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REVIEW ARTICLE

Sex Differences in Trimethylamine N-Oxide Levels as Cardiovascular Disease Risk Factors

Abdul Hafi Parimoo

Foundation World School, Mamath, Budgam, Jammu & Kashmir, 191111, India; a.hafiparimoo@gmail.com

ABSTRACT: Trimethylamine N-oxide (TMAO), a gut microbiota-derived metabolite, has been linked to various cardiovascular diseases (CVDs) and is an important component of understanding cardiovascular health. Despite being an important marker of cardiovascular health, the influence of sex-related differences on TMAO levels remains poorly understood. The primary objective of this review is to analyze sex-based hormonal influences on TMAO production, while also looking at interlinked mechanisms and contributors. This review reveals that increased TMAO levels are linked to greater risks of atherosclerosis, endothelial dysfunction, dyslipidemia, and inflammation; it discusses the variability of sex-based findings and emphasizes the need for further research to fill these gaps. An exhaustive review of available literature suggests TMAO as a promising biomarker and therapeutic target in cardiovascular health in male and female populations, with a potential for further studies into its advancement and health interventions.

KEYWORDS: Biomedical and Health Sciences, Genetics and Molecular Biology of Disease, Cardiovascular Disease, Gut Microbiota, Trimethylamine N-Oxide.

Introduction

Cardiovascular disease (CVD) is a collective term used to refer to a group of diseases affecting the heart ("cardio") and blood vessels ("vascular"). The most common types of CVDs include Coronary Artery Disease (CAD), Myocardial Infarction (MI), and Stroke.

CAD is caused by the buildup of atherosclerotic plaques in the coronary arteries, leading to narrowed blood vessels that restrict blood flow. This can cause chest pain (angina) and increase the risk of a heart attack (myocardial infarction) due to plaque rupture and clot formation. CAD remains a major cause of death worldwide.[1, 2] MI, more commonly known as a heart attack, occurs when blood flow to a part of the heart is obstructed, typically due to a blood clot in the coronary artery, leading to damage to the heart tissue. MIs can lead to the sudden onset of cardiac arrest, depending on the extent of the damage.[3] Stroke occurs when blood flow to the brain is disrupted, either due to a blocked artery (ischemic stroke) or a vessel rupture and bleeding in the brain (hemorrhagic stroke). Stroke can lead to long-term disability or death, depending on the affected brain area and its severity.[4]

The prevalence of CVDs differs greatly by region and is affected by the environment, lifestyle, genetics, and healthcare access. In high-income countries, such as the United States, Canada, and Western European countries, CVD has long been a leading cause of death.[5] Although CVD rates have decreased in recent years due to advanced healthcare and prevention strategies employed in these countries, CVD remains significant in countries with rising rates of obesity, diabetes, and hypertension due to lifestyle and diet. Additionally, in low-middle-income countries, such as India, China, and many in Sub-Saharan Africa, the prevalence of CVDs is sharply rising due to urbanization, changes in diet, and other increasing risk factors.[6] These regions face challenges in access to healthcare, early detection, and treatment strategies, leading to significantly delayed diagnosis and disease management. In Asia, countries such as Japan and South Korea have an increasing rate of CVD, mainly due to westernized lifestyle factors.[7] Southeast Asian countries, such as Indonesia and Vietnam, are also victims of this increase in CVDs due to urbanization. A primary contributor to this is the Western diet and high-fat diets, which are characterized by a high intake of red meat, processed foods, refined sugar, and saturated fats.[8]

Ongoing research explores the role of the gut microbiome as a primary factor in the pathophysiology of cardiovascular diseases. The gut microbiota is a vast microbiological community, full of millions of microorganisms, that plays an important role in the metabolism of dietary compounds. Dysbiosis, which is an imbalance in gut microbiota composition, has been related to systemic inflammation, metabolic disorders, and endothelial dysfunction.[9] Additionally, certain metabolites produced by the gut microbiome can have direct impacts on human health.[10] Trimethylamine N-oxide (TMAO), a metabolite of gut microbiota derived from dietary precursors such as choline, phosphatidylcholine, and carnitine, has come to light for its link with increased cardiovascular risk.[11] These precursors, typically found in foods like eggs, red meat, and dairy products, are metabolized by the gut microbiota into trimethylamine and subsequently oxidized to TMAO by flavin monooxygenase 3 (FMO3) in the liver.[12] In recent years, research has focused on sex-specific differences in gut microbiome, specifically TMAO production and its impact on CVD.[13] However, this area remains incompletely understood. Research suggests that differences in hormonal activity, metabolic pathways, and lifestyle choices may drive these sex-based differences in the generation of TMAO. This review will first discuss the metabolism

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of TMAO, subsequently explore its involvement in cardiovascular disease, and finally examine the sex-linked differences associated with the effects of TMAO.

■ Mechanisms of TMAO Production and Dietary Sources

Mechanisms:

Trimethylamine N-oxide (TMAO) is synthesized through a series of metabolic stages involving microbial activity in the gut and hepatic oxidation with the aid of host enzymes. TMAO production starts in the gastrointestinal tract, where specific strains of bacteria metabolize dietary precursor compounds such as choline, L-carnitine, lecithin, phosphatidylcholine, and betaine into trimethylamine (TMA).[14] Bacterial strains that produce TMA through choline metabolism are present in nearly all individuals.[15] These include *Anaerococcus hydrogenalis, Clostridium asparagiforme, Clostridium hathewayi, Clostridium sporogenes, Escherichia fergusonii, Proteus penneri, Providencia rettgeri*, and *Edwardsiella tarda*.[16] The genomes of these, and other bacteria that utilize alternative substrates, encode specific enzymes listed in Table 1, which are necessary for converting TMA-containing compounds to TMA.

Table 1: These are the primary microbial enzymes involved in the production of TMA. This table outlines the key microbial enzymes and their associated strains that are involved in the conversion of dietary precursors to TMA. The recurrence of specific strains, such as *Escherichia coli*, suggests their integral role in the pathway.

Enzyme	Common Strains	Gene	Substrate	Function	Reference
Choline-TMA lyase	*Clostridium sporogenes, Lachnoclostridium, L. saccharolyticum*	cutC/D	Choline	Converts choline to TMA by catalyzing the C-N bond cleavage	17
Carnitine monooxygenase	*Escherichia coli, Ralstonia eutropha, Clostridium sporogenes*	cntA/B	Carnitine	Converts carnitine to TMA	18
Betaine reductase	*Clostridium sporogenes,*	Unknown	Betaine	Reduces betaine to TMA	19, 20
TMAO reductase	*Escherichia coli, Klebsiella pneumoniae*	Unknown	TMAO	Reduces TMAO to TMA	21
YeaW/X (homologous to cntA/B)	*Escherichia coli, Acinetobacter baumannii*	yeaW/X	Various substrates (including choline, carnitine)	Utilizes a variety of substrates to promote TMA synthesis	22

The composition of TMA-producing bacteria varies significantly between individuals and is heavily influenced by external factors,[23] particularly sex, diet, age, and health. Diets rich in red meat, eggs, and dairy, which are sources of choline and L-carnitine, are associated with greater TMAO levels due to the increased TMA precursors and a gut microbiota composition favoring TMA-producing bacteria. Studies also suggest that dietary controls (such as plant-based diets) can effectively reduce TMAO production, suggesting that individuals at high risk for TMAO-related diseases may benefit from reducing their intake of TMA precursors.[23,24] This is supported by studies which show that the production of TMA and thereby TMAO is beneficial for the survival of several strains of bacteria, such as *Escherichia coli*.[25]

TMAO has the chemical formula $(CH_3)_3NO$ and is a tertiary amine oxide, a colorless organic compound that forms following the oxidation of the amino group of TMA.[26] After TMA is produced, it enters the hepatic portal and is transported to the liver, where it is oxidized to TMAO. This is catalyzed by hepatic flavin-containing monooxygenase enzymes, specifically FMO3, which oxidizes TMA to form TMAO. The excess TMA decomposes into dimethylamine or methane. This pathway by which TMAO is produced from dietary precursors of TMA is illustrated in Figure 1.

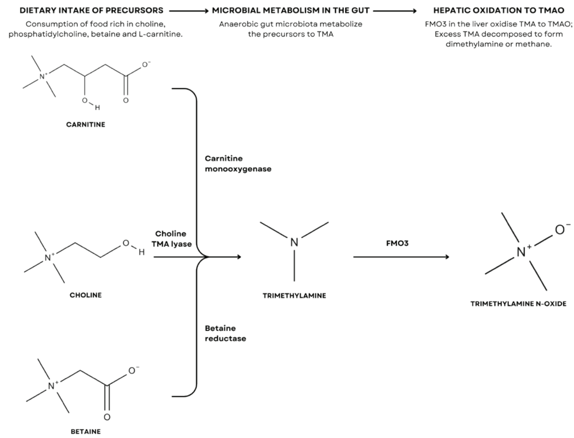

Figure 1: Summary of the primary pathway by which TMAO is produced. This figure represents the pathways followed in the conversion of dietary precursors to TMA by gut microbial enzymes, followed by the subsequent hepatic oxidation of TMA to TMAO via FMO3. *This is an original figure created using Canva and MolView.*

There are strong correlations between FMO3 activity and plasma TMAO levels.[27] Genetic variation influences the activity of the FMO3 enzyme, which is highly polymorphic.[28] Certain polymorphisms in FMO3 can lead to either a loss of function or increased enzyme activity, thereby altering the production of TMAO and giving rise to primary trimethylaminuria (TMAU), a disorder where the sweat, breath, saliva, and urine of a person smell like rotten fish. While this disorder does not have major health consequences, it can cause the mental health of the individual to deteriorate.[29,30]

The role of L-carnitine in TMAO synthesis is also a significant area of study; Koeth *et al.* shed light on an alternative pathway for TMAO production, in which L-carnitine is initially converted to an intermediate metabolite, γ-butyrobetaine (GBB), and then into TMA, which is then converted to TMAO by oxidation in the presence of FMO3.[22] This pathway may be relevant in individuals with high dietary L-carnitine intake, as it could be associated with increased TMAO levels beyond those produced through direct TMA synthesis from choline and other dietary precursors.

Sex also plays a significant role in TMAO production because of the differences in male and female hormones. Estrogen has been observed to increase FMO3 expression, and thereby TMAO metabolism. In one study, researchers administered 0.5mg estradiol pellets, a type of estrogen, in a murine model and compared the subsequent plasma TMAO levels to control groups, they found that the group with estradiol administration showed higher TMAO levels.[27] This is backed by a study that showed that plasma TMAO levels are reduced in menopausal women.[31] However, some studies suggest that males have higher TMAO levels than females, possibly due to hormonal levels, dietary habits, and microbiome composition.[32]

Age is another factor in TMAO metabolism, as it impacts gut microbiota composition and enzyme activity. Older adults often experience shifts in their gut microbiome and changes in liver and kidney function, which can both influence TMA production and TMAO elimination. One study found that increased age was associated with higher TMAO levels in males,[31] and proposed hormonal influences on the expression of the FMO3 gene as a potential explanation for this difference.

Once produced, TMAO is either excreted through renal elimination or accumulates in tissues where it acts as an osmolyte, helping maintain cellular homeostasis under osmotic stress.[33, 34] The contribution of TMAO to cardiovascular conditions remains an active area of study, with many studies suggesting enhanced cholesterol deposition in arterial walls, promoted platelet hyperreactivity, and impaired endothelial function as potential mechanisms.

Dietary Precursors:

Diet is a determinant of TMAO production as it supplies key precursors such as choline, L-carnitine, lecithin, phosphatidylcholine, and betaine.[35-37] Choline is an essential nutrient that is abundant in animal products such as eggs, meat, and fish. Phosphatidylcholine, a major component of cell membranes, is a key source of choline and is found in foods like egg yolks, soybeans, and organ meats.[38] High plasma TMAO concentrations, above 2 - 5 µM, have been linked with high choline intake.[39] However, some research shows that chronic intake of choline does not alter plasma TMAO levels, challenging the assumption that high choline consumption could directly contribute to CVDs. In Lemos *et al*., researchers supplemented the diet of 30 adults with 400 mg/day of choline through either 3 eggs or choline bitartrate; they observed no significant changes in fasting plasma TMAO levels. Researchers did, however, observe a significant increase in plasma choline after 4 weeks of consumption of 3 eggs daily without changes in TMAO levels. This suggests that a great proportion of phosphatidylcholine was absorbed before reaching the colon for microbial conversion to TMA,[40] highlighting the significance of metabolism and absorption rates in the production of TMAO.

L-carnitine, primarily found in red meat, poultry, and fish, is also a major dietary precursor of TMAO. It is synthesized endogenously from methionine and lysine.[41] While most individuals obtain sufficient amounts of L-carnitine through their diet and endogenous synthesis, vegetarians and vegans typically have much lower intakes, which contributes significantly to lower plasma TMAO levels when compared to omnivores.[42] In a study conducted by Samulak *et al*., supplementation with 1500 mg of L-carnitine L-tartrate for 24 weeks in healthy older women significantly increased plasma TMAO levels compared to a placebo group.[43] However, despite this increase, there were no significant changes in several markers of oxidative stress. In a follow-up study conducted 4 months after discontinuation, a reduction in plasma TMAO levels was observed, while lipid levels remained stable. These findings suggested that plasma TMAO concentrations can be modulated by L-carnitine supplementation and cessation, yet its direct impact on lipid metabolism and cardiovascular health remains uncertain.[44]

Fish and shellfish, which contain preformed TMAO, lead to the highest concentrations of TMAO among dietary sources.[45] In a study by Cho *et al*., fish consumption resulted in plasma TMAO levels that were ~50 times higher than eggs or beef.[36] A high-fat diet can influence plasma TMAO levels, with certain studies indicating that high-fat meals lead to increased postprandial TMAO levels. In one study involving healthy men, postprandial TMAO levels rose significantly after a high-fat meal, which consisted of 55% fat, with 50% of the fat being saturated.[46] This suggested that the fat content, particularly the saturated fat, may contribute to the observed rise in TMAO levels. In recent years, the Western diet (WD) has increasingly resembled high-fat diets, characterized by an excessive intake of processed foods, refined carbohydrates, and unhealthy fats.[47]

■ The Role of TMAO in Cardiovascular Diseases

Several clinical studies indicate that elevated plasma TMAO levels correlate with a higher risk of cardiovascular events, providing evidence for TMAO as an independent biomarker of cardiovascular risk.[48-52] Outlined below are the mechanisms by which TMAO plays a role in cardiovascular diseases, summarized visually in Figure 2.

Endothelial Function Disruption:

TMAO disrupts endothelial function by inhibiting endothelial cell proliferation during the G1 phase of the mitotic cell cycle and has cytotoxic effects on circulating endothelial progenitor cells.[53, 54] This impairs the regenerative processes of blood vessels, contributing to vascular dysfunction.

Pro-Inflammatory Mechanism:

A substantial body of research has shown that TMAO can accelerate atherosclerosis by diverse mechanisms that have been systematically investigated. Atherosclerosis is a condition where plaque buildup in blood vessels leads to narrowing of blood vessels and loss of the necessary elasticity needed to withstand and maintain blood pressure. TMAO can induce the up-regulation of CD36 and SR-A1 receptors on macrophages, which are responsible for lipid uptake into cells. This induces increased accumulation of lipids within macrophages, causing them to transition into foam cells.[55] The excessive lipid levels trigger the release of inflammatory cytokines that lead to vascular inflammation and subsequent damage to blood vessels.[56]

TMAO is known to activate nuclear factor kappa B (NF-κB), a transcription factor that up-regulates production of pro-inflammatory proteins,[57] and therefore induces the expression of adhesion molecules like vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). These molecules cause the accumulation of leukocytes on the endothelium of vessels and contribute to the development of plaque formation.[58-60]

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Mitochondrial Dysfunction, Oxidative Stress, and Vascular Physiology:

Mitochondria are the sites of oxidative phosphorylation; however, the superoxide radicals produced during this process are extremely reactive and dangerous. Mitochondrial superoxide dismutase 2 (SOD2) is responsible for converting free radicals into less harmful hydrogen peroxide. However, elevated TMAO levels have been found to interfere with this by suppressing the activity of mitochondrial sirtuin 3 (SIRT3), which is responsible for the activation of SOD2.[11] This results in elevated superoxide levels and increased oxidative stress.[61] Oxidative stress impairs endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) activity.[62,63] eNOS and iNOS are two essential enzymes for the production of nitric oxide (NO), a signaling molecule that is important for vasodilation and vascular health, in both murine cells and human endothelial cells.[64,65] Reduced bioavailability of NO causes vascular stiffness, resulting in hypertension.

Increased ROS triggers the release of calcium ions (Ca^{2+}) from intracellular stores through inositol 1,4,5-trisphosphate receptors (IP3Rs) on the endoplasmic reticulum. This change in calcium balance causes an impairment in the contractile response of the vascular endothelial cells and promotes vascular stiffness and atherosclerosis.[66,67]

Cholesterol Metabolism Disruption and Dyslipidemia:

Unchecked levels of cholesterol in the bloodstream elevate the risk of dyslipidemia and atherosclerosis.[68] TMAO is also known to suppress the activity of cholesterol 7-alpha hydroxylase (CYP7A1), an enzyme that aids in the breakdown of cholesterol into bile acids, which is important for cholesterol clearance from the liver.[69] Due to TMAO's suppression, the reduced activity of CYP7A1 leads to a decrease in bile acid production and impairment of the liver's ability to break down and excrete cholesterol.

Pro-thrombotic Effects and Platelet Activation:

TMAO-induced oxidative stress triggers the release of calcium from intracellular stores through IP3 receptors, which increases the propensity of platelet activation to prothrombotic stimuli.[70] This surge in intracellular calcium enhances the release of thromboxane A2 (TXA2), a vasoconstrictor and platelet activator that further supports thrombus formation.[71]

Moreover, TMAO promotes the expression of adhesive molecules, such as P-selectin and integrins (specifically $\alpha IIb\beta 3$) on the platelet surface.[72] These molecules cause platelets to adhere to the vascular endothelium and each other more readily, leading to increased clot stability.[73] P-selectin also interacts with P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes to enhance platelet-leukocyte aggregation. This aggregation is known to represent biomarkers of thrombo-inflammatory cardiovascular disease.[74]

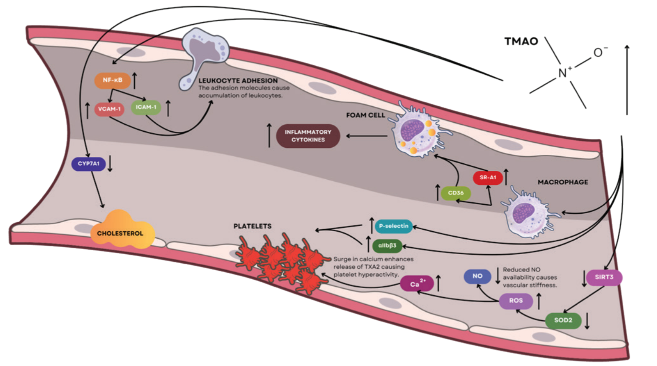

Figure 2: Summary of the mechanisms by which TMAO impacts cardiovascular disease. This figure illustrates the multiple pathways through which elevated TMAO levels promote the development of cardiovascular disease and atherogenesis via the mechanisms discussed in this section. *This is an original figure created using Canva and the NIH BioArt repository.*

Sex-Based Differences in TMAO Metabolism and Cardiovascular Health

While the relation between TMAO and CVDs is well-established, more recent research investigating the intersection of sex-based differences in TMAO metabolism and cardiovascular risk has yielded conflicting results. This variance may be due to the influence of confounding factors such as age, gut microbiome diversity, and hormonal status. Some studies, such as those by Manor *et al.*, Chen *et al.*, and Stubbs *et al.*, suggest that males have greater circulating TMAO levels.[32,75,76] On the contrary, studies by Bennett *et al.*, Obeid *et al.*, and Falls *et al.* demonstrate that females may have higher levels.[27,39,77]

A primary factor in these differences is the effect of sex hormones on hepatic FMO3 expression. A 1997 study by Falls *et al.* investigated the regulation of flavin-containing monooxygenase 1 (FMO1), a flavin-containing monooxygenase isoform found in mice, and FMO3 in mice using sex steroids.[77] They found that the FMO1 and FMO3 expression is downregulated by the high levels of serum testosterone in male mice and that after castration, these mice demonstrated levels of FMO3 that were similar to those of females. However, once treated with testosterone after castration, the FMO3 levels in male mice returned to baseline. When unspayed female mice were treated with testosterone, their FMO3 levels reached those of male mice, virtually undetectable. These findings suggest that testosterone suppresses the expression of FMO3. This study is supported by another one; Bennett *et al.* concluded that in a similar mouse model, females had much higher FMO3 expression when compared to their male counterparts.[27] This provides evidence for the positive relation between estrogen and FMO3 expression.

It is to be noted that estrogen levels in females vary with age, particularly due to menopause, which is a natural process that is characterized by the end of the monthly menstrual cycle due to ovarian follicular function. Menopause typically occurs in women between the ages of 45 to 55,[78] and causes a decline in estrogen. Post-menopausal women, therefore, have lower estrogen, and thereby lower FMO3 activity stimulation when compared to premenopausal women; this may lead to differences in TMAO levels, which is evident through a study by Spencer *et al.*, which found that plasma TMAO levels are reduced after menopause.[31]

Manor *et al.* conducted a cohort study with 52% females and 48% males, with an average age of 50 ± 13 years, and concluded that males showed significantly higher TMAO levels than females.[32] However, it can be speculated that the high proportion of elderly women, who may be postmenopausal, might explain the observed sex differences, as reduced estrogen levels can account for lower FMO3 activity and thereby a decrease in TMAO production. Another study in 2024 by Almer *et al.* had similar findings, with males showing significantly higher levels of serum TMAO concentrations than females.[79] The age group for this study was 11 to 26 years for both sexes. This can rule out our previous speculation that the results are being affected by postmenopausal hormonal changes. An alternative explanation for the observations in these studies is the dietary habits of males compared to females. Studies have previously found that men tend to have a higher consumption of red meat, fat, and a westernized diet than women;[80, 81] This may cause an increase in the TMA-precursors and thereby more synthesis of TMA, and by extension, TMAO.

Other studies have also shown evidence for higher levels of TMAO in females than in males. A study conducted by Veeravalli *et al.* in 2017 found that female mice had much greater TMAO levels than male mice. They proposed that this was due to the fact that when male mice reach 5-6 weeks of age, the expression of FMO3 is switched off, which does not occur in humans.[82] The study by Bennett *et al.*, which has been previously discussed, had also found that in the mouse model, there were much higher levels of plasma TMAO in females than in males, and higher levels of TMA in males than in females. This observation allowed them to conclude that the conversion of TMA to TMAO is more efficient in female mice. It also highlighted that the differences in TMAO levels were not replicated in human populations, causing them to believe that this may be due to the differences and variability in the diet consumption of humans. A visual summary of these key pathways through which sex differences affect plasma TMAO levels is provided in Figure 3.

However, not all studies abide by a sex-different hypothesis. Several studies did not detect significant sex-specific differences in TMAO levels; they did, however, find that the concentration of certain precursors is greater in males than in females. All three studies by Obeid *et al.*, Rohrmann *et al.*, and Mueller *et al.*, conducted on human models, found that betaine is significantly higher in males than in females; however, only Rohrmann *et al.* found that choline was higher in males than in females, where the other two did not correlate it significantly with sex.[83-85]

It is essential that we understand the variations in TMAO levels with sex that are observed before and after cardiac events to better inform treatment and rehabilitation plans in the future. Thus far, only one study has been conducted on TMAO levels following a cardiac event. This was a study by Baranyi *et al.* in 2022, which investigated the effect of sex-specific differences on TMAO concentrations before and after cardiac rehabilitation in patients who suffered an acute myocardial infarction (AMI).[86] Their sample had an average age of 57.9 (± 12.0) years and included 45 Caucasian males and 11 Caucasian females, all of whom had suffered an AMI. All patients underwent 4 weeks of cardiac rehabilitation. They found that female patients consistently had higher levels of TMAO blood concentration than males from the first day after AMI, and the difference was significant till the start of cardiac rehabilitation. By the end of the rehabilitation period, both males and females had had similar levels of TMAO concentration. This proves the efficacy of cardiac rehabilitation for patients who have suffered major adverse cardiovascular events (MACEs). They speculated that females may be at a greater thrombotic risk than men due to the higher TMAO concentrations and therefore higher levels of TMAO-induced platelet hyperactivity, as discussed in the preceding sections. This study, being the first of its kind, highlights the need for future studies that look at TMAO levels before and after MACEs, with a more generalizable sample and a multi-omic approach.

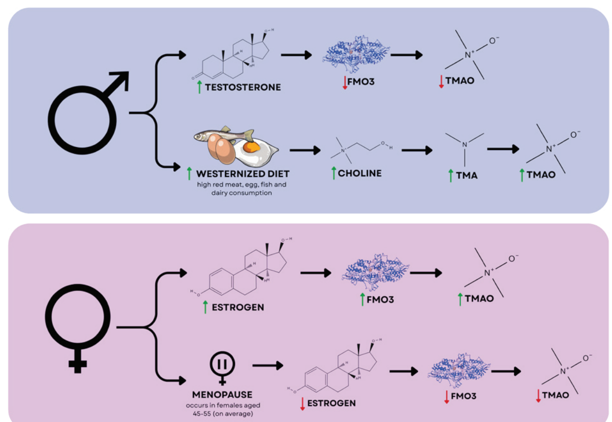

Figure 3: Summary of the pathways by which sex affects the TMAO levels. In both males and females, different routes can act to either elevate or reduce circulating TMAO levels, which may partially account for the inconsistent findings reported across previous studies. *This is an original figure created using Canva, MolView, and the SWISS MODEL database.*

Discussion

This analysis of decades of existing research has evaluated the complex role of TMAO in cardiovascular diseases and how it links to sex-based differences. While TMAO is a well-established biomarker for cardiovascular events,[87] its exact effect on the pathophysiological mechanisms remains to be fully explored by researchers due to its intersection with several factors, including sex-based differences.

Sex differences are essential in understanding the complete role of TMAO in cardiovascular physiology. It is possible that the variations in results that arise from different studies may exist due to several factors, such as focusing only on the postmenopausal or premenopausal phases, as estrogen levels in both are different and may act as an external variable in the studies.

When comparing mouse studies to humans, there are an array of factors that must be taken into account for better generalizability and applicability to humans, such as dietary intake, gut microbiome diversity, and even kidney function. The key matter of concern is the fact that FMO3 is, as previ-

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ously discussed, switched off in male mice following 5-6 weeks of age, which makes the model much less reliable. Apart from these, a key factor is also the presence of hepatic FMO1. Unlike mice, adult humans do not have the presence of the FMO1 enzyme in the liver, and it is only expressed in the kidney and small intestine; therefore, it cannot play an active role in the production of TMAO, as studies have found no production of TMAO in cells isolated from these tissues.[88, 89] Furthermore, mouse studies typically involve all mice kept on the same diet to avoid confounding variables; however, this does not follow the diet differences between male and female humans, raising concerns regarding the validity of the comparisons drawn between the two models. This may also be a possible explanation for why mouse model studies tend to find females with higher levels of TMAO. Figure 4 provides a detailed summary of the differences between murine and human models that must be taken into account when evaluating the applicability of murine model studies to human populations.

Future research in the area must consider studies that account for sex and hormonal status in greater detail. These studies must separate sex differences from other factors to reach a conclusive result on variations and cause-and-effect in TMAO with sex, while also including both pre- and postmenopausal women to make clear the impact of hormonal changes on TMAO metabolism and CVD risk. Studies should also focus on the dietary aspect and the sex differences in TMAO levels following a MACE to identify critical threats to individuals undergoing recovery from a cardiovascular event and create better strategies to tackle these threats. Further research should address the conflicting data and evaluate TMAO-focused interventions in diverse populations to improve cardiovascular health.

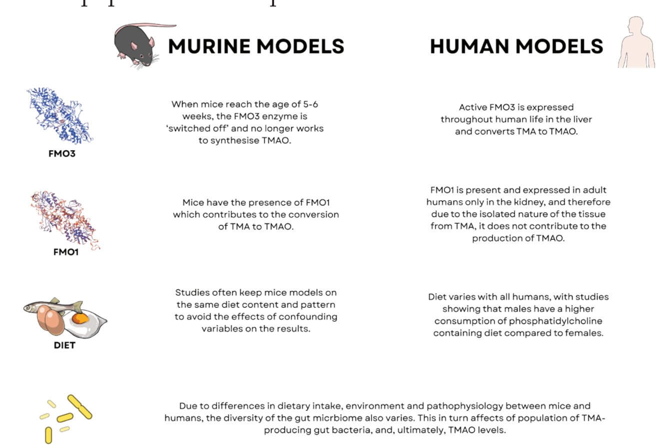

Figure 4: Differences between murine and human models that limit the direct applicability of findings on sex-linked differences in TMAO levels from mouse studies to human populations. *This is an original figure created using Canva, MolView, the NIH BioArt repository, and the SWISS MODEL database.*

Conclusion

This review has synthesized a vast body of literature on the sex differences in TMAO levels as CVD risk factors, outlining the complicated interactions between biological, dietary, sex, and age factors. While several studies suggest higher levels of TMAO in males relative to females, possibly due to dietary factors, others conclude that females have comparatively greater levels of TMAO than males, which could be caused by the upregulation of FMO3 by estrogen, especially in pre-menopausal women. In addition, research on TMAO levels post-cardiac events is scarce and should be the focus of future studies to allow effective treatment and rehabilitation.

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Author

Abdul Hafi Parimoo is an A Level student at Foundation World School, India, and has a passion for the field of Medicine.