with STEMI China age: 58.7 ±12.1 years male sex: 80.6%	N/A	SYNTAX Score ≥23 presence of multivessel disease	adjusted OR 1.16 [1.06; 1.29]; p = 0.001 r = 0.237, p < 0.001 AUC 0.656 [0.591; 0.722]; p < 0.001 adjusted OR 1.15 [1.01; 1.32]; p = 0.035 r = 0.192, p < 0.001	2.18 (1.34–3.90)
Senthong et al.[31]	prospective cohort study	n = 353; stable patients with CAD detected by elective coronary angiography USA age: 65.0 ±11.0 years male sex: 79%	N/A	SYNTAX Score SYNTAX Score II presence of diffuse lesions	SYNTAX Score (r = 0.61) p < 0.0001 adjusted OR 4.82; p < 0.0001 SYNTAX Score II (r = 0.62) p < 0.0001 adjusted OR = 1.88; p = 0.0001 8.4 [5.7; 14.0] compared to 4.4 [5.2; 13.5] adjusted OR 2.05 [1.45; 2.90], p = 0.0001	5.5 mM (3.4–9.8)
Fu et al.[32]	observational study	n = 26 patients with CAD who underwent optical coherence tomography China age: 60 ±10 years male sex: 77% n = 12 – plaque rupture group n = 14 – non-plaque rupture group	N/A	TMAO concentration in rupture compared to non-rupture TMAO concentration and plaque composition	TMAO level – lipid arc (r = 0.43, p = 0.031), lipid volume index (r = 0.39, p = 0.048)	rupture compared to no rupture 8.6 ±4.8 compared to 4.2 ±2.4; p = 0.011
Tan et al.[33]	prospective observational study	n = 146; STEMI with pre-intervention optical coherence tomography China age: 57.0 ±11.0 years male sex: 82.2% n = 77 – plaque rupture n = 69 – plaque erosion	N/A	TMAO rupture compared to erosion	rupture adjusted OR 4.06 [2.38; 6.91]; p < 0.001 AUROC 0.89, 1.95 μM sensitivity = 88.3%, specificity = 76.8%	rupture compared to erosion 3.33 (2.48–4.57) compared to 1.21 (0.86–1.91); p < 0.001
Heart failure						
Tang et al.[37]	single-center prospective cohort study	n = 720; stable subjects with HF, patients with ACS within the preceding 30 days excluded USA age: 66 ±10 years male sex: 59%	5 years	all-cause mortality	death n = 207 Q4 compared to Q1 adjusted for traditional risk factors and BNP HR 2.2 [1.42; 3.43]; p < 0.001 adjusted for renal function HR 1.75 [1.07; 2.86]; p < 0.001	5.0 (3.0–8.5)
Trøseid et al.[38]	prospective observational study	n = 155; patients with stable HF for >6 months (NYHA class II–IV) Europe age: 57 ±11 years male sex: 83% n = 73 – CAD n = 75 – DCM n = 7 – other	median: 5.2 years	all-cause and anticipated mortality, i.e., HTx	death (n = 39) HTx (n = 16) T3 compared to T1 unadjusted HR 2.24 [1.28; 3.92]; p = 0.005 adjusted HR 1.79 [0.90; 1.79]; p = 0.097 NYHA class II/III/IV r = 0.15, p < 0.05	CAD – 12.1 ±19.5 DCM – 9.2 ±8.5 healthy control – 7.9 ±8.9

Table 1. Results of studies on the association of TMAO with cardiovascular risk – cont.

Author	Type of study	Population characteristics	Follow-up	Study endpoint	Results HR/OR (95% CI)	TMAO [μmol/L], median (IQR)
Zhou et al.[39]	prospective cohort study	n = 1208; patients with chronic HF after MI China age: 73 (64–80) years male sex: 68.5%	median: 1.84 years	MACE, all-cause mortality, HF rehospitalization, recurrent MI all-cause mortality	Q4 compared to Q1 MACE n = 507 death n = 56 readmitted with HF n = 384 recurrent MI n = 67 unadjusted HR 3.15 [2.09; 4.73]; p < 0.01 adjusted HR 2.31 [1.42; 3.59]; p < 0.01 all-cause mortality HR 2.15 [1.37; 3.24]; p < 0.01	Q4 > 7.92 Q1 < 2.83
Schuett et al.[40]	retrospective study	n = 2490; LURIC population Europe n = 823 – HF patients: HFrEF (n = 428) and HFpEF (n = 395)	mean: 9.7 years	all-cause mortality death due to cardiovascular causes	all patients T3 compared to T1 death n = 728 1.70 [1.41; 2.04]; p < 0.001 cardiovascular death n = 446 1.87 [1.48; 2.38] HFrEF T3 compared to T1 death 2.33 [1.67; 3.24]; p < 0.001 cardiovascular death 2.27 [1.52; 3.37] HFpEF – ns	T3 ≥ 5.92 T1 ≤ 3.90
Suzuki et al.[41]	retrospective study	n = 972; patients with acute HF UK age: 78 (69–84) years male sex: 61%	1 year	all-cause mortality (death) composite death or rehospitalization due to HF (death/HF)	death n = 268 T3 compared to T1 univariate HR 1.35 [1.21; 1.51]; p < 0.0005 n = 384 death/HF HR 1.33 [1.20; 1.46]; p < 0.0005 adjusted for renal function – ns	5.6 (3.4–10.5) T3 = 14.2 (8.2–151.5) T1 = 2.9 (0.5–4.0)
Israr et al.[42]	retrospective study	n = 806; patients with acute HF UK age: 78 (69–84) years male sex: 61%	1 year	death at 30 days n = 62 death at 1 year n = 213 death/HF at 30 days n = 98 death/HF at 1 year n = 313	T3 compared to T1 HR 1.39 [1.05; 1.84]; p = 0.022 HR 1.26 [1.08; 1.47] p = 0.004 HR 1.38 [1.10; 1.73] p = 0.006 HR 1.25 [1.09; 1.42] p = 0.001 adjusted for renal function – ns	10.2 (5.8–18.7)
Suzuki et al.[43]	multicenter prospective study (BIOSTAT-CHF)	n = 2234; patients with progressive worsening or new-onset symptoms of HF UK age: 70 (61–78) years male sex: 74%	3 years	all-cause mortality (3 years) composite event of mortality combined with rehospitalization due to HF (3 years)	unadjusted HR 2.27 [1.90; 2.72]; p < 0.001 adjusted |HR 1.42 [1.13; 1.80]; p = 0.003 unadjusted HR 1.93 [1.66; 2.23]; p < 0.001 adjusted HR 1.21 [1.00; 1.46]; p = 0.054	5.9 (3.6–10.8)
Wei et al.[44]	prospective study	n = 915; chronic HF patients with reduced ejection fraction China age: 57.1 ±14.1 years male sex: 69.9%	median: 33 months, max. 7 years	cardiovascular death or HTx (n = 314) recurrence of HF + first rehospitalization for cardiovascular causes	T3 compared to T1 HR 1.47 [1.13; 1.91]; p = 0.004 adjusted HR 1.33 [1.01; 1.74]; p = 0.039 high dose-dependent association: first rehospitalization for cardiovascular causes (p = 0.002) recurrence of HF (p = 0.003)	2.52 (1.20–4.76) T3 > 3.770 T1 ≤ 1.574
Atrial fibrillation						
Svingen et al.[47]	retrospective cohort study	n = 3797; patients with suspected stable angina Europe n = 3143; community-based control population Europe	median: 7.3 years community control 10.8 years	diagnosis of AF during hospitalization	m = 412 Q4 compared to Q1 adjusted HR 1.16 [1.05; 1.28]; p = 0.0009 community control n = 484 adjusted HR 1.10 [1.004; 1.19] per 1 standard deviation increase in log-transformed plasma TMAO	Q4 > 15.8 (11.9–23.5) Q1 < 2.7 (2.2–3.2)

Table 1. Results of studies on the association of TMAO with cardiovascular risk – cont.

Author	Type of study	Population characteristics	Follow-up	Study endpoint	Results HR/OR (95% CI)	TMAO [µmol/L], median (IQR)
Gong et al.[49]	prospective observational cohort study	n = 117; consecutive rheumatic heart disease patients with AF age: 57 (50–64) years male sex: 41% n = 25 – patients with cardiac thrombi n = 92 – patients without cardiac thrombi	N/A	comparison of TMAO concentration between 2 groups	TMAO, group I compared to group II: 4.55 [3.19; 4.83] compared to 3.53 [2.96; 4.25]; p = 0.01	N/A

ACS – acute coronary syndrome; DCM – dilated cardiomyopathy; MACE – major adverse cardiac event; TMAO – trimethylamine oxide; a – age; m – male; Q – quartile; T – tertile; HR – hazard ratio; OR – odds ratio; 95% CI – 95% confidence interval; IQR – interquartile range; AUROC – area under the receiver operating characteristic curve; ASCVD – atherosclerotic cardiovascular disease; CAD – coronary artery disease; MI – myocardial infarction; HF – heart failure; HFrEF – heart failure with reduced ejection fraction; HFpEF – heart failure with preserved ejection fraction; BNP – brain natriuretic peptide; HTx – heart transplantation; LURIC – Ludwigshafen Risk and Cardiovascular Health; PCI – percutaneous coronary intervention; SYNTAX – synergy between percutaneous coronary intervention with taxus and cardiac surgery score; STEMI – ST-segment elevation myocardial infarction; NYHA – New York Heart Association; eGFR – estimated glomerular filtration rate; N/A – not available/not applicable; AUC – area under the curve; AF – atrial fibrillation; N/A – not applicable; ns – nonsignificant.

higher TMAO levels are associated with the risk of CAD in previously healthy individuals.[26]

The prognostic value of TMAO was also assessed in patients with acute coronary syndrome (ACS). Increased TMAO levels showed an association with the risk of death or recurrent myocardial infarction (MI) during a 2-year follow-up,[27] as well as with future cardiovascular events during a 5-year follow-up.[28] Importantly, the risk persisted despite the improvement regarding traditional cardiovascular risk factors such as hypertension, dyslipidemia or diabetes.[28] In another interesting study, Li et al. showed that increased TMAO levels were a risk factor for MACEs at 30 days and 6 months in patients presenting with chest pain of suspected cardiac origin.[29] The strong prognostic significance of TMAO was observed irrespective of baseline troponin levels and the final diagnosis of ACS. In the same study, in an independent cohort of patients with ACS who underwent coronary angiography due to ACS, higher TMAO levels were associated with an increased risk of MACEs at 1 year, independent of traditional risk factors.

In addition to predicting future MACEs, plasma TMAO levels were shown to correlate with the extent of CAD and atherosclerotic plaque stability. Studies conducted to date have revealed an association between TMAO levels and the SYNTAX Score, multivessel CAD[30,31] and the risk of atherosclerotic plaque rupture.[32,33] Finally, it was reported that TMAO could act as a mediator of clopidogrel resistance and inhibit clopidogrel effects, which has significant implications for the medical treatment of CAD.[34]

In the context of atherosclerosis, TMAO has also been investigated in patients with chronic kidney disease (CKD).[35,36] As renal function deteriorates, TMAO level increases and correlates with coronary atherosclerosis burden,[35] gut microbiota alterations, increased intestinal permeability, chronic inflammation, and endothelial dysfunction.[36] Chronic kidney disease has complex pathogenesis and dynamics. The higher risk of death and the extent of atherosclerosis are therefore the cumulative effect of many processes. Accordingly, TMAO is one of the bystanders rather than the single and direct causative agent of vascular complications of CKD.

Heart failure

There is a considerable body of evidence on the role of TMAO as a prognostic marker in patients with HF. These associations were studied in the chronic and acute settings as well as depending on the preserved or reduced ejection fraction and the burden of symptoms.

A significant association between increased TMAO levels and mortality risk in patients with chronic HF (CHF) was first described in 2014.[37] Subsequent studies reported associations with the New York Heart Association (NYHA) functional class and CHF after MI.[38,39] Schuett et al. demonstrated that increased TMAO levels were a strong predictor of mortality in patients with HF with reduced ejection fraction, but not in those with preserved ejection fraction, over a mean follow-up of 9.7 years.[40]

Several studies reported data on the effect of increased TMAO levels on disease course and prognosis in patients with acute HF. Suzuki et al. showed that elevated TMAO levels were a strong predictor of mortality or rehospitalization at 1 year.[41] However, after adjustment for renal confounders, the correlation was no longer significant,[41] which is in line with the results of a more recent study by Israr et al.[42] In a multicenter study including patients with new-onset or progressive HF, higher TMAO levels were strongly associated with an increased risk of mortality and/or rehospitalization during 1-, 2- and 3-year follow-up. In line with previous studies, TMAO levels were not reduced by optimal treatment.[43]

The association between elevated TMAO levels and poor prognosis in patients with HF has not been fully elucidated

so far. Apart from gut microbiota, the role of FMO3 polymorphism has been postulated.[44] Moreover, Li et al. suggested a potential inhibitory effect of loop diuretics on renal excretion of TMAO, resulting in its retention in tissues.[45]

Atrial fibrillation

The role of TMAO as a risk factor or mediator of atrial fibrillation (AF) has not been determined so far.[46] A study on 2 Norwegian cohorts indicated that high TMAO levels were associated with a risk of incident AF, independent of traditional risk factors.[47] In the AF-RISK study, higher TMAO levels were associated with progression to permanent AF.[48] Finally, the proarrhythmic and prothrombotic effects of TMAO can result in increased risk of thrombus formation in patients with AF.[49]

Results from meta-analyses

There are several meta-analyses assessing TMAO levels as a predictor of mortality or other adverse events in patients with CVD.[50–55] The results strongly indicate that elevated TMAO levels are a significant risk factor for death and MACEs. Selected meta-analyses are summarized in Table 2.

Interventions aimed at reducing TMAO levels and toxicity

The knowledge of TMAO synthesis, metabolism and excretion pathways allows investigators to study interventions aimed at reducing TMAO toxicity. Some of the conclusions were formulated on the basis of random findings derived from other studies. Examples include the association between statin use and reduced TMAO levels[56] or between aspirin use and reduced TMA synthesis.[57] In both cases, the lowering effect was probably due to drug-induced alterations in gut microbiota.

A standard targeted approach is to reduce the dietary intake of TMA precursors and TMAO. The elimination of red meat from diet results in reduced TMAO levels after 4 weeks,[5] while vegetarians and vegans have lower circulating TMAO levels and a lower capacity to synthesize TMA, probably due to changes in gut microbiota.[17] The beneficial effect of Mediterranean diet on reducing TMAO levels is primarily due to the high intake of plant foods,[58] although the results of studies are equivocal.[59]

The use of broad-spectrum antibiotics was shown to suppress gut microbiota and reduce TMAO levels. However, antibiotic therapy does not offer satisfactory long-term outcomes and is associated with a high risk of side effects.[11] Rifaximin has been reported to be a safer alternative, but the results of the recent GUTHEART study are insufficient to confirm this suggestion.[60] Other interventions include supplementation with probiotics,[61] gut bacteria transplant from healthy donors,[62] bariatric surgery,[63] resveratrol,[64] and meldonium.[65] Current interventions are summarized in Table 3 and Fig. 3.

Review limitations and gaps in knowledge

The possibility of discussing all ongoing studies in microbiota biomarkers is beyond the scope of this review. For now, to the best of our knowledge, despite the extensive literature supporting its usefulness, TMAO has not been identified as an established biomarker in CAD or HF guidelines. Perhaps, the ongoing research will consolidate the use of TMAO and other gut microbiota metabolites such as bile acids and short chain fatty acids in everyday practice. Linking the metabolism of the gut

Table 2. Results of meta-analyses on the association of TMAO with cardiovascular risk

Author	Population (number of studies included)	Endpoints	Results RR/HR (95% CI)
Heianza et al.[50]	n = 19,256 (19)	MACCE, death	MACE: RR 1.62 [1.45; 1.80]; p < 0.001, I^2 = 23.5% death: RR 1.63 [1.36; 1.95]; I^2 = 45.9%
Schiattarella et al.[51]	n = 26,167 (26)	MACCE, death	MACCE: RR 1.67 [1.33; 2.11]; p < 0.00001, I^2 = 46% death: RR 1.91 [1.40; 2.61]; p < 0.0001, I^2 = 94%
Qi et al.[52]	n = 7716 (11)	cardiovascular events, death	cardiovascular events: RR 1.23 [1.07; 1.42]; I^2 = 31.4% death: RR 1.55 [1.19; 2.02]; I^2 = 80.8%
Farhangi[53]	n = 31,230 (20)	death	death: RR 1.466 [1.291; 1.665]; p < 0.001, I^2 = 81.9%
Li et al.[54]	n = 6879 (7)	MACE, death	MACE: T3 compared to T1: HR 1.68 [1.44; 1.96] death: T3 compared to T1: HR 1.67 [1.17; 2.38]
Guasti et al.[55]	n = 923 (3)	MACE, death	MACE: RR 2.05 [1.61; 2.61]; I^2 = 50% death: RR 3.42 [2.27; 5.15]; I^2 = 0%

TMAO – trimethylamine oxide; MACE – major adverse cardiac events; MACCE – major adverse cardiac and cerebrovascular events; CVE – cardiovascular events; HR – hazard ratio; RR – risk ratio; T – tertile; CI – confidence interval.

Table 3. Results of studies assessing therapeutic strategies aimed at reducing TMAO levels

Author	Type of study	Population/ model	Intervention	Endpoint	Results	Comment
Li et al.[56]	retrospective study; 3-year follow-up	n = 4007; sequential patients undergoing elective diagnostic coronary angiography	statin use	MACE, defined as death, myocardial infarction, or stroke; reduction of TMAO concentration	n = 322 MACE by 3 years statin use associated with decreased MACE: HR 0.74, 95% CI: [0.60; 0.93]; p = 0.0089 plasma TMAO associated with increased MACE: HR 1.57, 95% CI: [1.40; 1.76]; p = 2.4e-14 statin use associated with decreased TMAO (3.9 compared to 4.3) p = 0.002	suspected mechanism: alteration in gut microbiome activity
Zhu et al.[57]	prospective study	healthy vegans/ vegetarians (n = 8); healthy omnivores (n = 10) orally supplemented with choline	aspirin 81 mg/day for 3 months	reduction of TMAO concentration	aspirin attenuated TMAO elevation TMAO: choline compared to choline + ASA 36.9 ±9.4 compared to 21.2 ±3.0; p = 0.009	suspected mechanism: alteration in gut microbiome activity
Wang et al.[5]	prospective study	n = 113; healthy adult participants all omnivores	discontinuation of red meat intake to non-meat or white meat	reduction of TMAO concentration	no meat – TMAO reduction; p < 0.0001 white meat – TMAO reduction; p < 0.0001	N/A
Awoyemi et al.[60]	prospective randomized, double-blind study	n = 151; patients with LVEF < 40%; NYHA class II–III despite optimal medical therapy	3 months: rifaximin, 550 mg twice daily, 250 mg 3 months: probiotic *Saccharomyces boulardii* NCM I-745 500 mg twice daily standard of care only	LVEF after 3 months of intervention baseline-adjusted NT-proBNP baseline-adjusted TMAO	LVEF: rifaximin compared to standard of care mean difference: –1.2 pp (3.2–0.7); p = 0.22 *Saccharomyces boulardii* –0.2 pp (2.2–1.9); p = 0.87 NT-proBNP: no significant effects rifaximin p = 0.28 *S. boulardii*: increase; p = 0.03 TMAO: no significant effects rifaximin; p = 0.8 *Saccharomyces boulardii* p = 0.16	patients low in baseline dysbiosis low dose of rifaximin
Boutagy et al.[61]	randomized double-blind, placebo-controlled	n = 19; healthy, non-obese males (18–30 years)	4-week hypercaloric (+1000 kcal day–1), high-fat diet (55% fat) + VSL#3 (900 billion live bacteria) orally placebo	reduction of TMAO concentration	plasma TMAO level increased significantly VSL#3 (89 ±66%); p < 0.05 placebo (115 ±61%); p < 0.05 VSL#3 compared to placebo: p > 0.05	VSL#3 does not influence plasma TMAO following a high-fat diet
Smits et al.[62]	double-blind randomized pilot study	n = 20; male patients with metabolic syndrome	vegan-donor FMT	conversion of choline and carnitine to TMA and TMAO fasting plasma TMAO level TMA/TMAO urinary excretion	no significant effect; p > 0.05	significant changes in intestinal microbiota composition did not affect TMAO metabolism; residual capacity to convert precursors to TMAO in vegans? short follow-up (2 weeks)
Trøseid et al.[63]	observational study	n = 34; obese patients (17 with and 17 without type 2 diabetes) undergoing bariatric surgery	bariatric surgery laparoscopic Roux-en-Y gastric bypass duodenal switch	abseline plasma TMAO level preoperatively (after 3 months of lifestyle intervention) 1 year after bariatric surgery	no significant effect of 3-month lifestyle intervention preoperatively; 1 year after bariatric surgery TMAO plasma levels more than doubled (HR 10.5, 95% CI: [7.5; 13.5]) compared to preoperative (HR 4.4, 95% CI: [2.8; 6.0]; p < 0.001) compared to baseline (HR 4.7, 95% CI: [3.7; 5.8]; p < 0.001), regardless of surgical method	mechanism: changes in gut microbiota profile

Table 3. Results of studies assessing therapeutic strategies aimed at reducing TMAO levels – cont.

Author	Type of study	Population/ model	Intervention	Endpoint	Results	Comment
Annunziata et al.[64]	double-blind, randomized, placebo-controlled study	n = 380; healthy individuals	grape pomace polyphenol nutraceutical (rich in resveratrol) 400 mg twice daily 4 weeks, 8 weeks	reduction of TMAO concentration	plasma TMAO reduction (–49.78%, $p < 0.0001$) 8 weeks – 75.85%; $p < 0.0001$	N/A
Dambrova et al.[65]	open label, interventional study	n = 8; healthy volunteers	meldonium orally, 500 mg twice daily, 7 days during TMAO-rich diet	reduction of TMAO concentration urine TMAO excretion	diet compared to diet + meldonium plasma: 81.5 ±8.6 mM compared to 43.0 ±3.8 mM; $p < 0.05$ excretion: 18.2 ±2.2 mmol/mg creatinine × 7 days compared to 24.3 ±1.5 mmol/mg creatinine × 7 days; $p < 0.05$	N/A

LVEF – left ventricular ejection fraction; MACE – major adverse cardiac event; N/A – not available/not applicable; NYHA – New York Heart Association functional classification; TMAO – trimethylamine oxide; NT-proBNP – N-terminal pro-B-type natriuretic peptide; FMT – fecal microbiota transplant; TMA – trimethylamine; ASA – acetylsalicylic acid (aspirin); HR – hazard ratio; 95% CI – 95% confidence interval.

microbiota to CVD is an attractive topic of ongoing research. It is worth mentioning the studies NCT04962763[66] and NCT02728154,[67] which will deepen the knowledge on the correlation of the intestinal microbiota with HF. The study NCT05014880 is going to assess the effectiveness of a dietary intervention reducing dietary TMAO levels during the rehabilitation of CAD patients.[68]

Conclusions

The TMAO is a biomarker that has been proven useful in a population of patients at higher cardiovascular risk. The use of TMAO in clinical practice requires confirmation in subsequent prospective interventional studies.

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