

Trimethylamine N-oxide—a marker for atherosclerotic vascular disease

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As a potential causative factor in various cardiovascular diseases, the gut microbe-generated metabolite trimethylamine N-oxide (TMAO) has courted considerable research interest as a potential biomarker. TMAO is a small molecule considered to be beneficial for the health of deep-water animals due to its ability to protect proteins against hydrostatic pressure stress. However, it may cause deleterious effects in humans as mounting evidence suggests that TMAO may enhance atherosclerosis, independent of traditional risk factors. This may be mediated by its capacity to enhance inflammation, platelet activation and thrombosis, and inhibit reverse cholesterol transport. In humans, circulating levels of TMAO have been found to be associated with increased risk of developing atherosclerotic diseases such as carotid atherosclerosis, coronary atherosclerotic heart disease, stroke, and peripheral arteriosclerosis. This review aims to discuss the current role of TMAO in the atherosclerosis process, using animal models and clinical studies, with special attention to determining whether TMAO could be used as a marker for monitoring severity and prognosis in atherosclerosis and to evaluate evidence for its role as a mediator in the pathogenesis of atherosclerotic vascular disease.

Keywords

Atherosclerosis; Trimethylamine N-oxide; Coronary heart disease; Cardiovascular risk

1. Introduction

Atherosclerosis is a chronic inflammatory state and the main pathological basis of cardiovascular disease (CVD) [1]. Within the past ten years, the intestinal flora has been recognized as an independent and important metabolic organ, which closely links the metabolism of nutrients with molecules that cause chronic diseases [2]. Intestinal micro-ecological imbalance can promote the development of atherosclerosis by increasing systemic inflammation [3–5] and recently, a metabolite of intestinal flora TMAO, has received widespread attention as a marker for and potential mediator of many diseases.

Professor Tang WH of the Cleveland Medical Center, was the first researcher to report a role for elevated TMAO lev-

els as a predictor of major adverse cardiac events such as myocardial infarction, stroke, and mortality [6]. Since then many comparable studies have shown that increased concentrations of TMAO and its precursors (L-carnitine, choline, and betaine) are associated with increased risks of cardiovascular adverse events and mortality [7–12]. Furthermore, TMAO is thought to be more accurate at predicting these events than traditional CVD risk factors such as blood lipid, C-reactive protein (CRP) levels, or renal function [13]. In a meta-analysis of 17 clinical studies, an every 10 $\mu\text{mol/L}$ increase in TMAO increased the risk of cardiovascular mortality by 7.6% [8]. High levels of TMAO are not only associated with the risk of CVD in humans, but also increase the risk of atherosclerosis in animal models. TMAO is believed to be involved in the complex pathological process of atherosclerotic lesions, such as the promotion of blood vessel inflammation [14], enhanced expression of macrophage scavenger receptors, and the formation of foam cells [6], as well as exacerbated platelet hyperreactivity enhanced thrombosis [15], and inhibition of bile acid synthesis [16].

Here we review the dietary sources, synthesis, and metabolic pathways of TMAO, and provided the latest evidence on the ability of TMAO to be used as a potential marker for atherosclerotic vascular disease, and finally tested TMAO as a target for the treatment of atherosclerosis.

2. Source, synthesis, and metabolism of TMAO

The main sources of TMAO are dietary choline, carnitine, and betaine. Choline is found in beef, chicken liver, bacon and soybeans, and betaine is found in wheat bran, wheat germ and spinach [17]. Meat products such as lean pork, lamb, and beef are the main sources of carnitine [18]. TMAO can also be obtained directly from fish [19] and high protein diets [20]; for example, two large eggs (65 grams) contain 320 mg of phosphatidylcholine, which represents the same amount of TMAO precursor as a 12-ounce beef burgers [21]. Trimethylamine (TMA) is an intermediate of TMAO which

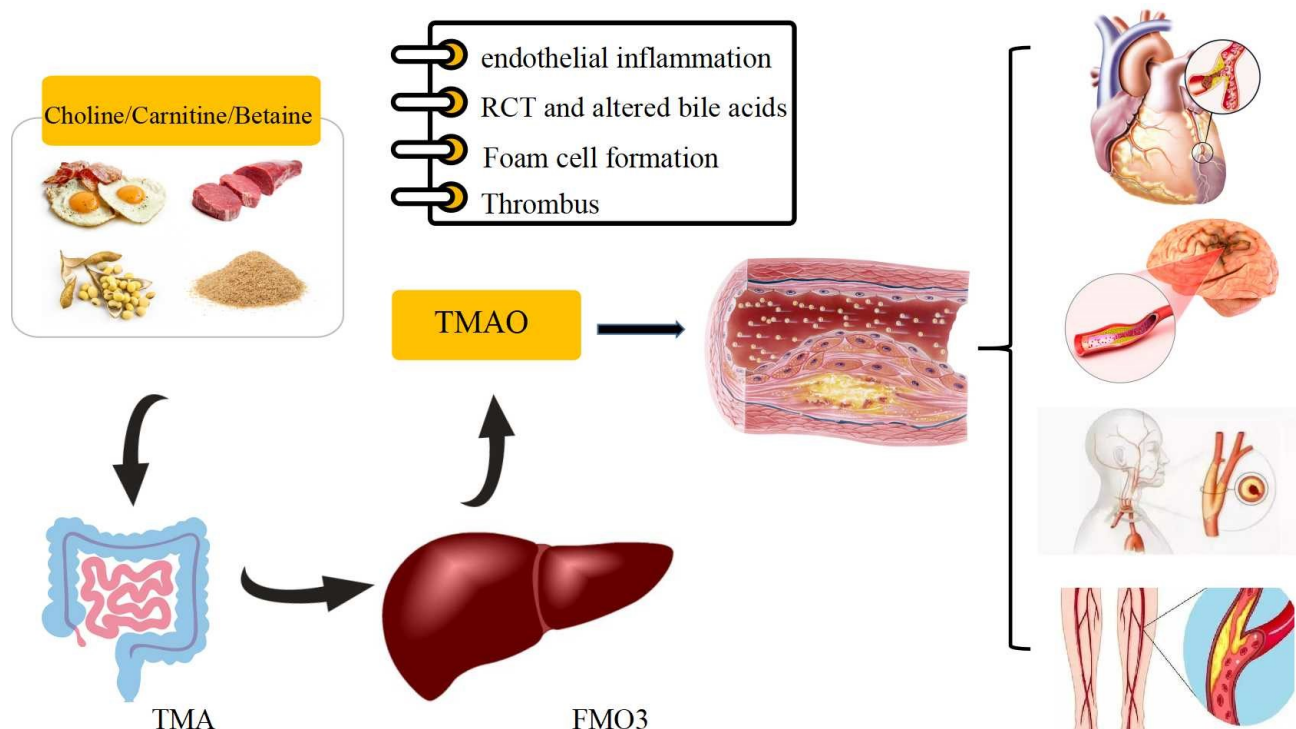


Fig. 1. Role of TMAO in atherosclerosis progression. Annotation: Trimethylamine (TMA) is generated by the action of TMA lyases in the gut microbiota from Choline, Carnitine and betaine. TMA quickly reaches the liver through the portal circulation, flavin-containing monooxygenase 3 (FMO3) significantly oxidize TMA to form TMAO into the circulatory system. TMAO promotes inflammation of blood vessels, enhances the formation of foam cells, exacerbates platelet hyperreactivity and thrombosis potential, alters bile acids and cholesterol transport. These factors are associated with increased risk of developing Atherosclerotic disease such as Carotid atherosclerosis, Coronary atherosclerotic heart disease, Stroke, and Peripheral arteriosclerosis.

is mainly metabolized by the gut microbiota from dietary nutrients having a TMA like structure. It quickly reaches the liver through the portal circulation and flavin-containing monooxygenase 3 (FMO3) can oxidize TMA to form TMAO where it is released into the circulatory system and finally excreted by the kidney [6, 22] (Fig. 1). Consuming 50 mg of deuterium-labeled methyl d₉-TMAO can be detected as early as 15 min in plasma, and 96% of the dose is excreted through urine instead of feces by 24 h [23]. The remaining TMAO is reduced to TMA by the action of TMAO reductase [24].

Microbial enzymes involved in the formation of TMA involve two main pathways, one is the TMA lyase system (CUTC/D) which degrades choline [25]. The pathway encoded by the CUTC gene cluster is the main pathway for TMA production from human choline [26, 27] and the identified CUTC amplicons are associated with various taxa especially *Clostridium* XIVa strains and *Eubacterium* sp [28]. Furthermore, Kymberleigh A. Romano identified eight species representing two different phyla (Firmicutes and Proteobacteria) that showed significant choline consumption and TMA accumulation [29]. The other pathway is the decomposition of carnitine (CntA/B and YeaW/X) [30]. Here, CntA amplicons displayed high identity (~99%) to Gammaproteobacteria-derived references, primarily from *Escherichia coli* [28]. Recently, Robert A Koeth proposed

that the conversion of dietary carnitine to TMAO also involves the L-carnitine→ γ BB→TMA/TMAO pathway [31]. However, only one type of *Clostridium* has been found to be able to convert γ BB to TMA [31]. Therefore, it is necessary to conduct further research to investigate the composition and activity of the intestinal flora to detect potential correlations.

3. TMAO as an enhancer of atherosclerosis-experimental model

3.1 TMAO and endothelial inflammation

Inflammatory injury of the vascular endothelium is widely regarded as the initial stage of atherosclerosis [32]. *In vitro* studies have shown an up-regulated IL-6, CRP, TNF- α and reactive oxygen species (ROS), and reduced Nitric Oxide (NO) production in TMAO-treated endothelial cells (ECs), suggesting that TMAO can directly impair NO-mediated endothelial function [33]. TMAO can also up-regulate the expression of vascular cell adhesion molecule 1 (VCAM-1), promote the adhesion of monocytes, and inhibit the self-repair capability of ECs through protein kinase C (PKC) and NF- κ B signaling, while enhancing macrophage adhesion [14, 34]. Furthermore, TMAO reduces the expression of the anti-inflammatory cytokine IL-10, which can protect ECs from damage caused by inflammation and increased oxidative stress [35] (Fig. 2).

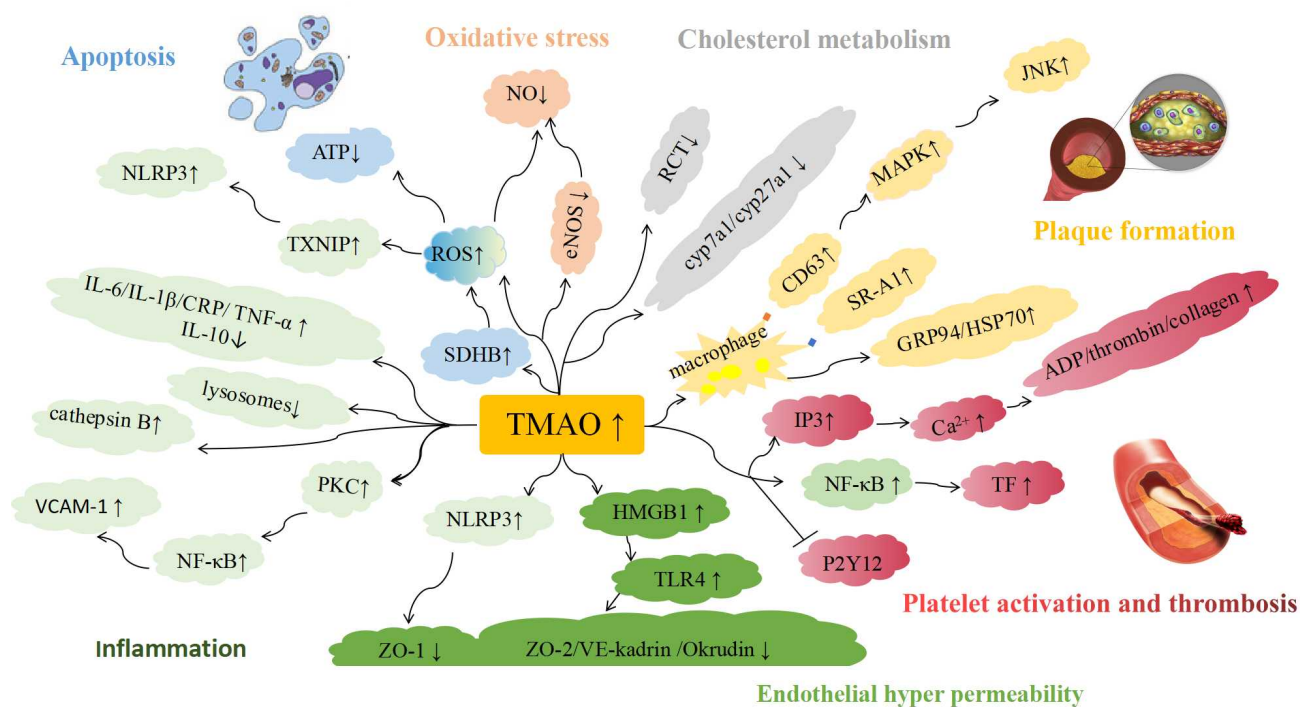


Fig. 2. Putative molecular mechanisms underlying TMAO-induced atherogenic effects. Annotation: High concentration of TMAO plays a vital role in endothelial hyper permeability and inflammation, oxidative stress, cholesterol metabolism, plaque formation, activation and thrombosis.

In young mice, six months of dietary supplementation with TMAO can induced aging of the arterial endothelium. This effect was accompanied by increased vascular nitrotyrosine, a marker of oxidative stress [36]. In a different study, aged rats exhibited higher plasma TMAO, TNF- α , and IL-1 β , and increased eNOS expression in the aorta when compared to young rats, all of these effects were restored to control levels by treatment with DMB (an inhibitor of TMA formation) [37]. These finding clearly indicated that the increase in circulating TMAO levels caused by aging can lead to endothelial dysfunction.

It has been reported that there are several mechanisms underlying the activation of inflammation, including lysosomal rupture, altered K⁺ channel gating, and activation of ROS [38]. Krishna proved that endotheliitis caused by TMAO is activated both *in vitro* and *in vivo* and in the presence of blockers or inhibitors of individual pathways, and found that the formation and activation of the NLRP3 inflammasomes induced by TMAO in ECs were markedly attenuated or abolished by the ROS scavenger, N-acetyl-L-cysteine and cathepsin B inhibitor. These results showed that TMAO can activate NLRP3 inflammasomes in ECs through at least two different pathways, involving increased ROS production, destabilized lysosomes, and enhanced cathepsin B activity [39]. Sun also confirmed that TMAO activates the thioredoxin interacting protein (ROS-TXNIP-NLRP3) signaling pathway through oxidative stress and promotes the expression of the inflammasome NLRP3 [40]. A different mechanism for TMAO action involves a reduction in Adeno-

sine triphosphate(ATP) production and induced endothelial cell apoptosis by damaging the structure and function of mitochondria, and the succinate dehydrogenase complex subunit B (SDHB)/ROS signaling pathway has been found to play an important role in this process [41].

It is well known that loss of integrity of tight junctions found in the endothelium helps to enhance the permeability of the quasi-cellular endothelium. Stimulating ECs with TMAO can reduce the expression of the tight junction protein ZO-1 in the ECs monolayer and change ECs permeability, which was prevented by silencing NLRP3 in the ECs [39]. The high-mobility basal box protein 1 (HMGB1) is also an inflammatory mediator, which can destroy cell-to-cell connections, resulting in vascular endothelial hyper permeability [42]. In a study conducted by Singh, it was discovered that TMAO significantly reduced the expression of junctional proteins such as ZO-2, VE-kadrlin, and Okrudin in the EC monolayer, while this effect was markedly attenuated by glycyrrhizin, an HMGB1 binding compound [42]. Furthermore, TMAO can upregulate HMGB1, and HMGB1 causing further activation of toll-like receptor 4(TLR4, an important receptor for HMGB1 binding where HMGB1 can activate inflammatory pathways). TLR4 siRNA can protect ECs from the interference by TMAO-related tight junction proteins [42] (Fig. 2).

3.2 TMAO and reverse cholesterol transport (RCT)

High TMAO levels were also found to be accompanied by low HDL levels in patients with cardiovascular disease [43]. The accumulation of oxidized low-density lipoprotein (ox-

LDL) in LDL cholesterol macrophages can be transported to the liver for recirculation or excreted in the form of bile acid [44]. Previous studies have confirmed that the RCT of choline-added APOE^{-/-} mice was reduced by about 30%, and total bile acids in mice and the expression of key bile acid synthases Cyp7a1 and Cyp27a1 in the liver were also significantly reduced relative to normal chow ($P < 0.05$) [6, 22], resulting in changes to the main mechanism of removing cholesterol in the body (Fig. 2).

3.3 TMAO and atherosclerotic plaque

Several experimental models have demonstrated a role for TMAO in the process of atherosclerosis. A TMAO-diet significantly promoted plaque progression in APOE^{-/-} mice fed with high-fat for 20 weeks when compared to C57BL/6J mice fed with a normal chow diet. However, the levels of blood sugar, cholesterol and triglycerides had no significant effect on the plaque area [6], and this effect could be reversed by reducing the level of TMAO [45]. TMAO can also increase the formation of foam cells by up-regulating the macrophage scavenger receptors CD36 and SR-A1, which promote the continuous development of plaques [6]. Furthermore, the CD36/MAPK/JNK pathway may play a crucial role in the TMAO-induced formation of foam cells [46]. In addition, it was also found that TMAO can induce the expression of heat shock proteins GRP94 and HSP70 in J774A.1 mouse macrophages, leading to endoplasmic reticulum stress [47] (Fig. 2). TMAO not only promotes plaque formation, but also contributes to plaque instability. In the tandem stenosis mouse model, which reflects plaque instability as typically seen in patients, TMAO levels correlated with several characteristics of plaque instability, such as markers of inflammation, platelet activation, and intraplaque hemorrhage [48].

3.4 TMAO and Thrombus

Platelets contribute to the formation of thrombus and foam cells and play an important role in the occurrence and development of atherosclerosis [49]. After 2 months of choline supplementation, healthy subjects showed a significant >10-fold increase in plasma TMAO levels at both 1- and 2-month periods, with a corresponding enhanced platelet aggregation response to submaximal adenosine diphosphate [50]. Furthermore, large scale clinical association studies ($n > 4000$ subjects) independently demonstrated that plasma TMAO levels are associated with risk of thrombotic events [15]. The exact mechanisms by which TMAO activates platelets however are still unclear.

TMAO can induce the release of calcium from platelets by changing the inositol 1,4,5-triphosphate (IP₃) signal transduction pathway, and directly enhance the platelet response to a variety of different agonists (ADP, thrombin and collagen) and promote platelet hyperresponsiveness by enhancing stimulus-dependent Ca²⁺ release [51]. Tissue factors are essential mediators of hemostasis and trigger thrombosis [52]. Xiaoye Cheng demonstrated that TMAO promoted

tissue factor (TF) expression via activation of the NF- κ B signaling pathway in primary HCAECs [53]. TMAO can also attenuate the inhibitory effect of clopidogrel on platelet aggregation by inhibiting P2Y₁₂ receptor inhibitors [54] (Fig. 2). Recently, Sarah M Skye found that the expression of the CutC gene from intestinal microbes is sufficient to transmit enhanced platelet reactivity and thrombosis potential in a host via TMA/TMAO generation [55]. These studies implicate TMAO as a potential molecular target for the treatment of atherosclerosis and thrombotic.

4. The role of TMAO in vasculopathy induced by atherosclerotic risk factors

The role of TMAO in atherosclerotic vasculopathy has been demonstrated in various models of atherosclerotic risk factors, namely: diabetes, hypertension, and chronic kidney disease.

4.1 Diabetes

High levels of circulating TMAO are associated with an increased risk of both type 1 and type 2 diabetes (T2DM) [56–58], as well as pre-diabetes prevalence [59], and diabetic complications such as retinal neuropathy [60]. Miao found that LIRKO mice suffered from severe hyperglycemia after 4 months Pigen diet, which was completely prevented by the knockdown of FMO3 (TMAO synthase) [61]. Similar results were obtained on obese/insulin resistant subjects, where FMO3 was significantly increased in the morbidly obese group ($P < 0.05$). However, this effect was smaller than that seen in the mouse model, possibly because diabetic patients were treated with insulin and/or other drugs to increase insulin sensitivity [61].

The exact biological mechanism of how plasma TMAO concentration is involved in glucose metabolism remains to be clarified. PI3K is a key protein that transduces insulin signals into the regulation of glucose metabolism, while Akt is an important molecule downstream of the PI3K pathway, and its stimulation will in turn activate glycogen synthetase (a critical regulatory enzyme for the regulation of hepatic glycogen storage) [62]. In a study conducted by Gao it was shown that dietary TMAO reduces the mRNA levels of PI3K and Akt, indicating that dietary TMAO may exacerbate the blocked insulin signaling pathway [63].

Another mechanism involving TMAO promotes the regulation of transcription factor FoxO1 caused by PERK, and inhibition of FMO3 or manipulation of the gut microbiota can hinder metabolic dysfunction mediated by the PERK-FoxO1 axis [64]. Specifically, FoxO1 drives glucose gene expression and inhibited by insulin, the deletion of Foxo1 in the liver can reduce excessive glucose production caused by general ablation of insulin receptors [65]. PERK is a key sensor of intracellular stress and has previously been shown to induce FoxO1 by phosphorylation on serine 298 [66]. These data indicate that TMAO may be central to the pathogenesis of metabolic syndrome.

4.2 Hypertension

Research conducted by Yang observed a significant decrease in microbial richness, diversity, and reproducibility in the spontaneously hypertensive rat [67], suggesting that the intestinal flora may be involved in the occurrence of hypertension, although the mechanism has not yet been fully elucidated. Meta-studies have shown that for every 5 $\mu\text{mol/L}$ and 10 $\mu\text{mol/L}$ increase in TMAO, the prevalence of hypertension will increase by 9% and 20% respectively, revealing a significant dose-dependent positive correlation between TMAO concentration and the risk of hypertension [68]. TMAO is an important metabolite of enteral malnutrition and may be involved in the pathogenesis of hypertension as a mediator.

A different study demonstrated that patients with hypertension have an increased abundance of the cutC gene in their intestines, which in turn produces more TMAO [69]. The integrity of the intestinal barrier is essential for maintaining host health and preventing inflammation and atherosclerotic processes. Kinga Jaworska confirmed that the permeability of the colon to TMA in hypertensive patients is increased and promotes its penetration into the circulatory system [70], representing a novel point of view that the high concentration of TMAO in cardiovascular diseases may depend upon the increased permeability of the colon to TMA, and changes in colon permeability rather than TMAO levels are the indicators of cardiovascular risk [70].

4.3 Chronic kidney disease (CKD)

The clearance of TMAO depends largely upon its excretion by the kidneys [71]. Serum TMAO concentrations substantially increase with deterioration of kidney function, and this effect is reversed by renal transplantation [72]. Meta-analyses of a total of 32 eligible clinical studies involving 42,062 participants showed that TMAO was strongly inversely correlated with glomerular filtration rate (GFR) and positively associated with the urine albumin-to-creatinine ratio, serum creatinine, and urine albumin excretion rates [73]. Jason R reported that the increase in TMAO concentration in CKD patients receiving coronary angiography was related to the load of coronary atherosclerosis and may be related to long-term mortality in CKD patients receiving coronary angiography [72]. Moreover, it was observed that TMAO predicts a poor overall survival rate in subjects with CKD [74–76].

TMAO is an independent predictor of CKD phase 3–5 mortality rate [77] and in animal models, TMAO levels significantly increase renal fibrosis compared with control ($P < 0.05$), while this result can be reversed by TMA lyase inhibitors [78]. This model was also demonstrated in ApoE KO mice, where the animals developed chronic kidney disease with elevated TMAO levels within 14 weeks of adenine administration, after using TMA inhibitors, and many markers of kidney injury (creatinine, cystatin C, FGF23, and urinary microalbumin) were significantly reduced [79]. This suggests that TMAO may represent a novel modifiable risk factor and

therapeutic target for improving atherosclerosis in patients with CKD.

5. Is TMAO a reliable marker for atherosclerosis?

As discussed so far TMAO has a role in the pathological process of atherosclerosis, but can it also be used to clinically monitor the development and prognosis of patients with atherosclerosis.

5.1 TMAO predicts carotid atherosclerosis

TMAO is a significant predictor of carotid plaque formation which is more significant than gender, diastolic blood pressure, total cholesterol, or diabetes mellitus [80]. A study from southern Germany showed that higher TMAO levels can predict thickness of the carotid intima-media (cIMT) [81]. TMAO is also an independent predictor of new lesions as seen by MRI after carotid artery stenting (CAS) [82]. In patients with severe carotid stenosis ($>70\%$) it was found that elevated plasma TMAO level were associated with the increased risk of new lesions on diffusion-weighted imaging (DWI) within 1–3 days of CAS1, and that a TMAO of 4.29 $\mu\text{mol/L}$ can better predict ischemic brain damage which is secondary to CAS [82].

5.2 TMAO is a predictive marker of coronary plaque development

In patients with non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI), TMAO was found to be independently related to the severity and prognosis of coronary atherosclerosis load [83–85]. TMAO is also significantly related to the severity of the culprit segment of calcification, including maximum calcification arc, maximum calcification thickness, and calcification length, and can be used as an indicator to the calcium load of the culprit plaque [86]. Studies have shown that repeated rupture or erosion of a plaque followed by healing, is related to the vulnerability of the plaque and a high level of inflammation, which helps to increase the high risk of coronary thrombosis [87, 88]. The study reported that in patients with STEMI, elevated TMAO levels indicated a higher SYNTAX score (response to coronary atherosclerosis load) and the presence of multivascular disease [89], and TMAO levels in patients with plaque rupture was significantly higher than those with no rupture [90]. While TMAO levels in patients with repaired plaques were significantly higher than those with unrepaired plaques, and can be used as a potential biomarker to predict healed plaque presence with a cut-off value of 2.9 $\mu\text{mol/L}$ [91]. The author believes that TMAO levels in the circulation may reflect the vulnerability and progression of coronary plaques.

TMAO not only plays a role in the process of primary atherosclerosis, but also contributes to late stent thrombosis after revascularization. A recent study found that an area under the curve (AUC) value for plasma TMAO of 0.85 can distinguish patients with new atherosclerosis and old atherosclerosis in STEMI patients who underwent PCI

surgery, by using OCT to evaluate the culprit plaque [92]. The above studies indicate that high TMAO levels can be used as a useful biomarker to distinguish between plaque instability and rupture and represents a promising diagnostic marker for the prediction of new atherosclerosis in a stent.

5.3 TMAO is an independent predictor of stroke development and prognosis

TMAO can cross the blood-brain barrier and cause oxidative stress, thus potentially leading to brain damage [93, 94]. Research has found that higher TMAO levels are associated with increased risk of a first stroke [95–97], and are closely related to a poor prognosis, such as post-stroke cognitive impairment (PSCI). When predicting moderate to severe stroke, a critical value for plasma TMAO levels has been found to be 4.95 $\mu\text{mol/L}$ [98]. Other studies however, suggest an optimal value of 6.6 $\mu\text{mol/L}$ [96]. PSCI is a common consequence of stroke, affecting approximately one-third of stroke survivors after one-year post stroke [99]. A study included 256 patients with ischemic stroke, suggesting that increases in plasma TMAO levels are related to poor cognitive function one year after ischemic stroke, and supports TMAO as a predictive biomarker of PSCI [100]. In patients with acute ischemic stroke, TMAO at a concentration of 5 $\mu\text{mol/L}$ can predict early deterioration in neurological function [101], and can be used as an independent predictor of stroke severity and infarct volume in patients with acute ischemia [102–104]. Furthermore, TMAO is also associated with poor prognosis in patients with cerebral hemorrhage at 3 months [105]. Su H suggested that TMAO can promote an increase in reactive astrocytes and glial scar formation through the Smurf2/ALK5 axis, thus aggravating nervous system damage after ischemic stroke [106]. Therefore, the study of TMAO can provide important predictive insights into the development and prognosis in stroke patients.

5.4 TMAO predicts the risk of peripheral arterial disease (PAD)

Dr. Vichai Senthong first reported that elevated plasma TMAO level are an important prognostic marker for patients with PAD [107]. In these patients, the increase of TMAO levels was related to an increased risk of death by 2.7 times, after adjusting for traditional risk factors, such as inflammatory biomarkers and a history of coronary artery disease, where the highest quartile for TMAO can still predict 5-year mortality [107]. PAD is characterized by atherosclerotic stenosis of the lower extremity vessels, leading to ischemic muscle pain in the elderly. Compared to patients with intermittent claudication, patients with advanced chronic severe limb ischemia (CLI) showed higher serum carnitine and TMAO levels and poorer long-term survival [108], whereas PAD patients with TMAO $>2.26 \mu\text{mol/L}$ exhibited higher risk of cardiovascular death [109]. To date potential mortality and pathophysiological predictors of PAD have not been clearly defined, this may be the reason why mortality and ischemic amputation rates in PAD patients are still very high [110]. These studies confirm the clinical prognostic value of TMAO levels in pa-

tients with PAD, and these findings may be used to develop a new prognostic indicator of risk stratification.

Taken together the experimental data on TMAO it might be suggested that TMAO is a potent atherogenic cytokine and increased risk of developing atherosclerotic diseases such as CAS, coronary atherosclerosis, stroke, and PAD (Table 1, Ref. [80–86, 89–92, 95–100, 102–105, 107–109]).

6. Treatment

Choline and carnitine are the main sources of TMAO production related to intestinal microbiota, therefore, diet regulation is a reasonable and cost-effective intervention strategy. Studies have shown that a healthy diet (vegetarian or low calorie diet) significantly improves TMAO levels [111–113]. Interestingly, microwave cooking can reduce the content of L-carnitine in some seafood [114]. In addition, exercise can change the diversity and distribution of the human microbial community [115], and voluntary exercise for 8 weeks can inhibit the increase in plasma TMAO in obese mice induced by a high-fat diet and prevent heart dysfunction also [116]. Some plant extracts and fruits, such as oolong tea [117], hawthorn [118], resveratrol [119], and ginkgolide B [120], have been shown to reduce the levels of circulating TMAO, which is beneficial for the improvement of atherosclerosis.

The use of prebiotics and probiotics may also help to produce a positive impact on the composition of the intestinal flora. Thus an in-depth study of the CutC/D gene responsible for the synthesis of TMA lyase and the development of a strain that antagonizes the synthesis pathway of TMAO represents a promising avenue of research [121]. For example, the TMA blockers DMB and IMC can inhibit the activity of microbial choline TMA enzymes in the body, increase the excretion of neutral sterols in mouse feces in the form of fecal sterols (bacterial metabolite of cholesterol), and prevent the accumulation of liver cholesterol driven by the diet [122]. *Akkermansia muciniphila* plays a protective role by improving intestinal barrier function to resist atherosclerosis [123]. Fecal microbiota transplantation (FMT) can reduce fecal TMA content and the content of serum TMAO [124]. These strategies that have focused on gut microbiota and microbial metabolites provide promising new insights into the treatment of atherosclerosis and CVDs.

7. Summary and future prospects

Emerging data has established a direct link between diet, the intestinal flora metabolite TMAO, and atherosclerosis. TMAO is involved in the complex process of atherosclerosis and plays an important role in the pathogenesis of atherosclerosis caused by various risk factors such as diabetes, hypertension, and chronic kidney disease. In humans, it has been proven that TMAO is a reliable marker for predicting the development and prognosis of arterial vascular disease. Taken together this convincing evidence has emerged from preclinical studies suggest a strong relationship between TMAO and atherosclerosis. Further studies have focused on molecu-

Table 1. TMAO plays a role in atherosclerosis studies.

	TMAO's significance	References
CAS	TMAO is a significant independent predictor of carotid plaque burden.	[80]
	Higher TMAO levels predicted increased cIMT.	[81]
	Plasma level of TMAO can be a predictor of ischemic brain injury secondary to CAS.	[82]
	The optimal cutoff value of TMAO in patients with >70% carotid artery stenosis was 4.29 $\mu\text{mol/L}$.	[82]
	High Plasma TMAO is associated with coronary atherosclerotic burden in patients with STIME or NSTEMI.	[83],[84],[85],[89]
Coronary atherosclerosis	TMAO is positively correlated with the incidence of calcification in the culprit lesion segment.	[86]
	Plasma TMAO level is associated with plaque rupture.	[85],[90],[92]
	Plasma TMAO level is associated with healed culprit plaques.	[91]
	A cutoff value of TMAO was 2.9 $\mu\text{mol/L}$ in patients with STEMI can be used as a potential biomarker to predict healed plaque presence.	[91]
	AUC value for plasma TMAO of 0.85 can distinguish patients with new atherosclerosis and old atherosclerosis in STEMI patients.	[92]
Stroke	Serum TMAO concentration exhibited higher risk of first Stroke in Chinese patients.	[95],[96],[97]
	TMAO level ≥ 4.95 (6.6) $\mu\text{mol/L}$ has high sensitivity and specificity for moderate to severe stroke.	[96],[98]
	Higher TMAO levels correlate with worse neurological deficit .	[96],[100]
	TMAO is an independent predictor for cognitive impairment in post-stroke patients.	[99]
	TMAO is an independent predictor of functional outcome and mortality of patients with ischemic stroke.	[102],[103],[104]
PAD	TMAO is also associated with poor prognosis in patients with cerebral hemorrhage at 3 months.	[105]
	TMAO is associated with PAD severity and prognosis.	[107],[108]
	PAD patients with TMAO >2.26 $\mu\text{mol/L}$ exhibited higher risk of cardiovascular death.	[109]

lar mechanisms and the development of safe, effective, and feasible dietary strategies to use as an anti-atherosclerotic, involving TMAO reduction and this has led to potentially significant public health benefits. Innovative therapeutic approaches targeting gut microbiota and TMAO, including lifestyle modifications, TMA inhibitors, prebiotics, probiotics, and Chinese herbs, have shed new light on the great potential of targeting TMAO to elucidate the fundamental mechanisms underlying the disease. We propose to test TMAO levels clinically to fully assess its role as a potential marker for atherosclerosis.

Author contributions

GX and AY conceptualized the study and prepared the original draft wrote; GX and PL revised the manuscript; PL, LG and YW provided language help and writing assistance.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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