

Role of Trimethylamine N-Oxide in Atherosclerosis: Inflammation, ER Stress and Beyond

Zeliha RUMANLI*, Aysun HACIŞEVKİ**

Role of Trimethylamine N-Oxide in Atherosclerosis: Inflammation, ER Stress and Beyond

Trimetilamin N-Oksit'in Aterosklerozdaki Rolü: Enflamasyon, ER Stresi ve Ötesi

SUMMARY

Cardiovascular diseases (CVD) account for a significant proportion of deaths worldwide. The common pathological feature underlying these diseases is atherosclerosis, which is characterized as an inflammatory condition driven by endothelial cell activation and dysfunction in its early stages. While the effects of the microbiota and its metabolites on various systems are being actively investigated, their impact on the cardiovascular system has attracted particular attention. Some of the most compelling data on the role of the intestinal microbiota in cardiovascular diseases have emerged from the identification of novel metabolites and pathways associated with cardiovascular risk, as well as metabolic analyses of plasma samples. However, the role of the microbiota in these diseases is still not fully understood. Through the digestion of certain foods, such as animal proteins, gut microbiota generate metabolites that can contribute to the development of several major diseases. TMAO is one such metabolite and has recently received considerable attention, as it may act as both a risk factor and a link between the gut microbiota and various diseases, including cardiovascular conditions. Elevated circulating TMAO levels have also been associated with chronic kidney disease (CKD), and impaired renal clearance is thought to contribute to TMAO accumulation, thereby further increasing the risk of cardiovascular disease. Recent evidence also suggests that TMAO may promote cardiovascular pathology through the activation of endoplasmic reticulum (ER) stress signaling pathways. ER stress, which occurs when protein folding homeostasis in the endoplasmic reticulum is disrupted, has been associated with endothelial dysfunction, inflammation, and apoptosis, processes that contribute to the development and progression of atherosclerosis. This review aims to address atherosclerosis from the perspective of TMAO and to highlight studies examining the relationship between TMAO, CKD, ER stress, and atherosclerosis.

Keywords: TMAO, CKD, atherosclerosis, inflammation, microbiota, ER stress.

ÖZ

Kardiyovasküler hastalıklar (KVH), dünya genelinde ölümlerin önemli bir bölümünü oluşturmaktadır. Bu hastalıkların altında yatan ortak patolojik özellik, erken evrelerinde endotel hücre aktivasyonu ve disfonksiyonu tarafından yönlendirilen inflamatuvar bir durum olarak tanımlanan aterosklerozdur. Mikrobiyota ve metabolitlerinin çeşitli sistemler üzerindeki etkileri aktif olarak araştırılırken, bunların kardiyovasküler sistem üzerindeki etkisi özellikle dikkat çekmiştir. Bağırsak mikrobiyotasının kardiyovasküler hastalıklardaki rolüne ilişkin en ikna edici verilerden bazıları, kardiyovasküler riskle ilişkili yeni metabolitlerin ve yolların tanımlanmasının yanı sıra plazma örneklerinin metabolik analizlerinden ortaya çıkmıştır. Bununla birlikte, mikrobiyotanın bu hastalıklardaki rolü hala tam olarak anlaşılamamıştır. Hayvansal proteinler gibi bazı gıdaların sindirimi yoluyla, yaygın bağırsak bakterileri (bağırsak mikrobiyotası), çeşitli önemli hastalıkların gelişimine katkıda bulunabilecek metabolitler üretmektedir. TMAO, bu metabolitlerden biri olup son zamanlarda hem risk faktörü niteliği taşıması hem de bağırsak mikrobiyotası ile kardiyovasküler durumlar da dahil olmak üzere çeşitli hastalıklar arasında bir bağlantı görevi görebileceği için önemli ölçüde ilgi görmüştür. Artmış dolaşımdaki TMAO düzeyleri ayrıca kronik böbrek hastalığı (KBH) ile de ilişkilendirilmiş olup, bozulmuş renal klirensin TMAO birikimine katkıda bulunarak kardiyovasküler riski daha da artırabileceği düşünülmektedir. Son kanıtlar, TMAO'nun endoplazmik retikulum (ER) stres sinyali yollarını aktive ederek kardiyovasküler patolojiyi destekleyebileceğini göstermektedir. Endoplazmik retikulumda protein katlanma homeostazının bozulması sonucu ortaya çıkan ER stresi; endotel disfonksiyonu, inflamasyon ve apoptoz ile ilişkilendirilmiş olup, bu süreçlerin aterosklerozun gelişim ve ilerlemesine katkıda bulunduğu belirtilmiştir. Bu derlemede, aterosklerozun TMAO perspektifinden ele alınması ve TMAO, KBH, ER stresi ve ateroskleroz arasındaki ilişkiyi inceleyen çalışmaların değerlendirilmesi amaçlanmıştır.

Anahtar kelimeler: TMAO, KBH, ateroskleroz, inflamasyon, mikrobiyota, ER stresi.

Received: 16.04.2026

Revised: 24.05.2026

Accepted: 18.06.2026

* ORCID: 0000-0003-0550-6281, University of Health Sciences, Gülhane Faculty of Pharmacy, Department of Pharmacology, Ankara, Türkiye

** ORCID: 0000-0002-3844-5772, Gazi University, Faculty of Pharmacy, Department of Biochemistry, and Gazi University, Institute of Health Sciences, Sports Pharmacy Program, Ankara, Türkiye.

* Corresponding Author; Aysun HACIŞEVKİ
E-mail: abozkir@gazi.edu.tr

INTRODUCTION

Atherosclerosis, a major contributor to global mortality and morbidity, is linked to hyperlipidemia and inflammation, and its chronic progression is driven by metabolites derived from diverse metabolic pathways (Din et al., 2019). The etiology of atherosclerotic vascular disease is generally classified into three major categories: genetic factors, environmental influences, and the microbiome (Brown & Hazen, 2014; Loscalzo, 2011). Numerous studies have demonstrated a link between genetic and environmental factors and cardiovascular risk. However, interest in the microbiome's specific relationship with cardiovascular diseases is growing globally, attracting the attention of health systems as a potential target for new preventive strategies (Brown & Hazen, 2014). Recent research has focused on the role of commensal gut microorganisms, also known as gut flora, in the metabolism of foods containing trimethylamine N-oxide (TMAO) precursors, which produce proteinogenic metabolites with potential effects on the pathogenesis of atherosclerosis (Mohammadi et al., 2018). Collectively, these findings suggest that the gut microbiota may act not only as a passive metabolic environment but also as an active regulator of cardiovascular homeostasis and disease progression.

Disruptions in the human gut microbiota are considered a critical factor in the development of atherosclerosis. A key aspect underlying this concept is the pivotal role of TMAO in the pathogenesis of atherosclerosis (Din et al., 2019). Evidence supporting the proatherogenic role of the microbiota has been demonstrated through studies on phosphatidylcholine metabolism. Specifically, TMAO, a metabolite produced in the liver from trimethylamine (TMA), is directly involved in atherosclerosis, inflammation, and platelet hyperreactivity. Recent findings have also emphasized the influence of diet, as the composition of the gut microbiota is strongly shaped by dietary intake (Li & Tang, 2017). Taken together, current evidence supports a complex interaction among diet, gut microbiota composition, and TMAO metabolism in

the progression of atherosclerosis. However, the relative contribution of TMAO as a direct pathogenic mediator versus a biomarker reflecting broader metabolic disturbances remains controversial.

In humans, TMAO is a derivative of TMA, which is produced in the intestine from various nutrient precursors containing the TMA moiety, such as choline, L-carnitine, γ -butyrobetaine, and betaine. The TMA generated in the gut then enters the circulation and is subsequently converted into TMAO, predominantly in the liver by flavin-containing monooxygenase 3 (FMO3) (Li & Tang, 2017). Studies have shown that the associations between TMAO precursor molecules, such as choline and L-carnitine, and disease risk are highly dependent on circulating TMAO levels, which serve as an indicator of alterations in the human gut microbiota (Fu et al., 2020; Wang et al., 2014). These observations indicate that cardiovascular risk associated with TMAO precursors may depend not only on dietary intake but also on interindividual differences in gut microbial composition and hepatic TMAO metabolism. Therefore, this review aims to provide an overview of the current evidence regarding the role of TMAO in the development and progression of atherosclerosis, with particular emphasis on the contribution of the gut microbiota.

TMAO, inflammation, and cardiovascular effects

TMAO was first identified as an independent risk factor for cardiovascular disease (CVD) in a study (Wang et al., 2011). Subsequent studies have confirmed the role of this metabolite as a risk factor for atherosclerosis, as well as the clinical prognostic significance of its circulating levels for cardiovascular disease and related complications (Canyelles et al., 2023; Thomas & Fernandez, 2021; Zhu, Li, & Jiang, 2020). Other studies have demonstrated that TMAO disrupts the reverse cholesterol transport system and/or affects various aspects of cellular cholesterol metabolism, thereby facilitating foam cell formation and promoting atherosclerosis and other vascular events (Koeth et al., 2013; Oktaviono et al., 2023). It has been

suggested that the intestinal microbiota-driven TMA/FMO3/TMAO pathway plays a key role in the regulation of lipid metabolism and inflammation (Warrier et al., 2015). It has been suggested that circulating TMAO levels may serve as a biomarker for renal dysfunction, as elevated plasma concentrations have been observed in individuals with end-stage renal disease (Bain, Faull, Fornasini, Milne, & Evans, 2006; Ren, Wang, & Xue, 2022). Collectively, these findings suggest that TMAO may represent a mechanistic link between gut microbiota dysbiosis, renal dysfunction, and cardiovascular disease. However, whether elevated TMAO levels act primarily as a causal mediator or reflect underlying metabolic and renal disturbances remains controversial.

The increased plasma TMAO levels varies according to the composition of the gut microbiota. In germ-free mice fed predominantly with L-carnitine, plasma TMAO levels remain undetectable or significantly decreased (Koeth et al., 2013).

TMAO promotes vascular inflammation, modulates platelet function, and induces a prothrombotic phenotype *in vivo*, which is believed to contribute to the pathogenesis of cardiovascular disease (Liabeuf, Cheddani, & Massy, 2018). A study demonstrated that CKD elevates circulating TMAO levels, which in turn contribute to endothelial dysfunction and cardiovascular disease associated with CKD by increasing vascular oxidative stress and inflammation (Li, Gua, Wu, & Chen, 2018). Another study showed that TMAO promotes atherosclerosis by inhibiting reverse cholesterol transport, activating macrophage, and enhancing platelet activity and thrombosis in mice. Furthermore, inhibition of TMAO production reduced the formation of atherosclerotic lesions. The study also found that elevated TMAO levels independently predicted worse cardiovascular outcomes and mortality in cardiology patients, beyond traditional risk factors (Yu et al., 2019). Two prospective clinical studies investigated the association between elevated fasting plasma TMAO levels and cardiovascular disease history. In one study, healthy volunteers received dietary

phosphatidylcholine before and after a one-week antibiotic regimen. TMAO was detected post-diet but was suppressed during antibiotic treatment. Following microbiota recovery one month later, TMAO and labeled TMAO reappeared after phosphatidylcholine intake (Wang et al., 2014). In the second study, oral phosphatidylcholine intake was linked to TMAO production by the gut microbiota in humans. Additionally, elevated fasting plasma TMAO levels were associated with a doubled risk of major adverse cardiovascular events (MACE), including death, myocardial infarction, or stroke. These findings demonstrate that TMAO, derived from dietary phosphatidylcholine and the intestinal microbiota, acts as an atherogenic precursor (Tang et al., 2014). Multiple studies have consistently shown the contribution of TMAO to atherogenesis (Nie et al., 2018). Taken together, both experimental and clinical studies support the hypothesis that TMAO contributes to cardiovascular pathology through interconnected mechanisms involving inflammation, oxidative stress, endothelial dysfunction, and altered cholesterol metabolism.

While the exact mechanisms remain unclear, it is possible that TMAO promotes the production of inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6, potentially aggravating myocardial fibrosis and microvascular dysfunction independent of its proatherogenic effects (Bozkurt et al., 1998; Stefania, Ashok, Geena, Katarina, & Isak, 2024), thereby worsening patient prognosis (Brown & Hazen, 2015). Moreover, it is known that TNF- α increases the overall intestinal permeability to IL-1 β and IL-6, further enhancing the translocation of metabolites such as TMA and bacterial fragments such as lipopolysaccharide (LPS), which exert deleterious effects on the myocardium (Katsimichas et al., 2019). In heart failure, microbiota-related shifts at the phylum level have been shown to promote not only TMAO production but also endotoxin (LPS) production. Similar to TMAO, LPS exacerbates heart failure progression (Desai et al., 2023; Lu, Yeh, & Ohashi, 2008). These observations further support the concept that cardiovascular effects attributed to TMAO

may occur within a broader microbiota-derived inflammatory network rather than through a single isolated pathway. Many different studies associate high TMAO levels with endothelial dysfunction, platelet hyperactivity and thrombosis, increase inflammatory biomarkers, and affect the cardiovascular system (Barrea et al., 2018; Trøseid, Andersen, Broch, & Hov, 2020; Winther et al., 2019). Some studies have also noted a link between poor prognosis and high TMAO in heart failure patients (Liabeuf et al., 2018; Wang et al., 2014). Nevertheless, variability among clinical findings suggests that the prognostic value of TMAO may be influenced by factors such as renal function, dietary habits, comorbidities, and interindividual differences in gut microbiota composition.

TMAO and atherosclerosis

Since its discovery, TMAO has attracted considerable attention from researchers due to its association with metabolic disorders. Strong positive associations have been reported between plasma TMAO levels and various cardiovascular conditions, including major adverse cardiovascular events (Guasti et al., 2021), acute coronary syndrome (Kong, Ma, Lin, & Chen, 2022), heart failure (Jarmukhanov et al., 2024), early atherosclerosis (Wang, Qiu, Lian, Yang, & Zhou, 2021), and renal failure in chronic kidney disease (Tang et al., 2015).

Recent studies emphasize the critical role of TMAO and hepatic FMO3 as key regulators of host lipid and sterol metabolism, as well as contributors to the development of atherosclerosis (Shih et al., 2015; Wang et al., 2011). Taken together, these findings suggest that the gut microbiota–TMA–FMO3–TMAO axis may represent a central mechanistic pathway linking dietary factors to atherosclerotic cardiovascular disease.

In addition, recent microbial transfer studies have confirmed that both the choline diet–enhanced susceptibility to atherosclerosis and the formation of TMA/TMAO are transmissible traits (Gregory et al., 2015). Accordingly, recent studies targeting the metaorganismal pathway responsible for TMAO pro-

duction have demonstrated that inhibition of TMAO generation through antisense oligonucleotide approaches results in reduced circulating TMAO levels and attenuated diet-induced atherosclerosis in animal models (Miao et al., 2015; Shih et al., 2015). The microbiota harbors enzymatic capacities that allow it to generate phosphatidylcholine, choline, carnitine, and TMA from dietary sources. The TMA produced through this process is subsequently oxidized in the liver and distributed throughout the host's body via the bloodstream. TMAO has been shown to be atherogenic and to influence not only cardiovascular risk but also the likelihood of developing heart failure (Tang, Li, & Hazen, 2019). Moreover, elevated circulating TMAO levels have been found to predict long-term mortality in patients with heart failure, independent of traditional cardiovascular risk factors (Wang et al., 2014) and B-type natriuretic peptide (BNP) levels (Suzuki, Heaney, Bhandari, Jones, & Ng, 2016). These observations further support the hypothesis that TMAO may function not only as a metabolic byproduct but also as a clinically relevant mediator associated with adverse cardiovascular outcomes.

TMAO has been identified in several studies as a biomarker for atherosclerosis, thrombosis, heart failure, and insulin resistance (Brial, Le Lay, Dumas, & Gauguier, 2018; Tang et al., 2013; Wang et al., 2011). Metabolomic screening in a large cohort has shown that three important phosphatidylcholine derivative metabolites (betaine, choline, and TMAO) can be used as potential biomarkers in predicting cardiovascular disease risk (Tang et al., 2013). It has been observed that TMAO production and atherosclerosis progression increase in response to a choline-rich diet, underscoring the role of the intestinal microbiota in CVD. Furthermore, antibiotic administration has been shown to suppress TMAO production by altering gut microbiota composition, further supporting the microbiota-dependent nature of TMAO metabolism (Hayashi et al., 2018). In a study examining the levels of various metabolites, including TMAO, in patients either protected from atherosclerosis or presenting with unexplained atherosclerosis, lower

levels of these metabolites—including TMAO—were observed in the protected group. In contrast, patients with unexplained atherosclerosis exhibited elevated levels of gut microbiome-derived metabolites (Bogiatzi et al., 2018).

Another aspect addressed in relation to atherosclerosis is its connection to the endoplasmic reticulum. ER stress, triggered by various risk factors, is thought to be involved in all stages of atherosclerosis and is characteristic of lipid-laden macrophages within atherosclerotic lesions (Feng et al., 2003; Hotamisligil, 2010; Myoishi et al., 2007; Zhou, Lhoták, Hilditch, & Austin, 2005). Furthermore, ER stress has been identified as a key trigger for abnormal macrophage activation, pathological inflammatory responses, and ultimately macrophage apoptosis, all of which play critical roles in the pathogenesis of atherosclerosis (Coope et al., 2012). Considering the proatherogenic potential of TMAO as a risk factor, a study was conducted to assess possible TMAO-induced ER stress by measuring GRP94 and HSP70 protein levels following trimethylamine N-oxide exposure in J774A.1 murine macrophage cell. The findings demonstrated that TMAO, a potent contributor to atherosclerosis, significantly enhanced GRP94 uptake and HSP70 expression, suggesting that TMAO can act as a stress-inducing agent in J774A.1 macrophage (Mohammadi et al., 2018). Consequently, it has been proposed that TMAO may contribute to abnormal macrophage activation and immunoinflammatory responses involved in foam cell formation (Din et al., 2019). To further demonstrate the transmissibility of TMAO-induced effects, fecal transplantation experiments in mice have shown that traits such as atherosclerosis and platelet hyperreactivity can indeed be transmitted (Zhu et al., 2016; Zhu, Wang, Tang, & Hazen, 2017).

TMAO is thought to be produced through a three-step process: first, the dietary intake of carnitine or phosphatidylcholine; second, the microbial conversion of these compounds into TMA by the intestinal microbiota; and third, the hepatic oxidation of TMA into TMAO by liver-expressed monooxygenases

(Wang et al., 2011). Recently, an alternative microbiota-dependent pathway involving the intermediate metabolite γ -butyrobetaine (γ BB) in the conversion of carnitine to TMAO has also been proposed. In that study, animals fed with γ BB exhibited increased atherosclerotic lesion formation (Koeth et al., 2014). Given the proposed proatherogenic properties of γ BB and its biochemical relationship with carnitine and TMAO, a group of researchers hypothesized that γ BB might be associated with the presence and complications of carotid atherosclerosis. Their findings indicated that patients with carotid atherosclerosis exhibited elevated levels of γ BB and its precursor, trimethyllysine, which were also associated with increased cardiovascular mortality (Skagen et al., 2016). A study investigating the oxidation of TMA by flavin monooxygenases demonstrated that FMO3 plays a predominant role in TMAO formation. Increased FMO3 expression elevated plasma TMAO levels, whereas FMO3 silencing reduced TMAO concentrations and was associated with differences in atherosclerosis susceptibility among mouse strains (Bennett et al., 2013).

Although numerous studies have shown a positive association between TMAO and cardiometabolic risk factors, particularly large population-based studies have reported inconsistencies in the association between circulating TMAO or its dietary precursors, choline and betaine, and increased cardiovascular risk (Cho et al., 2017; Janeiro, Ramírez, Milagro, Martínez, & Solas, 2018). It has been proposed that these relationships should be evaluated in a dose-dependent manner. One study reported a nonlinear, dose-dependent association between rising TMAO concentrations and heart failure mortality (Farhangi, 2020), a finding supported by additional investigations (Nagata et al., 2015; Skagen et al., 2016). Furthermore, some studies suggest that TMAO may function more as a potential disease biomarker rather than a direct pathogenic agent. Indeed, it has been proposed that TMAO accumulation in disease states could reflect a cellular adaptive response to stress (Barrea et al., 2019; Friedrich et al., 2015) (Table 1.) shows some important studies from the past five years related to TMAO

and cardiovascular processes. These inconsistencies may be explained by differences in renal function, dietary habits, gut microbiota composition, population heterogeneity, and methodological variability among studies. Therefore, the biological significance of elevated TMAO levels likely depends on the clinical and metabolic context in which they are evaluated.

TMAO and renal diseases

TMAO, a metabolite produced by gut microbiota, has garnered significant attention due to its association with various chronic diseases, including cardiovascular disease, type 2 diabetes, and particularly CKD. Elevated TMAO levels are commonly observed in CKD patients and are believed to contribute to disease progression. Recent studies have increasingly focused on elucidating the pathogenic role of TMAO in CKD and on exploring therapeutic strategies that target TMAO metabolism. This review outlines the link between TMAO and CKD and highlights advances in the development of TMAO-targeting agents as potential treatments for CKD (Zixin et al., 2022). Collectively, current evidence suggests a bidirectional relationship between TMAO and CKD, in which impaired renal clearance promotes TMAO accumulation, while elevated TMAO levels may further aggravate renal and cardiovascular pathology.

Plasma TMAO levels are elevated in patients with CKD and are associated with poorer long-term outcomes. Moreover, chronic dietary exposure leading to increased TMAO has been shown to directly promote renal fibrosis and dysfunction in animal models, further supporting its pathogenic role in CKD progression (Tang et al., 2015).

In a study investigating renal handling of TMAO, plasma TMAO levels were found to inversely correlate with measured glomerular filtration rate (mGFR), confirming their elevation in CKD. The findings indicated that TMAO is primarily eliminated via glomerular filtration, with minimal tubular secretion or reabsorption, and is efficiently cleared by hemodiafiltration (Pelletier et al., 2019).

In another study, a mouse model of CKD induced by adenine feeding, elevated plasma TMAO levels were associated with renal dysfunction and cardiac hypertrophy. Treatment with iodomethylcholine, a gut microbial TMA formation inhibitor, significantly reduced TMAO levels, improved renal injury markers, and prevented cardiac hypertrophy, suggesting that targeting TMAO production may offer therapeutic benefit in CKD and its cardiovascular complications (Zhang et al., 2021).

In a prospective cohort study of 5,469 individuals followed for a median of 8.3 years, higher plasma TMAO levels were associated with increased all-cause mortality. This association was particularly significant in individuals with impaired renal function (eGFR <90 mL/min/1.73 m²), suggesting that reduced kidney function modifies the mortality risk linked to elevated TMAO levels (Gruppen et al., 2017). Nevertheless, it remains unclear whether elevated TMAO levels directly contribute to CKD progression and mortality or primarily reflect impaired renal clearance and broader metabolic disturbances. Therefore, further mechanistic and longitudinal studies are needed to clarify the causal role of TMAO in CKD.

TMAO and ER stress

The endoplasmic reticulum (ER), a vital organelle of the cell, plays essential roles in protein folding, synthesis, translocation, and lipid metabolism (Stevens & Argon, 1999). Disruption of ER homeostasis can lead to misfolded or improperly synthesized proteins, thereby increasing ER stress (Baba, Caliskan, Boyuk, & Hacısevki, 2021). Studies have demonstrated a link between ER stress pathways and various conditions such as cardiovascular diseases, diabetes mellitus, obesity and neurodegenerative disorders (Baba et al., 2021; Minamino & Kitakaze, 2010; Oyadomari et al., 2002). Prolonged ER stress contributes to cardiomyocyte dysfunction and apoptosis, ultimately resulting in cardiovascular diseases. Understanding the role of ER stress in the pathophysiology of these diseases has spurred therapeutic research in this area, which continues to evolve (Wang et al., 2018).

TMAO is known to promote the progression of cardiovascular and metabolic disorders by activating ER stress signaling, particularly through its interaction with the ER stress sensor protein PERK (Chen et al., 2019). These observations suggest that activation of ER stress signaling may constitute one of the central molecular pathways through which TMAO contributes to cardiovascular and metabolic disorders. In a study, TMA produced by gut microbiota was shown to be oxidized into TMAO in non-liver tissues, where TMAO promoted inflammation by activating ER stress and trained immunity pathways via the PERK receptor. TMAO induced endothelial cell activation, metabolic reprogramming, and innate immune transdifferentiation, contributing to vascular inflammation. These findings highlight new therapeutic targets for cardiovascular diseases, chronic kidney disease-related complications, and other inflammatory conditions (Saaoud et al., 2023). In a study, researchers showed that dietary TMAO supplementation accelerates atherosclerosis in ApoE^{-/-} mice by upregulating the enzyme MBOAT2, which disrupts glycerophospholipid metabolism and induces endothelial cell pyroptosis through ER stress. This work highlights the role of TMAO-induced ER stress in cardiovascular disease progression (Yu et al., 2024). In another study using a murine model of flow-induced atherosclerosis, researchers investigated the effect of elevated TMAO on vascular injury and neointimal formation. Mice fed high-TMAO or high-choline diets showed increased atherosclerosis, inflammation, ER stress, and activation of NF- κ B and the NLRP3 inflammasome, while treatment with 3,3-Dimethyl-1-butanol (DMB) reduced TMAO levels and attenuated these effects. Experiments with human aortic smooth muscle cells confirmed that TMAO-induced inflammation is mediated by ER stress, highlighting TMAO's role in vascular remodeling and suggesting ER stress inhibition as a therapeutic strategy for atherosclerosis (Chen et al., 2023).

ER stress, triggered by elevated levels of TMAO, plays a critical role in the development of cardiovascular diseases (CVD) as well as other complications such as cognitive decline. For example, in a study, increased

TMAO levels were detected in both the plasma and brain of Alzheimer's (3 $\times$ Tg-AD) and insulin-resistant (db/db) mice compared to wild-type controls, with levels rising further with age. The study also demonstrated that TMAO impairs synaptic plasticity by activating the ER stress-mediated PERK signaling pathway, suggesting a potential mechanism underlying TMAO-induced cognitive decline (Govindarajulu et al., 2020). These findings further suggest that the biological effects of TMAO-induced ER stress may extend beyond the cardiovascular system and contribute to multisystem inflammatory and degenerative processes.

However, despite growing experimental evidence, the precise causal relationship between TMAO-induced ER stress and human cardiovascular disease progression remains to be fully clarified.

CONCLUSION

Growing evidence indicates that TMAO, a metabolite derived from gut microbiota, plays an important role in the development and progression of atherosclerosis. Through its effects on lipid metabolism, inflammatory signaling pathways, platelet activation, and endothelial dysfunction, TMAO has emerged as a potential contributor to cardiovascular pathology. Recent studies further suggest that TMAO may promote vascular injury and metabolic dysfunction through the activation of ER stress pathways, linking microbiota-derived metabolites to cellular stress responses involved in atherogenesis.

Despite the increasing number of studies supporting the association between circulating TMAO levels and cardiovascular risk, the causal role of TMAO in atherosclerosis remains a subject of ongoing debate. Some findings indicate that TMAO may function primarily as a biomarker reflecting alterations in gut microbiota composition, dietary patterns, or renal function rather than as a direct pathogenic factor. The biological significance of elevated TMAO levels likely depends on the interaction among renal function, dietary habits, gut microbiota composition, and overall cardiometabolic status.

Future research should therefore focus on clarifying the mechanistic pathways underlying TMAO-mediated cardiovascular effects and determining whether modulation of gut microbiota, dietary interventions, or pharmacological inhibition of TMAO production may represent effective strategies for reducing cardiovascular risk. In addition, further large-scale

longitudinal and mechanistic studies are needed to clarify whether TMAO represents a modifiable therapeutic target or primarily a biomarker associated with disease progression. A better understanding of the microbiota–TMAO–ER stress axis may provide new insights into the prevention and treatment of atherosclerotic cardiovascular diseases.

Table 1. Summary of studies from the last five years investigating the role of TMAO in cardiovascular and renal outcomes

Year	Author(s)	Population / Model	Key Findings		Notes
2025	Zhong et al., 2025	Aged rats (normal physiology), young rats with 5/6 nephrectomy (CKD model); HUVECs and vascular smooth muscle cells	TMAO induces vascular aging and exacerbates it in CKD model rats; antibiotic treatment reduces vascular aging by lowering TMAO; TMAO inhibits GATA4 degradation, activating NF-κB signaling and promoting cellular senescence	TMAO is a key contributor and potential therapeutic target in CKD-associated vascular aging	Blocking the GATA4 pathway attenuates TMAO-induced senescence a promising therapeutic approach
2025	Obeid et al., 2025	Patients from 27 SRs/meta-analyses covering stroke, CVD, and mortality outcomes	Most included studies reported higher TMAO levels in patients with CVD, stroke, or mortality; however, causality remains unproven due to poor methodological quality	TMAO may be a prognostic marker, but evidence is insufficient to confirm causal relationship	Serious methodological and reporting flaws in existing SRs; no prospective studies in healthy populations
2025	Cheng, Hung, & Lin, 2025	152 nondialysis CKD patients (stages 3–5); median follow-up: 3.3 years	Higher plasma TMAO levels independently associated with rapid eGFR decline; other metabolites (TMA, choline, carnitine) not associated	TMAO linked to accelerated kidney function loss in CKD	Distinct gut microbiota in rapid decliners; increased TMA-producing bacteria and enzymes (e.g., cutC, bbuA proteins)
2025	Zhang et al., 2025	Experimental (<i>in vivo/in vitro</i>) Male mice (APAP- or MCT-induced liver injury), human DILI samples	FMO3 expression is elevated in DILI; overexpression worsens liver injury by inducing ER stress via CREB3/P4HB axis. TMAO, as an FMO3 metabolite, triggers ER stress through the PERK pathway; inhibiting TMAO mitigates injury.	Indirect (via TMAO → ER stress)	Highlights a novel TMAO-mediated mechanism of drug-induced liver injury; ER stress as key pathway.
2024	Aleksova et al., 2024	149 patients with acute myocardial infarction (AMI)	Patients with persistently high or rising TMAO during hospitalization had >2x higher MACE risk over 30 months vs. those with decreasing/stable levels	Elevated in-hospital TMAO dynamics predict worse cardiovascular outcomes (MACE)	Independent predictors of MACE included age, poor LV function, and TMAO trajectory

2024	Zou et al., 2024	100 patients undergoing partial nephrectomy (PN)	TMAO, IS, and PCS levels increased post-PN; TMAO showed strongest and sustained rise; TMAO and reduced GFR independently associated with increased 10-year CV event risk (Framingham score)	TMAO is an independent predictor of CV risk post-partial nephrectomy	RPVP loss (not ischemia) predicted elevated post-PN TMAO; TMAO remained elevated up to 5 years
2024	Obeid et al., 2024	478 CKD patients (stages G2–G4), mean follow-up: 5.1 years	TMAO levels were higher in CKD and in patients with existing CVD, but not independently predictive of CVD or renal outcomes after adjustment	TMAO elevated due to lower GFR; not an independent predictor of CVD or kidney outcome in CKD	Betaine (not TMAO) was associated with worse cardiovascular and renal outcomes; challenges role of TMAO as therapeutic target
2024	Wiese et al., 2024	Controlled feeding study (secondary analysis). 8 adults with moderate CKD matched with 8 non-CKD controls (age, sex, race); 6-day diet	Serum TMAO, IS, PCS levels 2.8–4.9× higher in CKD; no major differences in gut microbiota composition; only urine TMAO significantly higher in CKD	Elevated TMAO in CKD linked to reduced eGFR; independent of microbiota diversity	Uniform diet controlled confounding suggests microbiota-independent accumulation of TMAO and other URS in CKD
2024	Shea et al., 2024	Prospective cohort (CARDIA) 3444 adults (Black and White, aged 33–45), free of CVD at baseline; 19-year follow-up; U.S. population-based cohort	Plasma choline positively associated with incident CVD; TMAO and betaine not associated overall. However, at lower eGFR, TMAO positively and betaine negatively associated with CVD	Kidney function modifies the relationship: low eGFR enhances TMAO-CVD link and reverses betaine's effect	Long-term data; adds nuance on choline metabolism and renal modulation of CVD risk
2023	Yuan et al., 2023	Retrospective observational study. 228 maintenance hemodialysis patients	TMAO levels significantly higher in malnourished patients; independent risk factor for malnutrition (OR = 2.07); TMAO predicts major CV events over 2 years (AUC = 0.68–0.74)	TMAO levels significantly higher in malnourished patients; independent risk factor for malnutrition (OR = 2.07); TMAO predicts major CV events over 2 years (AUC = 0.68–0.74)	TMAO levels significantly higher in malnourished patients; independent risk factor for malnutrition (OR = 2.07); TMAO predicts major CV events over 2 years (AUC = 0.68–0.74)
2023	Huang, Zhu, Huang, & Zhou, 2023	108 T2DM patients (DKD and non-DKD) + 33 healthy controls	TMAO significantly higher in DKD group; negatively correlated with eGFR, positively with ACR and urea; independent risk factor for DKD (AUC = 0.691)	TMAO significantly higher in DKD group; negatively correlated with eGFR, positively with ACR and urea; independent risk factor for DKD (AUC = 0.691)	TMAO significantly higher in DKD group; negatively correlated with eGFR, positively with ACR and urea; independent risk factor for DKD (AUC = 0.691)
2023	Korytowska-Przybylska, Michorowska, Wyczalkowska-Tomasik, Pączek, & Giebułtowicz, 2023	CKD patients	Strong correlation between serum and saliva levels of TMAO ($r = 0.81$, $p = 0.008$) and creatinine ($r = 0.72$, $p = 0.029$); saliva valid for TMAO detection	Strong correlation between serum and saliva levels of TMAO ($r = 0.81$, $p = 0.008$) and creatinine ($r = 0.72$, $p = 0.029$); saliva valid for TMAO detection	Strong correlation between serum and saliva levels of TMAO ($r = 0.81$, $p = 0.008$) and creatinine ($r = 0.72$, $p = 0.029$); saliva valid for TMAO detection

2022	Amrein et al., 2022	1726 patients with suspected fCAD	TMAO and its precursors were higher in fCAD patients, but diagnostic accuracy was low (AUC=0.56). TMAO was a strong independent predictor of death and CVD over 5 years, even after adjusting for renal function. The prognostic accuracy was robust (AUC=0.73 for CVD at 2 years).	Strong (CV mortality & events)	Demonstrates prognostic rather than diagnostic utility of TMAO and its precursors in coronary artery disease.
2022	Dai et al., 2022	737 elderly patients (≥ 65 y) with advanced CKD (eGFR ≤ 20 mL/min/1.73 m ² in last 6 months), EQUAL Study	Higher TMAO independently predicted increased all-cause mortality (HR: 1.46), while higher CMPF (fish biomarker) was associated with lower all-cause mortality (HR: 0.79) and lower KRT risk (HR: 0.80). Patients with low TMAO and high CMPF had significantly reduced mortality risk (HR: 0.49). High CMPF seemed to attenuate the negative effect of TMAO.	Strong (mortality in CKD)	CMPF may indicate protective effect of fish intake It highlights to potential dietary modulation of TMAO-related risk. Further causality needs investigation.
2022	Zhang et al., 2022	Human (PD cohort), <i>in vivo</i> (CKD rats), and <i>in vitro</i> study Peritoneal dialysis (PD) patients (28.3 $\pm$ 8.0 months follow-up), CKD rat model, and primary peritoneal mesothelial cells	Higher serum TMAO levels (≥ 50 μ M) in PD patients were associated with increased CRP and dialysate IL-6 and PAI-1. High TMAO significantly raised risk of new-onset peritonitis (HR: 3.60). In CKD rats, TMAO exacerbated peritoneal inflammation. <i>In vitro</i> , TMAO induced mesothelial cell necrosis and increased production of inflammatory cytokines (CCL2, TNF- α , IL-6, IL-1 β) and adhesion molecules.	CKD (PD-related inflammation)	TMAO may directly contribute to peritonitis in PD patients via mesothelial necrosis and cytokine induction. Suggests local inflammatory risk beyond systemic CV effects.
2022	Xie et al., 2022	<i>In vivo</i> (mouse model) CD1 mice with 5/6 nephrectomy to induce CKD + choline diet	CKD increased TMAO; choline diet further elevated TMAO and worsened cardiac dysfunction. Medicinal charcoal (TMAO-lowering) improved heart function. TMAO inhibited Hif-1 α and reduced cardiac capillary density. FG-4592 (Hif-1 α stabilizer) reversed these effects. TMAO thus impairs cardiac angiogenesis by suppressing Hif-1 α , aggravating CKD-related heart damage.	Both CKD and CV (via angiogenesis)	Demonstrates mechanistic link between gut-derived TMAO, impaired Hif-1 α signaling, and worsened cardiac outcomes in CKD. Supports targeting TMAO in therapy.

2021	Zhang et al., 2021	<i>In vivo</i> (mouse model) Female ApoE KO mice, 0.2% adenine diet for CKD ± IMC treatment	Adenine diet induced CKD and elevated TMAO; IMC (TMA formation inhibitor) reduced renal injury markers, fibrosis, and cardiac hypertrophy. IMC had no effect on atherosclerosis but mitigated kidney and cardiac complications. Surprisingly, CKD mice had reduced aortic inflammation unrelated to TMAO. Overall, lowering TMAO via IMC may protect against CKD and cardiovascular outcomes, especially myocardial hypertrophy.	Both CKD and CV (via heart hypertrophy)	Suggests gut-microbiota-targeted TMAO suppression (IMC) may offer dual protection against renal fibrosis and cardiac remodeling in CKD context.
2021	Winther et al., 2021	Prospective cohort 311 T2D patients with albuminuria	TMAO pathway metabolites (TMAO, choline, carnitine, betaine) were not associated with all-cause mortality or CVD. However, higher choline, carnitine, and the combined metabolite score were significantly associated with ≥30% eGFR decline, indicating renal deterioration risk.	CKD only (not CVD)	Suggests that TMAO pathway metabolites may be early renal risk markers but not independent CV risk factors.
2021	Yang & Zhang, 2021	<i>In vitro</i> study MPC-83 pancreatic acinar cells	TMAO decreased cell viability, induced apoptosis, and increased oxidative stress markers in a dose-dependent manner. TMAO activated IRE1α/XBP-1 ER stress pathway, linking it to cellular dysfunction.	No direct link (pancreas)	Suggests a role of TMAO in hyperlipidemic acute pancreatitis via oxidative and ER stress; potential diagnostic and therapeutic target
2021	Boini, Singh, & Koka, 2021	<i>In vitro</i> study Endothelial cells	TMAO (30μM) induced ER stress via PERK-eIF2α-ATF4 pathway, increased CHOP, IRE1α, and mitochondrial ROS, caused loss of mitochondrial membrane potential and increased endothelial permeability. PERK inhibition reversed these effects.	CV risk	Demonstrates that TMAO contributes to endothelial dysfunction—a key factor in CVD—through PERK-mediated ER stress. Suggests PERK inhibitors as a potential therapeutic strategy.

AUTHOR CONTRIBUTION STATEMENT

Literature screening, investigation, visualization and writing—original draft (ZR), concept, design and writing—review & editing (AH).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Aleksova, A., Fluca, A. L., Stornaiuolo, M., Barbati, G., Pierri, A., Zwas, D. R., ... Janjusevic, M. (2024). Intra-hospital variation of gut microbiota product, trimethylamine N-oxide (TMAO), predicts future major adverse cardiovascular events after myocardial infarction. *Hellenic Journal of Cardiology*, 86, 4-16. <https://doi.org/10.1016/j.hjc.2024.05.007>

1123

- Amrein, M., Li, X. S., Walter, J., Wang, Z., Zimmermann, T., Strebel, I., ... Mueller, C. (2022). Gut microbiota-dependent metabolite trimethylamine N-oxide (TMAO) and cardiovascular risk in patients with suspected functionally relevant coronary artery disease (fCAD). *Clinical Research in Cardiology*, 111(6), 692–704. <https://doi.org/10.1007/s00392-022-01992-6>
- Baba, B., Caliskan, M., Boyuk, G., & Hacisevki, A. (2021). Chemical Chaperone PBA Attenuates ER Stress and Upregulates SOCS3 Expression as a Regulator of Leptin Signaling. *Biochemistry (Moscow)*, 86(4), 480–488. <https://doi.org/10.1134/S0006297921040088>
- Bain, M. A., Faull, R., Fornasini, G., Milne, R. W., & Evans, A. M. (2006). Accumulation of trimethylamine and trimethylamine-N-oxide in end-stage renal disease patients undergoing haemodialysis. *Nephrology Dialysis Transplantation*, 21(5), 1300–1304. <https://doi.org/10.1093/ndt/gfk056>
- Barrea, L., Annunziata, G., Muscogiuri, G., Di Somma, C., Laudisio, D., Maisto, M., ... Savastano, S. (2018). Trimethylamine-N-oxide (TMAO) as Novel Potential Biomarker of Early Predictors of Metabolic Syndrome. *Nutrients*, 10(12), 1971. <https://doi.org/10.3390/nu10121971>
- Barrea, L., Annunziata, G., Muscogiuri, G., Laudisio, D., Di Somma, C., Maisto, M., ... Savastano, S. (2019). Trimethylamine N-oxide, Mediterranean diet, and nutrition in healthy, normal-weight adults: also a matter of sex? *Nutrition*, 62, 7–17. <https://doi.org/10.1016/j.nut.2018.11.015>
- Bennett, B. J., Vallim, T. Q. de A., Wang, Z., Shih, D. M., Meng, Y., Gregory, J., ... Lusis, A. J. (2013). Trimethylamine-N-Oxide, a Metabolite Associated with Atherosclerosis, Exhibits Complex Genetic and Dietary Regulation. *Cell Metabolism*, 17(1), 49–60. <https://doi.org/10.1016/j.cmet.2012.12.011>
- Bogiatzi, C., Gloor, G., Allen-Vercoe, E., Reid, G., Wong, R. G., Urquhart, B. L., ... Spence, J. D. (2018). Metabolic products of the intestinal microbiome and extremes of atherosclerosis. *Atherosclerosis*, 273, 91–97. <https://doi.org/10.1016/j.atherosclerosis.2018.04.015>
- Boini, K. M., Singh, A., & Koka, S. S. (2021). Abstract 14071: Gut Microbial Metabolite Trimethylamine N-oxide Enhances Endoplasmic Reticular Stress and Promotes Endothelial Dysfunction. *Circulation*, 144(Suppl_1). https://doi.org/10.1161/circ.144.suppl_1.14071
- Bozkurt, B., Kribbs, S. B., Clubb, F. J., Michael, L. H., Didenko, V. V., Hornsby, P. J., ... Mann, D. L. (1998). Pathophysiologically Relevant Concentrations of Tumor Necrosis Factor- α Promote Progressive Left Ventricular Dysfunction and Remodeling in Rats. *Circulation*, 97(14), 1382–1391. <https://doi.org/10.1161/01.CIR.97.14.1382>
- Brial, F., Le Lay, A., Dumas, M.-E., & Gauguier, D. (2018). Implication of gut microbiota metabolites in cardiovascular and metabolic diseases. *Cellular and Molecular Life Sciences*, 75(21), 3977–3990. <https://doi.org/10.1007/s00018-018-2901-1>
- Brown, J. M., & Hazen, S. L. (2014). Metaorganismal nutrient metabolism as a basis of cardiovascular disease. *Current Opinion in Lipidology*, 25(1), 48–53. <https://doi.org/10.1097/MOL.0000000000000036>
- Brown, J. M., & Hazen, S. L. (2015). The Gut Microbial Endocrine Organ: Bacterially Derived Signals Driving Cardiometabolic Diseases. *Annual Review of Medicine*, 66(1), 343–359. <https://doi.org/10.1146/annurev-med-060513-093205>
- Canyelles, M., Borràs, C., Rotllan, N., Tondo, M., Escalà-Gil, J. C., & Blanco-Vaca, F. (2023). Gut Microbiota-Derived TMAO: A Causal Factor Promoting Atherosclerotic Cardiovascular Disease? *International Journal of Molecular Sciences*, 24(3), 1940. <https://doi.org/10.3390/ijms24031940>
- Chen, S., Henderson, A., Petriello, M. C., Romano, K. A., Gearing, M., Miao, J., ... Biddinger, S. B. (2019). Trimethylamine N-Oxide Binds and Activates PERK to Promote Metabolic Dysfunction. *Cell Metabolism*, 30(6), 1141–1151.e5. <https://doi.org/10.1016/j.cmet.2019.08.021>

- Chen, C.-Y., Leu, H.-B., Wang, S.-C., Tsai, S.-H., Chou, R.-H., Lu, Y.-W., ... Lin, S.-J. (2023). Inhibition of Trimethylamine N-Oxide Attenuates Neointimal Formation Through Reduction of Inflammasome and Oxidative Stress in a Mouse Model of Carotid Artery Ligation. *Antioxidants & Redox Signaling*, 38(1-3), 215-233. <https://doi.org/10.1089/ars.2021.0115>
- Cheng, E., Hung, S.-C., & Lin, T.-Y. (2025). Association of trimethylamine N-oxide and metabolites with kidney function decline in patients with chronic kidney disease. *Clinical Nutrition*, 44, 239-247.
- Cho, C. E., Taesuwan, S., Malysheva, O. V., Bender, E., Tulchinsky, N. F., Yan, J., ... Caudill, M. A. (2017). Trimethylamine- N -oxide (TMAO) response to animal source foods varies among healthy young men and is influenced by their gut microbiota composition: A randomized controlled trial. *Molecular Nutrition & Food Research*, 61(1). <https://doi.org/10.1002/mnfr.201600324>
- Coope, A., Milanski, M., Arruda, A. P., Ignacio-Souza, L. M., Saad, M. J., Anhê, G. F., & Velloso, L. A. (2012). Chaperone Insufficiency Links TLR4 Protein Signaling to Endoplasmic Reticulum Stress. *Journal of Biological Chemistry*, 287(19), 15580-15589. <https://doi.org/10.1074/jbc.M111.315218>
- Dai, L., Massy, Z. A., Stenvinkel, P., Chesnaye, N. C., Larabi, I. A., Alvarez, J. C., ... Evans, M. (2022). The association between TMAO, CMPE, and clinical outcomes in advanced chronic kidney disease: results from the European QUALity (EQUAL) Study. *The American Journal of Clinical Nutrition*, 116(6), 1842-1851. <https://doi.org/10.1093/ajcn/nqac278>
- Desai, D., Desai, A., Jamil, A., Csendes, D., Gutlapalli, S. D., Prakash, K., ... Khan, S. (2023). Re-defining the Gut Heart Axis: A Systematic Review of the Literature on the Role of Gut Microbial Dysbiosis in Patients With Heart Failure. *Cureus*, 15(2): e34902. <https://doi.org/10.7759/cureus.34902>
- Din, A. U., Hassan, A., Zhu, Y., Yin, T., Gregersen, H., & Wang, G. (2019). Amelioration of TMAO through probiotics and its potential role in atherosclerosis. *Applied Microbiology and Biotechnology*, 103(23-24), 9217-9228. <https://doi.org/10.1007/s00253-019-10142-4>
- Farhangi, M. A. (2020). Gut microbiota-dependent trimethylamine N-oxide and all-cause mortality: Findings from an updated systematic review and meta-analysis. *Nutrition*, 78, 110856. <https://doi.org/10.1016/j.nut.2020.110856>
- Feng, B., Yao, P. M., Li, Y., Devlin, C. M., Zhang, D., Harding, H. P., ... Tabas, I. (2003). The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. *Nature Cell Biology*, 5(9), 781-792. <https://doi.org/10.1038/ncb1035>
- Friedrich, N., Budde, K., Suhre, K., Völker, U., John, U., Felix, S. B., ... Wallaschofski, H. (2015). Sex differences in urine metabolites related with risk of diabetes using NMR spectroscopy: results of the study of health in pomerania. *Metabolomics*, 11(5), 1405-1415. <https://doi.org/10.1007/s11306-015-0795-6>
- Fu, B. C., Hullar, M. A., Randolph, T. W., Franke, A. A., Monroe, K. R., Cheng, I., ... Lampe, J. W. (2020). Associations of plasma trimethylamine N-oxide, choline, carnitine, and betaine with inflammatory and cardiometabolic risk biomarkers and the fecal microbiome in the Multiethnic Cohort Adiposity Phenotype Study. *The American Journal of Clinical Nutrition*, 111(6), 1226-1234. <https://doi.org/10.1093/ajcn/nqaa015>
- Govindarajulu, M., Pinky, P. D., Steinke, I., Bloemer, J., Ramesh, S., Kariharan, T., ... Amin, R. H. (2020). Gut Metabolite TMAO Induces Synaptic Plasticity Deficits by Promoting Endoplasmic Reticulum Stress. *Frontiers in Molecular Neuroscience*, 13. <https://doi.org/10.3389/fnmol.2020.00138>

- Gregory, J. C., Buffa, J. A., Org, E., Wang, Z., Levison, B. S., Zhu, W., ... Hazen, S. L. (2015). Transmission of Atherosclerosis Susceptibility with Gut Microbial Transplantation *. *Journal of Biological Chemistry*, 290(9), 5647–5660. <https://doi.org/10.1074/jbc.M114.618249>
- Gruppen, E. G., Garcia, E., Connelly, M. A., Jeyarajah, E. J., Otvos, J. D., Bakker, S. J. L., & Dullaart, R. P. F. (2017). TMAO is Associated with Mortality: Impact of Modestly Impaired Renal Function. *Scientific Reports*, 7(1), 13781. <https://doi.org/10.1038/s41598-017-13739-9>
- Guasti, L., Galliazzo, S., Molaro, M., Visconti, E., Pennella, B., Gaudio, G. V., ... Squizzato, A. (2021). TMAO as a biomarker of cardiovascular events: a systematic review and meta-analysis. *Internal and Emergency Medicine*, 16(1), 201–207. <https://doi.org/10.1007/s11739-020-02470-5>
- Hayashi, T., Yamashita, T., Watanabe, H., Kami, K., Yoshida, N., Tabata, T., ... Hirata, K. (2018). Gut Microbiome and Plasma Microbiome-Related Metabolites in Patients With Decompensated and Compensated Heart Failure. *Circulation Journal*, 83(1), 182–192. <https://doi.org/10.1253/circj.CJ-18-0468>
- Hotamisligil, G. S. (2010). Endoplasmic reticulum stress and atherosclerosis. *Nature Medicine*, 16(4), 396–399. <https://doi.org/10.1038/nm0410-396>
- Huang, Y., Zhu, Z., Huang, Z., & Zhou, J. (2023). Elevated serum trimethylamine oxide levels as potential biomarker for diabetic kidney disease. *Endocrine Connections*, 12(8). <https://doi.org/10.1530/EC-22-0542>
- Janeiro, M., Ramírez, M., Milagro, F., Martínez, J., & Solas, M. (2018). Implication of Trimethylamine N-Oxide (TMAO) in Disease: Potential Biomarker or New Therapeutic Target. *Nutrients*, 10(10), 1398. <https://doi.org/10.3390/nu10101398>
- Jarmukhanov, Z., Mukhanbetzhanov, N., Kozhakhmetov, S., Nurgaziyev, M., Sailybayeva, A., Bekbossynova, M., & Kushugulova, A. (2024). The association between the gut microbiota metabolite trimethylamine N-oxide and heart failure. *Frontiers in Microbiology*, 15. <https://doi.org/10.3389/fmicb.2024.1440241>
- Katsimichas, T., Antonopoulos, A. S., Katsimichas, A., Ohtani, T., Sakata, Y., & Tousoulis, D. (2019). The intestinal microbiota and cardiovascular disease. *Cardiovascular Research*, 115(10), 1471–1486. <https://doi.org/10.1093/cvr/cvz135>
- Koeth, R. A., Wang, Z., Levison, B. S., Buffa, J. A., Org, E., Sheehy, B. T., ... Li, L. (2013). Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nature Medicine*, 19(5), 576–585.
- Koeth, R. A., Levison, B. S., Culley, M. K., Buffa, J. A., Wang, Z., Gregory, J. C., ... Hazen, S. L. (2014). Butyrobetaine Is a Proatherogenic Intermediate in Gut Microbial Metabolism of L-Carnitine to TMAO. *Cell Metabolism*, 20(5), 799–812. <https://doi.org/10.1016/j.cmet.2014.10.006>
- Kong, W., Ma, J., Lin, Y., & Chen, W. (2022). Positive Association of Plasma Trimethylamine-N-Oxide and Atherosclerosis in Patient with Acute Coronary Syndrome. *Cardiovascular Therapeutics*, 2022, 1–9. <https://doi.org/10.1155/2022/2484018>
- Korytowska-Przybylska, N., Michorowska, S., Wyczalkowska-Tomasik, A., Pączek, L., & Giebułtowicz, J. (2023). Development of a novel method for the simultaneous detection of trimethylamine N-oxide and creatinine in the saliva of patients with chronic kidney disease – Its utility in saliva as an alternative to blood. *Journal of Pharmaceutical and Biomedical Analysis*, 234, 115519. <https://doi.org/10.1016/j.jpba.2023.115519>
- Li, D. Y., & Tang, W. H. W. (2017). Gut Microbiota and Atherosclerosis. *Current Atherosclerosis Reports*, 19(10), 39. <https://doi.org/10.1007/s11883-017-0675-9>

- Li, T., Gua, C., Wu, B., & Chen, Y. (2018). Increased circulating trimethylamine N-oxide contributes to endothelial dysfunction in a rat model of chronic kidney disease. *Biochemical and Biophysical Research Communications*, 495(2), 2071–2077. <https://doi.org/10.1016/j.bbrc.2017.12.069>
- Liabeuf, S., Cheddani, L., & Massy, Z. (2018). Uremic Toxins and Clinical Outcomes: The Impact of Kidney Transplantation. *Toxins*, 10(6), 229. <https://doi.org/10.3390/toxins10060229>
- Loscalzo, J. (2011). Lipid Metabolism by Gut Microbes and Atherosclerosis. *Circulation Research*, 109(2), 127–129. <https://doi.org/10.1161/RES.0b013e3182290620>
- Lu, Y.-C., Yeh, W.-C., & Ohashi, P. S. (2008). LPS/TLR4 signal transduction pathway. *Cytokine*, 42(2), 145–151. <https://doi.org/10.1016/j.cyto.2008.01.006>
- Miao, J., Ling, A. V., Manthena, P. V., Gearing, M. E., Graham, M. J., Crooke, R. M., ... Biddinger, S. B. (2015). Flavin-containing monooxygenase 3 as a potential player in diabetes-associated atherosclerosis. *Nature Communications*, 6(1), 6498. <https://doi.org/10.1038/ncomms7498>
- Minamino, T., & Kitakaze, M. (2010). ER stress in cardiovascular disease. *Journal of Molecular and Cellular Cardiology*, 48(6), 1105–1110. <https://doi.org/10.1016/j.yjmcc.2009.10.026>
- Mohammadi, A., Vahabzadeh, Z., Jamalzadeh, S., & Khalili, T. (2018). Trimethylamine-N-oxide, as a risk factor for atherosclerosis, induces stress in J774A.1 murine macrophages. *Advances in Medical Sciences*, 63(1), 57–63. <https://doi.org/10.1016/j.advms.2017.06.006>
- Myoishi, M., Hao, H., Minamino, T., Watanabe, K., Nishihira, K., Hatakeyama, K., ... Kitakaze, M. (2007). Increased Endoplasmic Reticulum Stress in Atherosclerotic Plaques Associated With Acute Coronary Syndrome. *Circulation*, 116(11), 1226–1233. <https://doi.org/10.1161/CIRCULATIONAHA.106.682054>
- Nagata, C., Wada, K., Tamura, T., Konishi, K., Kawachi, T., Tsuji, M., & Nakamura, K. (2015). Choline and Betaine Intakes Are Not Associated with Cardiovascular Disease Mortality Risk in Japanese Men and Women. *The Journal of Nutrition*, 145(8), 1787–1792. <https://doi.org/10.3945/jn.114.209296>
- Nie, J., Xie, L., Zhao, B., Li, Y., Qiu, B., Zhu, F., ... Qin, X. (2018). Serum Trimethylamine N-Oxide Concentration Is Positively Associated With First Stroke in Hypertensive Patients. *Stroke*, 49(9), 2021–2028. <https://doi.org/10.1161/STROKEAHA.118.021997>
- Obeid, R., Awwad, H., Heine, G. H., Emrich, I. E., Fliser, D., Zawada, A. M., & Geisel, J. (2024). Plasma Concentrations of Trimethylamine-N-Oxide, Choline, and Betaine in Patients With Moderate to Advanced Chronic Kidney Disease and Their Relation to Cardiovascular and Renal Outcomes. *Journal of Renal Nutrition*, 34(6), 530–538. <https://doi.org/10.1053/j.jrn.2024.03.009>
- Obeid, R., Mohr, L., White, B. A., Heine, G. H., Emrich, I., Geisel, J., & Carter, R. C. (2025). Circulating trimethylamine N-oxide and cardiovascular, cerebral, and renal diseases including mortality: Umbrella review of published systematic reviews and meta-analyses. *Nutrition, Metabolism and Cardiovascular Diseases*, 35(8), 103908. <https://doi.org/10.1016/j.numecd.2025.103908>
- Oktaviono, Y. H., Dyah Lamara, A., Saputra, P. B. T., Arnindita, J. N., Pasahari, D., Saputra, M. E., & Suasti, N. M. A. (2023). Roles of Trimethylamine-N-oxide in Atherosclerosis and Its Potential Therapeutic Aspect: A Literature Review. *Biomolecules and Biomedicine*, 23(6):936-948. <https://doi.org/10.17305/bb.2023.8893>
- Oyadomari, S., Koizumi, A., Takeda, K., Gotoh, T., Akira, S., Araki, E., & Mori, M. (2002). Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. *Journal of Clinical Investigation*, 109(4), 525–532. <https://doi.org/10.1172/JCI14550>

- Pelletier, C. C., Croyal, M., Ene, L., Aguesse, A., Billion-Crossouard, S., Krempf, M., ... Soulage, C. O. (2019). Elevation of Trimethylamine-N-Oxide in Chronic Kidney Disease: Contribution of Decreased Glomerular Filtration Rate. *Toxins*, 11(11), 635. <https://doi.org/10.3390/toxins11110635>
- Ren, Y., Wang, Z., & Xue, J. (2022). [Gut-derived uremic toxin trimethylamine-N-oxide in cardiovascular disease under end-stage renal disease: an injury mechanism and therapeutic target]. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi = Journal of Biomedical Engineering = Shengwu Yixue Gongchengxue Zazhi*, 39(4), 848–852. <https://doi.org/10.7507/1001-5515.202110017>
- Saaoud, F., Liu, L., Xu, K., Cueto, R., Shao, Y., Lu, Y., ... Yang, X. (2023). Aorta- and liver-generated TMAO enhances trained immunity for increased inflammation via ER stress/mitochondrial ROS/glycolysis pathways. *JCI Insight*, 8(1). <https://doi.org/10.1172/jci.insight.158183>
- Shea, J. W., Jacobs, D. R., Howard, A. G., Lulla, A., Lloyd-Jones, D. M., Murthy, V. L., ... Meyer, K. A. (2024). Choline metabolites and incident cardiovascular disease in a prospective cohort of adults: Coronary Artery Risk Development in Young Adults (CARDIA) Study. *The American Journal of Clinical Nutrition*, 119(1), 29–38. <https://doi.org/10.1016/j.ajcnut.2023.10.012>
- Shih, D. M., Wang, Z., Lee, R., Meng, Y., Che, N., Charugundla, S., ... Lusi, A. J. (2015). Flavin containing monooxygenase 3 exerts broad effects on glucose and lipid metabolism and atherosclerosis [S]. *Journal of Lipid Research*, 56(1), 22–37. <https://doi.org/10.1194/jlr.M051680>
- Skagen, K., Trøseid, M., Ueland, T., Holm, S., Abbas, A., Gregersen, I., ... Skjelland, M. (2016). The Carnitine-butyrobetaine-trimethylamine-N-oxide pathway and its association with cardiovascular mortality in patients with carotid atherosclerosis. *Atherosclerosis*, 247, 64–69. <https://doi.org/10.1016/j.atherosclerosis.2016.01.033>
- Stefania, K., Ashok, K. K., Geena, P. V., Katarina, P., & Isak, D. (2024). TMAO enhances TNF- α mediated fibrosis and release of inflammatory mediators from renal fibroblasts. *Scientific Reports*, 14(1), 9070. <https://doi.org/10.1038/s41598-024-58084-w>
- Stevens, F. J., & Argon, Y. (1999). Protein folding in the ER. *Seminars in Cell & Developmental Biology*, 10(5), 443–454. <https://doi.org/10.1006/scdb.1999.0315>
- Suzuki, T., Heaney, L. M., Bhandari, S. S., Jones, D. J. L., & Ng, L. L. (2016). Trimethylamine N -oxide and prognosis in acute heart failure. *Heart*, 102(11), 841–848. <https://doi.org/10.1136/heart-jnl-2015-308826>
- Tang, W. H. W., Wang, Z., Levison, B. S., Koeth, R. A., Britt, E. B., Fu, X., ... Hazen, S. L. (2013). Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk. *New England Journal of Medicine*, 368(17), 1575–1584. <https://doi.org/10.1056/NEJMoa1109400>
- Tang, W. H. W., Wang, Z., Fan, Y., Levison, B., Hazen, J. E., Donahue, L. M., ... Hazen, S. L. (2014). Prognostic Value of Elevated Levels of Intestinal Microbe-Generated Metabolite Trimethylamine-N-Oxide in Patients With Heart Failure. *Journal of the American College of Cardiology*, 64(18), 1908–1914. <https://doi.org/10.1016/j.jacc.2014.02.617>
- Tang, W. H. W., Wang, Z., Kennedy, D. J., Wu, Y., Bufo, J. A., Agatista-Boyle, B., ... Hazen, S. L. (2015). Gut Microbiota-Dependent Trimethylamine N -Oxide (TMAO) Pathway Contributes to Both Development of Renal Insufficiency and Mortality Risk in Chronic Kidney Disease. *Circulation Research*, 116(3), 448–455. <https://doi.org/10.1161/CIRCRESAHA.116.305360>
- Tang, W. H. W., Li, D. Y., & Hazen, S. L. (2019). Dietary metabolism, the gut microbiome, and heart failure. *Nature Reviews Cardiology*, 16(3), 137–154. <https://doi.org/10.1038/s41569-018-0108-7>

- Thomas, M. S., & Fernandez, M. L. (2021). Trimethylamine N-Oxide (TMAO), Diet and Cardiovascular Disease. *Current Atherosclerosis Reports*, 23(4), 12. <https://doi.org/10.1007/s11883-021-00910-x>
- Trøseid, M., Andersen, G. Ø., Broch, K., & Hov, J. R. (2020). The gut microbiome in coronary artery disease and heart failure: Current knowledge and future directions. *EBioMedicine*, 52. <https://doi.org/10.1016/j.ebiom.2020.102649>
- Wang, B., Qiu, J., Lian, J., Yang, X., & Zhou, J. (2021). Gut Metabolite Trimethylamine-N-Oxide in Atherosclerosis: From Mechanism to Therapy. *Frontiers in Cardiovascular Medicine*, 8. <https://doi.org/10.3389/fcvm.2021.723886>
- Wang, S., Binder, P., Fang, Q., Wang, Z., Xiao, W., Liu, W., & Wang, X. (2018). Endoplasmic reticulum stress in the heart: insights into mechanisms and drug targets. *British Journal of Pharmacology*, 175(8), 1293–1304. <https://doi.org/10.1111/bph.13888>
- Wang, Z., Klipfell, E., Bennett, B. J., Koeth, R., Levinson, B. S., DuGar, B., ... Hazen, S. L. (2011). Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*, 472(7341), 57–63. <https://doi.org/10.1038/nature09922>
- Wang, Z., Tang, W. H. W., Buffa, J. A., Fu, X., Britt, E. B., Koeth, R. A., ... Hazen, S. L. (2014). Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-N-oxide. *European Heart Journal*, 35(14), 904–910. <https://doi.org/10.1093/eurheartj/ehu002>
- Warrier, M., Shih, D. M., Burrows, A. C., Ferguson, D., Gromovsky, A. D., Brown, A. L., ... Brown, J. M. (2015). The TMAO-Generating Enzyme Flavin Monooxygenase 3 Is a Central Regulator of Cholesterol Balance. *Cell Reports*, 10(3), 326–338. <https://doi.org/10.1016/j.celrep.2014.12.036>
- Wiese, G. N., Biruete, A., Stremke, E. R., Lindemann, S. R., Jannasch, A., Moorthi, R. N., ... Hill Gallant, K. M. (2024). Gut Microbiota and Uremic Retention Solutes in Adults With Moderate CKD: A 6-Day Controlled Feeding Study. *Journal of Renal Nutrition*, 34(1), 26–34. <https://doi.org/10.1053/j.jrn.2023.06.011>
- Winther, S. A., Øllgaard, J. C., Tofte, N., Tarnow, L., Wang, Z., Ahluwalia, T. S., ... Rossing, P. (2019). Utility of Plasma Concentration of Trimethylamine N-Oxide in Predicting Cardiovascular and Renal Complications in Individuals With Type 1 Diabetes. *Diabetes Care*, 42(8), 1512–1520. <https://doi.org/10.2337/dc19-0048>
- Winther, S. A., Øllgaard, J. C., Hansen, T. W., von Scholten, B. J., Reinhard, H., Ahluwalia, T. S., ... Rossing, P. (2021). Plasma trimethylamine N-oxide and its metabolic precursors and risk of mortality, cardiovascular and renal disease in individuals with type 2-diabetes and albuminuria. *PLOS ONE*, 16(3), e0244402. <https://doi.org/10.1371/journal.pone.0244402>
- Xie, F., Zhen, X., Liu, Z., Chen, X., Liu, Z., Zhou, M., ... Nie, J. (2022). Dietary choline, via gut microbe-generated trimethylamine-N-oxide, aggravates chronic kidney disease-induced cardiac dysfunction by inhibiting hypoxia-induced factor 1α. *Frontiers in Physiology*, 13. <https://doi.org/10.3389/fphys.2022.996166>
- Yang, G., & Zhang, X. (2021). TMAO promotes apoptosis and oxidative stress of pancreatic acinar cells by mediating IRE1α-XBP-1 pathway. *Saudi Journal of Gastroenterology*, 27(6), 361–369. https://doi.org/10.4103/sjg.sjg_12_21
- Yu, B., Yuan, C., Chen, J., Zhou, Z., Zhang, Y., Su, M., ... Wu, P. (2024). TMAO induces pyroptosis of vascular endothelial cells and atherosclerosis in ApoE–/– mice via MBOAT2-mediated endoplasmic reticulum stress. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, 1869(8), 159559. <https://doi.org/10.1016/j.bbalip.2024.159559>

- Yu, D., Shu, X., Rivera, E. S., Zhang, X., Cai, Q., Calcutt, M. W., ... Zheng, W. (2019). Urinary Levels of Trimethylamine-N-Oxide and Incident Coronary Heart Disease: A Prospective Investigation Among Urban Chinese Adults. *Journal of the American Heart Association*, 8(1). <https://doi.org/10.1161/JAHA.118.010606>
- Yuan, X., Wei, X., Liu, J., Tang, C., Peng, L., Wang, H., & Huang, Y. (2023). Association of Circulating Trimethylamine-N Oxide With Malnutrition and the Risk of Coronary Artery Disease in Patients With Maintenance Hemodialysis. *Journal of Renal Nutrition*, 33(3), 465–471. <https://doi.org/10.1053/j.jrn.2022.11.001>
- Zhang, H., Zhao, S., Fang, R., Wang, X., Chen, H., Cai, Z., ... Xiao, J. (2025). FMO3 exacerbates hepatic endoplasmic reticulum stress in drug-induced liver injury by inhibiting CREB3/P4HB axis and activating TMAO-mediated PERK pathway. *Life Sciences*, 374, 123699. <https://doi.org/10.1016/j.lfs.2025.123699>
- Zhang, L., Xie, F., Tang, H., Zhang, X., Hu, J., Zhong, X., ... Nie, J. (2022). Gut microbial metabolite TMAO increases peritoneal inflammation and peritonitis risk in peritoneal dialysis patients. *Translational Research*, 240, 50–63. <https://doi.org/10.1016/j.trsl.2021.10.001>
- Zhang, W., Miikeda, A., Zuckerman, J., Jia, X., Charugundla, S., Zhou, Z., ... Shih, D. M. (2021). Inhibition of microbiota-dependent TMAO production attenuates chronic kidney disease in mice. *Scientific Reports*, 11(1), 518. <https://doi.org/10.1038/s41598-020-80063-0>
- Zhong, X., Li, Z., Liu, H., Huang, L., Yang, F., & Wang, H. (2025). Trimethylamine-N-oxide promotes vascular aging via inhibiting GATA4 degradation in chronic kidney disease rats. *Journal of Cardiology*. <https://doi.org/10.1016/j.jcc.2025.06.011>
- Zhou, J., Lhoták, S., Hilditch, B. A., & Austin, R. C. (2005). Activation of the Unfolded Protein Response Occurs at All Stages of Atherosclerotic Lesion Development in Apolipoprotein E-Deficient Mice. *Circulation*, 111(14), 1814–1821. <https://doi.org/10.1161/01.CIR.0000160864.31351.C1>
- Zhu, W., Gregory, J. C., Org, E., Buffa, J. A., Gupta, N., Wang, Z., ... Hazen, S. L. (2016). Gut Microbial Metabolite TMAO Enhances Platelet Hyperactivity and Thrombosis Risk. *Cell*, 165(1), 111–124. <https://doi.org/10.1016/j.cell.2016.02.011>
- Zhu, W., Wang, Z., Tang, W. H. W., & Hazen, S. L. (2017). Gut Microbe-Generated Trimethylamine N -Oxide From Dietary Choline Is Prothrombotic in Subjects. *Circulation*, 135(17), 1671–1673. <https://doi.org/10.1161/CIRCULATIONHA.116.025338>
- Zhu, Y., Li, Q., & Jiang, H. (2020). Gut microbiota in atherosclerosis: focus on trimethylamine N-oxide. *APMIS*, 128(5), 353–366. <https://doi.org/10.1111/apm.13038>
- Zixin, Y., Lulu, C., Xiangchang, Z., Qing, F., Binjie, Z., Chunyang, L., ... Dongsheng, O. (2022). TMAO as a potential biomarker and therapeutic target for chronic kidney disease: A review. *Frontiers in Pharmacology*, 13. <https://doi.org/10.3389/fphar.2022.929262>
- Zou, X., Xiong, L., Guo, Z., Li, Y., Ning, K., Liu, X., ... Zhang, Z. (2024). Trimethylamine-N-oxide (TMAO) and the predicted risk of cardiovascular events after partial nephrectomy. *Asian Journal of Surgery*, 47(1), 281–288. <https://doi.org/10.1016/j.asjsur.2023.08.104>