REVIEW ARTICLE

Research Progress in the Relationship Between Trimethylamine Oxide and Coronary Heart Disease

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ABSTRACT

Objective • To provide a theoretical basis for intestinal intervention in the treatment of coronary heart disease.

Methods • Summarizing the mechanism of trimethylamine oxide (TMAO) inducing coronary heart disease and discussing the target of clinical intervention including TMAO generation, metabolism, and other links. The authors also clarified the potential clinical value of TMAO as a predictor of cardiovascular disease.

Results • The intestinal microbiota metabolite TMAO is closely related to the occurrence and development of coronary heart disease. TMAO can induce the development of coronary heart disease by promoting endothelial cell dysfunction, promoting foam cell formation, affecting cholesterol and bile acid metabolism, and promoting platelet activation and thrombosis. Diet, physical exercise, and other ways can reshape intestinal flora, inhibit TMAO generation, and help to prevent and cure coronary heart disease. In addition, TMAO has important clinical value in predicting risk stratification and evaluating the prognosis of coronary heart disease.

Conclusion • TMAO can induce and assist in the development of coronary heart disease by promoting endothelial cell dysfunction, foam cell formation, and other mechanisms. At present, diet and physical exercise can reduce the production of TMAO to a certain extent, to prevent the occurrence and development of coronary heart disease. Furthermore, TMAO is a promising predictive marker for risk stratification and evaluating the prognosis of coronary heart disease.

TMAO can not only directly induce coronary heart disease by promoting endothelial cell dysfunction, foam cell formation and other mechanisms, but also promote the occurrence and development of coronary heart disease by affecting the risk factors related to coronary heart disease (such as hypertension and diabetes). It has been confirmed that diet and physical exercise can reduce the production of TMAO to a certain extent and prevent the occurrence and development of coronary heart disease. In addition, TMAO is a valuable indicator for assessing risk stratification and prognosis of coronary heart disease. (*Altern Ther Health Med.* [E-pub ahead of print.])

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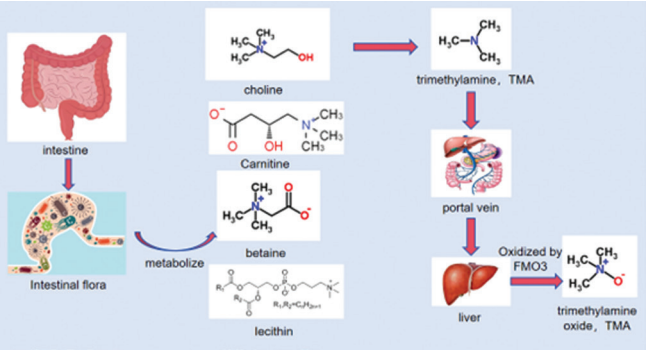
INTRODUCTION

Atherosclerosis (AS) and coronary heart disease (CHD) are both atherosclerotic cardiovascular diseases and in fact, atherosclerosis is considered a cause of CHD. CHD is the most common type of organ lesion caused by AS and is diagnosed when one to two coronary arteries show more than 50 percent narrowing of the vessel diameter.[1] Coronary atherosclerosis (CAS) refers to the condition where one to two coronary arteries show less than 50 percent narrowing of the vessel diameter. In other words, CHD is a more severe form of CAS. Sudden cardiac death event caused by acute myocardial infarction is a leading threat to people's lives and rank first among the risks of mortality from cardiovascular diseases.

It is now possible to rapidly diagnose CHD by coronary angiography (CAG). Patients diagnosed with CHD may receive percutaneous transluminal coronary angioplasty (PTCA) with the assistance of optical coherence tomography (OCT), intravenous ultrasound (IVUS), and fractional flow reserve (FFR). Several PTCA procedures (e.g., drug-coated

Figure 1. Trimethylamine Oxide Production and Metabolism Flow Chart



balloons, drug-eluting stents, and rotablation) have found widespread clinical use but treatments for early CHD or CAS are still limited. Trimethylamine N-oxide (TMAO), an intestinal flora metabolite, has been found capable of inducing the occurrence and development of CHD. TMAO may serve as a target for CHD treatment and an indicator for prognostic assessment, and thus opens up a new pathway for CHD prevention and treatment.

TMAO production and metabolism

TMAO can come from various sources. It is naturally present in fish and seafood. In human bodies, TMAO is primarily produced by the oxidation of trimethylamine (TMA) by the intestinal flora. The intestinal microorganisms produce TMA lyase from the choline, L-carnitine, betaine, lecithin, etc., which are found in meat (mainly red meat), eggs, and dairy products; and this in turn, produces TMA (Figure 1). Intestinal microorganisms that produce TMA lyase can metabolize choline, L-carnitine, betaine, lecithin, etc. from meat (mainly red meat), eggs and dairy products to produce TMA.

TMA then enters the liver via the portal vein and is oxidized into TMAO in the presence of flavin-containing monooxygenase-3 (FMO3), as shown in Figure 1. Blood then carries TMAO to every part of the human body. The majority of TMAO (about 95%) is excreted via urine, and only a small portion is excreted via feces (about 4%) and respiration (about 1%). Al-Waiz et al.[2] confirmed the above metabolic pathways of TMAO by radiolabeling TMA and TMAO. Plasma TMAO level is mainly influenced by diet, intestinal flora, drugs, activity of FMO3 in the liver, and renal excretion rate.

TMAO and CHD

A large number of studies have proved that an elevated plasma level of TMAO is closely related to various human chronic diseases (e.g., cardiovascular diseases, chronic kidney disease, type 2 diabetes mellitus, and cancers). Also, TMAO is closely related to the occurrence of several cardiovascular diseases (e.g., hypertension, AS/CHD, heart failure, nonvalvular atrial fibrillation, and cardiomyopathy) and the mortality risks posed by them. Back in 2011, Wang et al.[3] induced AS in healthy mice by supplementing their diet with choline, TMAO, and trimethylglycine, and found that the severity of AS in mice was related to the doses of choline, TMAO, and trimethylglycine supplements. Animal experiments have already demonstrated the role of an elevated plasma level of TMAO in facilitating AS development. Similar reports have been published based on human subjects, particularly females.

Similarly, a similar pattern has been reported in healthy women. A 10-year follow-up study[4] involving 760 healthy subjects showed that ΔTMAO (an increase or decrease in the TMAO level) had a strong linear correlation with the risk of CHD ($P = .016$). Compared to females with persistently low TMAO levels, those with persistently high TMAO levels had a significantly increased risk of CHD. Eating an unhealthy diet further enhanced the positive correlation between ΔTMAO and the risk of CHD, while the correlation between the two was dramatically weakened if females ate a healthy diet. The above results indicated that healthy females with an elevated blood level of TMAO had a significantly increased probability of AS and CHD. Furthermore, even in the populations already affected by AS/CHD, the high TMAO levels in the blood also increase the risk of acute coronary syndrome (ACS, including STEMI, NSTEMI, and sudden cardiac death), resulting in a higher death risk caused by CHD in the short term.

Tan et al.[5] studied the relationship between the morphology of culprit plaques and plasma TMAO levels in STEMI patients. Their study included 211 ST-elevation myocardial infarction (STEMI) patients, who received OCT of the culprit lesions (plaque rupture in 77 patients and plaque erosion in 69 patients). The results showed that the plasma TMAO levels were significantly higher in patients with plaque rupture than in those with plaque erosion (3.33 μm vs.1.21 μm). After an adjustment for conventional risk factors, elevated TMAO levels remained independently correlated with plaque rupture (adjusted odds ratio (OR): 4.06, 95% CI: 2.38-6.91, $P < .001$). The area under the receiver operating characteristic (ROC) curve of this indicator was 0.89 for plaque rupture and plaque erosion. At the critical TMAO level of 1.95 μm, the sensitivity and specificity of TMAO in discriminating between plaque rupture and plaque erosion were 88.3% and 76.8%, respectively. According to the above-mentioned study, an elevated plasma level of TMAO was independently correlated to the risk of plaque rupture in STEMI patients. Hence, TMAO may be a biomarker for plaque rupture.

Sheng et al.[6] conducted a prospective study, which recruited two cohorts, including 335 patients with STEMI and 53 healthy controls. The coronary artery atherosclerotic burden was measured by the number of coronary artery lesions and the Synergy Between PCI with Taxus and Cardiac Surgery (SYNTAX) score. The results showed that an elevated plasma level of TMAO was an independent predictor of a higher SYNTAX score, and also a predictor of multivessel disease (MVD). It was inferred that TMAO might be used as a quantitative biomarker for CAS. Waleed et al.[7] divided the non–non-ST-segment elevation myocardial infarction (NSTEMI) patients (n = 73) and the age- and gender-

matched healthy controls (n = 35) into two groups in a prospective cohort study. They were stratified based on coronary atherosclerotic burden measured by the number of coronary artery lesions and clinical risk scores (SYNTAX and Gensini). In the NSTEMI group, MVD patients had a higher TMAO level than those with single-vessel disease (SVD) (P = .002). Besides, patients falling in the medium to high-risk group (score ≥23) based on SYNTAX (P = .003) and Gensini (P = .005) had a higher TMAO level than those in the low-risk group (score <23). After adjustment for conventional risk factors, TMAO level remained an independent predictor for MVD (OR: 5.94, P = .005), medium to high SYNTAX score (OR: 3.61, P = .013), and medium to high Gensini score (OR: 4.60, P = .008). The area under the ROC curve (AUC) was used to measure the ability of TMAO to predict MVD (AUC: 0.73, 95% confidence interval [CI]: 0.60-0.86, P = .002), SYNTAX score (AUC: 0.70, 95% CI: 0.58-0.82, P = .003), and Gensini score (AUC: 0.70, 95% CI: 0.57-0.83, P = .005). The above studies consistently indicated that the plasma TMAO level was significantly correlated with the severity of CAS in NSTEMI patients. However, for chronic coronary syndrome (CCS), the high plasma TMAO level was similarly correlated to the prognosis. Yao et al.[8] performed a meta-analysis using the hazard ratio (HR) data from 2,369 studies in 10,301 CHD patients, who were subjected to prospective observations on the relationship between the plasma TMAO level and the incidence of major adverse cardiovascular events (MACEs). The statistics showed that an elevated plasma level of TMAO increased the risk of MACEs by 58% in CHD patients (HR: 1.58; 95% CI = 1.35-1.84, P = .000). These patients received a follow-up for at least one year. It was found that the HR of MACE in the long term was higher by 62% compared with that in the short term (HR for follow-up for ≥4 years: 1.96; 95% CI = 1.52-2.52 vs 1 - 3 years: 1.34; 95% CI = 1.26-1.43, P = .004). These results suggested that the plasma TMAO level was significantly correlated with the long-term risk of MACEs in CHD patients (including ACS and CCS patients). Besides, the plasma TMAO level was more predictive of the long-term risk than the short-term risk of MACEs. The critical plasma level of TMAO for prognostic assessment was 5.1 μmol/L. Therefore, TMAO increases the risk of CHD in healthy populations and may serve as a novel predictive biomarker for plaque rupture and death risks in ACS/CCS patients. TMAO can be used as an important indicator for risk stratification and also as a target for therapeutic intervention in CHD.

Mechanism of TMAO inducing CHD

In recent years, many studies have been conducted concerning the mechanism of TMAO inducing CHD, and CAS is considered to be one important cause of CHD. The induction of Coronary atherosclerosis CAS (a milder form of CHD) by TMAO can be summarized in four mechanisms: promoting endothelial cell dysfunction, promoting foam cell formation, interfering with cholesterol and bile acid metabolism, and causing platelet activation and thrombosis. These four mechanisms do not work separately but mutually influence each other, resulting in complex cascade reactions and facilitating the development of CHD.

Inducing vascular endothelial dysfunction

In the cardiovascular field, vascular endothelial dysfunction has long been considered an initiating factor for the occurrence and development of AS. The formation of CAS plaques is an outcome of vascular endothelial dysfunction, which primarily manifests as vascular endothelial damage, self-repair disorder, and dysfunction in endothelial cell secretion. A growing number of experimental studies have shown that high plasma TMAO level causes vascular endothelial damage by inducing oxidative stress, inflammatory response, and endothelial cell pyroptosis and autophagy.

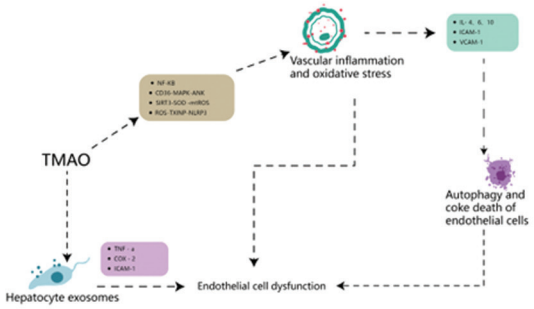
First of all, oxidative stress is one of the leading reasons for vascular endothelial damage. Oxidative stress can disrupt the redox balance in endothelial cells, causing the oxidative denaturation of proteins and DNA. In some severe situations, cell membrane lipid peroxidation, protein enzymolysis, and cell apoptosis may occur. Brunt et al.[9] built models of vascular endothelial damage in mice and healthy subjects. The feed was supplemented with TMAO for young mice to induce ACh-induced endothelium-dependent dilation of the carotid artery (peak dilation: 79 ± 3% vs. 95 ± 3%, P < .01). The dilation was accompanied by an increase in the vascular nitrotyrosine level (an indicator of oxidative stress). On the contrary, supplementation with 2,3-dimethyl-1-butene (DMB), a TMA inhibitor, inhibited TMAO production and improved the above-mentioned TMAO-induced phenotype. This effect might be explained by the normalization of vascular superoxide production and the restoration of NO-mediated vasodilation to improve endothelial-dependent vasodilation in aging mice. In the healthy populations, the plasma TMAO level was higher in the middle-aged and elderly people (64 ± 7 years old) than in the young people (22 ± 2 years old) (i.e., 6.5 ± 0.7 vs.1.6 ± 0.2 μmol/L). The plasma TMAO level was negatively correlated with brachial artery flow-mediated dilation (r^2 = 0.29, P < .00001), and related to the higher nitrotyrosine level in endothelial cells. The blood flow-mediated vasodilation could be restored in healthy subjects by taking an ascorbic acid supplement (vitamin C), indicating that chronic oxidative stress induced by high TMAO levels inhibited endothelial cell function. By constructing human umbilical vein endothelial cells (HUVECs) and mouse models, Ke et al.[10] confirmed that high concentration of circulating TMAO can exacerbate the aging and dysfunction of vascular endothelial cells, which may be related to the inhibition of Sirtuin 1 (SIRT1) expression and the increase of oxidative stress, thus activating the p53/p21/Rb pathway. The above studies have fully demonstrated that TMAO promotes age-related endothelial dysfunction by inducing oxidative stress.

Secondly, some researchers believe that as the TMAO level increases, the level of nitrotyrosine, an oxidative stress marker, also increases. The reason may be that the high

TMAO level induces the inactivation of endothelial nitric oxide synthase (eNOS), SIRT1 downregulation, activation of nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasomes, and activation of the NF-κB/MAPK pathway. As a result, the reactive oxygen species (ROS) production in endothelial cells increases, which further aggravates arterial endothelial cell dysfunction. Therefore, inflammatory response is another important reason for endothelial cell damage. High plasma TMAO levels can activate several inflammatory signaling pathways, including NF-κB, CD36-MAPK-JNK, SIRT3-SOD2-mtROS, and ROS-TXNIP-NLRP3. This further promotes inflammatory cell activation and migration, overexpression of inflammatory factors (IL-4, IL-6, and IL-10), and adhesion factors (ICAM-1, VCAM-1, and E-selectin), causing chronic vascular inflammation and vascular endothelial damage. Chen et al.[11] performed experiments using liquid chromatography-tandem mass spectrometry, Western Blot, and fluorescence probes and found that TMAO induced inflammatory response in human umbilical vein endothelial cells (HUVECs) and in the aorta of ApoE−/− mice. They proved for the first time that TMAO promotes vascular inflammation by activating NLRP3 inflammasomes, while the activation of NLRP3 inflammasomes is partially mediated by inhibiting the SIRT3-SOD2-mtROS signaling pathway. Similarly, Seldin et al.[12] increased inflammatory gene expression by feeding choline (a precursor of TMAO) to LDLR knockout mice or injecting TMAO into mice, and the same changes in inflammatory signal expression were measured by fluorescent PCR in cultured human aortic endothelial cells and vascular smooth muscle cells after supplementation with TMAO. This activation may be mediated by nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways. Besides, TMAO causes endothelial dysfunction by promoting hepatocytes' production of exosomes with unique activity. Liu et al.[13] found that exosomes produced by hepatocytes in the presence of TMAO were taken up by cardiac myocytes, hepatocytes, aorta, and endothelial cells of gastrocnemius, and promoted the mRNA expressions of TNF, Icam1, Sele, and COX-2 in the aorta, which finally led to inflammation and endothelial dysfunction.

Besides, oxidative stress and vascular inflammation may induce pyroptosis and autophagy of endothelial cells. Studies have shown that TMAO induces pyroptosis of vascular endothelial cells via several pathways, including ALDH2/ROS/NLRP3/GSDMD[14] and TET2-CYTB-mtROS,[15] and by upregulating the succinate dehydrogenase complex subunit B (SDHB).[16] For example, Li et al.[14] found that TMAO induces the pyroptosis of endothelial cells via the ALDH2/ROS/NLRP3/GSDMD pathway, which typically manifests as increased cell death, extravasation of lactate dehydrogenase, production of protein gasdermin D (GSDMD), and plasma membrane pore formation. First, TMAO induces an upregulation of the NLRP3 inflammasomes, apoptosis-associated speck-like protein containing CARD (ASC), and cysteine protease caspase-1. Meanwhile, TMAO inhibits the activity of mitochondrial aldehyde dehydrogenase 2 (ALDH2) and increases intracellular ROS production. TMAO-induced activation of inflammasomes and production of GSDMD occur, which finally leads to the pyroptosis of endothelial cells. Wu et al.[16] proved that TMAO upregulated SDHB expression, thereby promoting ROS production and inflammatory factor (e.g., TNF-α) release, resulting in AS in mice. The above-mentioned pathways all implicate ROS, indicating that oxidative stress, vascular inflammation, and endothelial cell pyroptosis complement and reinforce each other, leading to CHD as shown in Figure 2.

Figure 2. TMAO can Release Inflammatory Mediators and Cytokines Through Various Inflammatory Signaling Pathways, Causing Oxidative Stress and Vascular Inflammation, Directly Leading to Vascular Endothelial Dysfunction, and Indirectly Inducing Endothelial Cell Pyroptosis in the Context of the Above Oxidative Stress and Vascular Inflammation, Thereby Leading to Vascular Endothelial Dysfunction. In Addition, it can also Increase the Expression of Inflammatory Factors Through the Hepatocyte Exosome Pathway, and Eventually Cause Vascular Endothelial Dysfunction and Promote the Occurrence and Development of Coronary Heart Disease.



Promoting foam cell formation

Existing studies have demonstrated that once vascular endothelial damage occurs, LDL-C enters the vascular wall via the damaged endothelium and undergoes oxidative modification to become ox LDL-C. In the meantime, a large number of monocytes are recruited due to inflammation and extravasate beneath the endothelium to become macrophages. The macrophages engulf ox LDL-C via the scavenger receptor class A (SR-A1) to become foam cells, resulting in fatty streaks, an early sign of AS. Wang et al.[3] found that the expressions of macrophage-associated receptors CD36 and SR-A1 increased after three weeks of supplementing the feed with choline or TMAO in ApoE−/− mice. This resulted in intracellular accumulation of cholesterol and foam cell formation. On the contrary, antibiotics were administered to inhibit intestinal flora, or DMB, a TMA inhibitor, which dramatically reduced the foam cells derived from macrophages. The above findings indicated that TMAO may upregulate two AS-associated macrophage scavenger

receptors, CD36 and SR-A1, to mediate macrophages' phagocytosis of the accumulated cholesterol, thereby promoting foam cell formation. Likewise, Mohammadi et al.[17] found that TMAO not only upregulated the expression of SR-A1 but also downregulated the ATP binding cassette transporter, A1, to promote foam cell formation.

Cholesterol and bile acid metabolism disorders

Hypercholesterolemia is a major risk factor for AS, and an inducer of the occurrence and development of AS. Studies have shown that TMAO reduces the expressions of two key enzymes required for bile acid synthesis, namely, CYP7α1 and CYP27α1, and the expressions of several bile acid transporters (OATP1, OATP4, MRP2, and NTCP) in the liver. As a result, the bile acid content decreases, which further reduces the reverse transport of cholesterol, leading to lipid overload of the arterial wall and the occurrence of AS. Ding et al.[18] found that mice eating a diet supplemented with TMAO had a two-fold increase in aortic plaque area compared with normal controls. Furthermore, the serum TG, TC, and LDL-C levels increased by 25.5%, 31.2%, and 28.3%, respectively. The above results indicated that TMAO inhibits the expression of the key enzyme in bile acid synthesis, that is, cholesterol 7α-hydroxylase (CYP7A1), and increases the expressions of short heterodimer partner (SHP) and farnesoid X receptor (FXR). This mechanism ultimately inhibits bile acid synthesis, aggravating atherosclerotic plaque formation. Similarly, Koeth et al.[19] found that the mRNA expressions of CYP7α1 and CYP27α1, the key enzymes in bile acid synthesis in the liver, decreased dramatically in mice eating a TMAO-supplemented diet compared with the normal controls. Besides, the expressions of several bile acid transporters (OATP1, OATP4, MRP2, and NTCP) also decreased significantly in the liver ($P < .05$). This indicated that TMAO inhibited the synthesis of several bile acid transporters, thereby reducing reverse cholesterol transport and leading to AS.

Causing platelet activation and thrombosis

Platelet activation and accumulation are downstream of vascular inflammatory response, serving as a core process in atherosclerotic plaque formation. They are also key factors involved in plaque rupture inducing severe complications of AS (e.g., STEMI). Zhu et al.[20] found that TMAO, a metabolite of intestinal flora, directly induced platelet hyperresponsiveness and thrombosis. TMAO promoted platelet hyperresponsiveness mainly by enhancing the stimulus-dependent release of intracellular Ca^{2+} reserve. However, Subramaniam et al.[21] believed that TMAO had no specific impact on platelet activation and systemic coagulation activation in mice, indicating that TMAO did not directly induce platelet activation, but acted on the phosphatidylinositol-3 kinase (PI3K) and MAPK pathways to activate NF-κB. As a result, the procoagulant activity of platelets was increased, and the thrombomodulin in human endothelial cells was downregulated. These processes initiated the coagulation cascade, thereby promoting platelet activation and accumulation, and leading to thrombosis. Cheng et al.[22] found that in STEMI patients, the plasma TMAO level was positively correlated with tissue factor (TF) activity. They further proved experimentally that TMAO upregulated TF expression by activating the NF-κB pathway in human coronary artery endothelial cells (HCAECs), thereby significantly increasing TF activity and thrombin production, and promoting arterial thrombosis. However, whatever the pathway, it has been demonstrated that TMAO has the potential to promote platelet activation or thrombosis. In the field of clinical pharmacy, a study in animal model[23] found that TMAO partially antagonized the effects of clopidogrel (Clo, P2Y12 receptor antagonist), inducing Clo resistance and increasing the risk of recurrence of thrombosis in post-PCI patients receiving long-term medication treatment. This result indicates that a high plasma TMAO level may be a potential reason for Clo resistance.

Correlation between TMAO and risk factors for CHD

The risk factors for CHD include hypertension, hyperlipidemia/obesity, and diabetes/insulin resistance. A growing number of studies have shown that TMAO is implicated in the effects of these risk factors to varying degrees.

TMAO and hypertension

Existing evidence has indicated that hypertension is a risk-independent factor for CHD. Hypertension induces AS by causing arterial endothelial damage and accelerating the progression of AS into CHD. The higher the blood pressure level, the more severe the AS, and the higher the risk of death caused by CHD. If there are severe lesions in the coronary arteries, an abrupt increase in blood pressure may trigger the rupture of the atherosclerotic plaques, resulting in thrombosis and blocked coronary arteries, and hence acute myocardial infarction. A growing body of evidence has shown that TMAO, the metabolite of intestinal flora, is significantly correlated to hypertension. Ge et al.[24] demonstrated through a meta-analysis that there is a significant positive dose-dependent relationship between circulating TMAO concentrations and the risk of hypertension. Circulating high concentrations of TMAO were associated with a higher prevalence of hypertension compared to low concentrations of TMAO (RR: 1.12; 95% ci: 1.06, 1.17; $P < .0001$; $I^2 = 64\%$; p heterogeneity P heterogeneity = .007; Stochastic model). TMAO itself promotes AS by inducing hypertension through several pathways.

Jiang et al.[26] found that in mice with Ang-II-induced hypertension, TMAO aggravated Ang-II-induced acute pressor response, which manifested as a sudden rise in systolic blood pressure caused by vasoconstriction. Antibiotics reduced TMAO generation and improved Ang-II-induced hypertension in mice, indicating that TMAO promoted Ang-II-induced hypertension by facilitating Ang-II-induced vasoconstriction. This process might involve the PERK/ROS/CaMKII/ plc-β3 axis. Liu et al.[27] built a mouse model, in which an elevated

plasma TMAO level led to an increase in plasma osmolality, triggering the release of arginine vasopressin (AVP). As a result, the aquaporin-2 (AQP-2) expression was upregulated, causing an increase in water reabsorption and blood volume, which elevated the blood pressure. Naqvi et al.[28] reported the interactions between high-salt diet, hypertension, and intestinal flora. More specifically, short-chain fatty acids (SCFAs), which are the metabolites of the intestinal flora, negatively regulated blood pressure elevation, and AS, while TMAO exerted the opposite effect. High-salt diet may increase the TMAO level and decrease SCFAs in the gut, which further promotes hypertension.

TMAO and hyperlipidemia/obesity

Hyperlipidemia, also known as dyslipidemia, may be caused by congenital factors (e.g., familial hypercholesterolemia) or acquired factors (e.g., diet, diseases, medications). Hyperlipidemia is usually asymptomatic and an occult risk factor for CHD. Among various risk factors, hypercholesterolemia, especially high LDL-C, is related to the occurrence of CHD. As mentioned above, TMAO promotes hypercholesterolemia by affecting bile acid metabolism. Hypertriglyceridemia is another common form of hyperlipidemia and is mainly seen in patients with abdominal obesity. Obese patients are usually combined with varying degrees of dyslipidemia. Dehghan et al.[29] performed a meta-analysis of 12 observational studies (involving over 17 thousand subjects) and found a positive correlation between the TMAO level in the blood and obesity. That is, the TMAO level in the blood causes an increase in body mass index (BMI) in a dose-dependent manner (P = .007). The highest TMAO level was correlated to a BMI increase of 0.56 kg/m^2 (weighted mean difference [WMD], 0.563; CI, 0.026-1.100; P = .04). Similarly, Schugar et al.[30] found in a mouse model that flavin-containing monooxygenase 3 (FMO3) converted TMA to TMAO. Mice fed with a high-fat, high-calorie diet would not become obese if the FMO3 gene was deleted or knocked out. Besides, FMO3-negative mice had higher expressions of genes associated with brown fat cells. Since brown fat cells are more easily metabolized than white fat cells, the above finding implied the potential role of the MA/FMO3/TMAO pathway.

TMAO and diabetes/insulin resistance

Diabetes mellitus (DM) is a metabolic disease characterized by elevated levels of blood glucose and is caused by insulin deficiency and ineffective use of insulin due to various reasons. As a common disease, DM has become a global public health challenge that threatens human health. The risk of CHD complication in DM patients increases by more than 4-fold compared to a healthy population. CHD occurs at a younger age and with a greater severity in the former population than in the latter. Moreover, the prognosis of CHD is worse and the mortality is higher in DM patients. The reason is that those with type 2 DM complicated by CHD usually have diffuse stenosis or obstruction affecting more than one coronary artery. Zhuang et al.[31] conducted a meta-analysis of 12 clinical studies (involving 15,314 subjects) and found that a high TMAO level in the blood was related to an increase in DM risk. The plasma TMAO level in DM patients was higher than that in non-DM patients. For every increase of the plasma TMAO level by 5 μmol/L, the incidence of DM increased by 54% (OR = 1.54). The plasma TMAO level was positively correlated with the risk of DM in a dose-dependent manner. As seen from above, TMAO may be an independent risk factor for predicting DM. Type 2 DM is featured by the ineffective use of insulin, that is, insulin resistance. Heianza et al.[32] found that fat intake and ΔTMAO in overweight/obese patients were significantly correlated to fasting blood glucose, insulin level, and HOMA-IR (Homeostasis Model Assessment-Insulin Resistance) index (P < .05). Among the subjects eating a high-fat diet, the greater the increase in TMAO, the smaller the improvement of insulin resistance. It is inferred that high-fat diets may reduce insulin sensitivity (increasing insulin resistance) by changing the TMAO level, thus inducing type 2 DM. Besides, Gao et al.[33] found in a mouse model that increasing TMAO intake reduced the gene expressions of insulin receptor substrate 2 (IRS2), phosphatidylinositol 3-kinase (PI3Krl), glycogen synthase 2 (GYS2), and glucose transporter 2 (GLUT2). The above results indicated that TMAO blocks the insulin signaling pathway to reduce liver glycogen synthesis and glycogen transport. Meanwhile, the upregulation of Forkhead box protein O1 (FOXO1) and glucose-6-phosphatase G-6-pase (G6pase) promotes gluconeogenesis, thereby aggravating impaired glucose tolerance. To conclude, an elevated TMAO level causes impaired glucose tolerance and promotes DM.

Prevention and treatment of TMAO and CHD

Existing treatment strategies. The causal relationship between TMAO and CHD has already been demonstrated. Current interventions targeting TMAO to treat CHD mainly include early dietary intervention and physical exercise. As mentioned above, choline, L-carnitine, and trimethylglycine are essential nutrients required for the body to function. They are also important sources of TMAO. Wang et al.[34] found that the long-term consumption of red meat (e.g., beef) increased the production of TMA/TMAO, while the amount of TMAO excreted via the kidney decreased, leading to an elevated TMAO level in the blood. Some statistics show that some fish (e.g., saltwater fish, octopus, and cod fish) or seafood (shellfish) are rich in TMAO. Yoo et al.[35] found that long-term high-fat diets altered the intestinal epithelial physiology and accelerated choline catabolism by *Escherichia coli*, leading to an increased TMAO level in the plasma. According to Argyridou et al.,[36] eight weeks of a vegetarian diet dramatically reduced the plasma TMAO level and improved glucose tolerance in patients with obesity or abnormal blood glucose, indicating that a vegetarian diet is an effective strategy for reducing the plasma TMAO level and improving glucose tolerance in patients with obesity or abnormal blood glucose. In another study, high dairy diets were related to an elevated TMAO level and inflammation.

According to some studies, dietary fibers nourish intestinal probiotics and inhibit the growth of harmful bacteria, thereby inhibiting TMAO production. As given above, changing dietary structures or dietary habits can reduce the TMAO level in the human body to some degree. For those with underlying heart and kidney diseases, such as type 2 DM, obesity, and hyperlipidemia, a vegetarian diet or diets low in TMAO and animal fat but high in high-quality proteins and dietary fibers can prevent atherosclerotic cardiovascular disease (ASCVD) to a lesser or greater degree. Individuals with long-term needs for dairy products should choose skimmed milk and preferably animal milk, cheese, yogurt, and other dairy products that are low in fat. They can have their TMAO level tested at professional agencies, if necessary. Besides, appropriate physical exercise can promote metabolism and reshape intestinal flora, thereby reducing the TMAO level and preventing cardiovascular diseases. Clarke et al.[37] found that professional rugby players had a higher α-diversity of intestinal flora and a decreased abundance of phyla Firmicutes and Bacteroidetes. Compared with sedentary women, females taking long-term physical exercise had a higher abundance of health-promoting intestinal microflora. As mentioned above, endogenous TMAO is primarily produced by TMA lyase secreted by Firmicutes and Bacteroidetes, indicating that physical exercise may inhibit the occurrence and development of CHD, by reshaping intestinal flora and reducing TMAO production.

Potential clinical values

Interventions on intestinal flora. According to existing evidence,[38] intestinal flora imbalance resulting in excessive TMAO production is an important reason for AS/CHD. Therefore, probiotic treatment is an essential auxiliary intervention for CHD. A large number of clinical trials and experiments of drugs using animal models have indicated that probiotics are active microorganisms, capable of regulating the host metabolism. Particularly, the effects of probiotics in controlling blood lipid and body weight, and resisting AS, inflammation, and oxidation have been generally accepted. Qiu et al.[39] found in the mouse model that probiotics (e.g., *Lactobacillus plantarum* ZDY04) reduced the TMAO level and the formation of atherosclerotic plaques in ApoE−/− mice. These results indicate the possibility of probiotics inhibiting or delaying the occurrence and development of CHD by reducing the TMAO level. According to other studies, some substances may delay the occurrence and development of CHD by competitively inhibiting or antagonizing the key enzymes in TMAO synthesis. Tsutsumi et al.[40] found that the long-chain monounsaturated fatty acids (LCMUFAs) from cod liver oil improved the intestinal flora, stimulated SCFA production, and induced GLP-1 secretion. Meanwhile, LCMUFAs reduced atherosclerotic lesions and the plasma levels of inflammatory cytokines (e.g., IL-6 and TNF-α), thereby improving endothelial functions and maintaining a low TMAO level in the plasma, which was conducive to preventing cardiovascular diseases. It can be concluded that cod liver oil brings cardiovascular benefits by improving intestinal flora. As mentioned above[9], DMB, the TMA inhibitor, can delay or improve AS progression by reducing the TMAO level in mice, thereby alleviating oxidative stress. Similar compounds[41] include resveratrol[15], berberine[42], and iodomethylcholine[43], all of which can inhibit TMAO production and reduce the TMAO level. Other studies show that antibiotics inhibit the conversion of TMA into TMAO by the intestinal flora. Wang et al.[3] performed a perfusion of broad-spectrum antibiotic mixture in mice and observed a reduction in the plasma TMAO level, accompanied by a reduction of foam cells. Nevertheless, it is generally believed that antibiotics act on nearly all intestinal bacteria without discriminating between probiotics and pathogens. Antibiotic use is likely to cause secondary infections (secondary fungal infections) and therefore is not recommended as a treatment for CHD. Some researchers propose "fecal microbiota transplantation,"[44] which involves the isolation of intestinal flora from a healthy human gut and transplantation to the gut of CHD patients. The purpose is to alter the intestinal flora structure, reshape the biological microenvironment, and finally delay or improve CHD. However, this method is only recommended by the guidelines for recurrent or refractory pseudomembranous colitis. Previous drug studies for CHD were usually concerned with a specific enzyme in the host, which is considered a target for intervention. The novel treatments under investigation at present are innovative, in that they target specific intestinal bacteria or enzymes of the intestinal bacteria, to inhibit diet-induced cardiovascular diseases. Such treatments open up a new pathway for cardiovascular disease prevention and treatment. The limitations lie in the fact that systematic and accurate clinical evidence in favor of these treatments is still lacking. We need to conduct clinical trials and long-term follow-ups to verify their efficacy in CHD.

TMAO as a predictive marker for risk stratification of CHD

It has been repeatedly mentioned in this study that TMAO can predict the occurrence and development of cardiovascular diseases. Tang et al.[45] proved through a prospective clinical study that in a community-based EPIC-Norfolk middle-aged population, the plasma levels of TMAO and its precursor, TMA, increased in healthy subjects, which predicted the risk of CVD events independently from conventional risk factors. More importantly, the TMAO level can be used to guide clinical treatments for suspected ACS patients. In a study conducted by American researchers,[46] TMAO predicted the occurrence of MACE at 30 days and 6 months more effectively than conventional risk factors and ECG data, even among subjects initially negative for troponin T, a marker for composite CVD risk. The above evidence fully demonstrates that compared with conventional testing methods, a high TMAO level in the blood is more sensitive than cardiac troponin T (cTnT), and predicts the acute stage of CHD (in 2 months) and sub-acute stage of CHD (6 weeks to 6 months) in a fast and accurate manner. Meanwhile,

clinicians can choose coronary angiography and/or dual platelet inhibition based on stratification of the plasma TMAO levels of the high-risk candidate population.

CONCLUSION

At present, the potential relationship between TMAO and CHD has been proved by a large number of studies and experiments. However, the specific mechanism and metabolic pathways of TMAO remain to be understood. Fortunately, the use of plasma TMAO levels to delay the progression of AS in CHD has become a reality. The plasma TMAO level may be included in the diagnostic and therapeutic guidelines for CHD/acute myocardial infarction in the future. This article discusses the potential relationship between TMAO and CHD, based on existing clinical and experimental evidence. More clinical trials are needed for validation, to inform the search for safer, faster, and more accurate intervention measures for CHD.

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