Chapter

Gut Microbial Metabolite Trimethylamine-N-Oxide and its Role in Cardiovascular Diseases

Shubham Barik, Aritra Mukherjee, Angelina Job Kolady, Boda Karunakar and Tony Grace

Abstract

Atherosclerosis (AS) is the common pathological underpinning of numerous cardiovascular illnesses (CVDs), and it is the leading cause of death worldwide. In recent years, researchers have begun to recognize the importance of gut microbiota in AS. Gut microbial dysbiosis has been reported to be connected with various CVDs. Moreover, dietary choline, betaine, and L-carnitine produce trimethylamine N-oxide (TMAO), a key gut microbe-dependent metabolite. Multiple studies have found a link between plasma TMAO levels and the likelihood of developing AS. The mechanism underlying this link, however, is still unknown. In this chapter, we discuss the TMAO-mediated mechanisms of atherosclerotic CVD from the perspectives of dietary patterns and gut microbial metabolism. Finally, we explain how TMAO has emerged as a novel therapeutic target for CVDs, as well as many treatment options for lowering TMAO levels that are currently being investigated, such as medications, dietary changes, probiotics, and so on.

Keywords: cardiovascular disease, gut microbiota, trimethylamine (TMA), trimethylamine-N-oxide (TMAO), diet, metabolism

1. Introduction

Cardiovascular disease or *CVD* is a term for disorders affecting the heart or blood vessels. Except in Africa, cardiovascular illnesses are the main cause of mortality globally, resulting in 17.9 million deaths (32.1%) in 2015, an increase from 12.3 million (25.8%) in 1990 [1]. CVD deaths are more widespread and have been growing in most developing countries, whereas rates in most developed countries have declined since the 1970s [2]. Coronary artery disease and stroke account for 80% of CVD deaths in males and 75% in females. The majority of cardiovascular disease affects older people. In the United States, 11% of adults between the ages of 20 y and 40 y have CVD, 37% between the ages of 40 y and 60 y, 71% between the ages of 60 y and 80 y, and 85% above the age of 80 y have CVD. In developed countries, the average age of death from coronary artery disease is over 80 y, while it is roughly 68 y in the developing world [2]. Diagnosis of diseases typically occurs seven to ten years earlier in men than in women.

The underlying processes differ according to the illness. Dietary risk factors are responsible for 53% of CVD fatalities [3]. Atherosclerosis is a common factor in coronary artery disease, stroke, and peripheral artery disease [4]. High blood pressure, smoking, diabetes mellitus, lack of exercise, obesity, high blood cholesterol, poor food, excessive alcohol consumption, and poor sleep, among other factors, may contribute to this [5]. High blood pressure contributes around 13% of CVD fatalities, whereas tobacco accounts for 9%, diabetes accounts for 6%, lack of exercise factors for 6%, and obesity accounts for 5%. Untreated strep throat can potentially cause rheumatic heart disease. Up to 90% of cardiovascular disease is thought to be preventable [6]. Lowering risk factors through good food, exercise, avoiding cigarette smoke, and limiting alcohol use are all part of CVD prevention. It also treats risk factors such as high blood pressure, lipids, and diabetes. In those with strep throat, antibiotics can lower the risk of rheumatic heart disease [7].

The microbiome plays a beneficial role in the homeostatic regulation of different body tissues of the host [8]. The overall relationship between humans and their microbiota can be described as a mutualistic symbiosis, also known as eubiosis [9]. This healthy balance of gut bacteria can be disrupted, leading to the onset of a variety of chronic diseases with an underlying inflammatory condition [10]. A large population of microbiota, predominantly bacteria, that populate the human gut have a symbiotic connection with the host, and imbalances in host-microbial interaction (dysbiosis) hamper these homeostatic systems that govern health and activate numerous pathways that contribute to advancing CVD risk factors [11]. Dysbiosis is related to intestinal inflammation and decreased gut barrier integrity, which raises circulating levels of bacterial structural components and microbial metabolites such as trimethylamine-N-oxide and short-chain fatty acids, which may aid in the development of CVDs [11].

Trimethylamine-N- oxide (TMAO) is a type of osmolyte found in the tissues of marine crustaceans and fish, where it prevents protein distortion and, therefore, the animal's death [12]. The concentration of TMAO increases as the animal's depth in the seas increases [13]. It is a protein stabilizer that counteracts the protein-destabilizing effects of pressure. In general, the bodies of animals living at great depths are adapted to high-pressure environments by having pressure-resistant biomolecules and small organic molecules present in their cells, known as piezolytes, of which TMAO is the most abundant. These piezolytes give the proteins the flexibility to function properly under great pressure [13–15]. However, more importantly, TMAO has emerged not only as an important metabolite in the human diet but also as a major cardiometabolic risk factor. It has been associated with many cardiovascular complications including foam cell formation [16], endothelial dysfunction [17], acute heart failure [18], infracted coronary artery [19], inflammation [20] and vascular aging [21].

2. Role of gut microbes in regulating cardiovascular health and disease

The gut microbiome has emerged as a critical factor in human health and disease [22, 23] and cardio-metabolic diseases are no exception. Obesity and insulin resistance are serious cardiometabolic risk factors [24–27], and gut microbial composition is a major regulator of these conditions. Changes in fecal microbial community composition have been linked to the development of obesity and insulin resistance, and microbial transplantation has been shown to transmit increased adiposity in

the host [28–30]. Disruptions to the microbiota early in life have since been identified to induce increased obesity [31]. Koren et al. [32] argued that microbiota could be associated with atherosclerosis since human atherosclerotic plaques were found to contain bacterial DNA, albeit it was unclear if the DNA came from live bacteria within the arterial wall. The initial research into a possible link between the gut microbiome and cardiovascular disease (CVD) focused on trimethylamine N-oxide (TMAO), a metaorganismal metabolite generated after ingestion of food substances plentiful in a Western diet (eg, carnitine, lecithin, choline) [16, 33, 34]. TMAO has swiftly established itself as a biomarker for human CVD risk as well as a promoter of atherothrombotic disease [35, 36]. In fact, a Western-style diet deficient in microbiota-accessible carbohydrates (MACs) may cause irreversible microbial diversity loss and the extinction of particular bacterial species in the digestive system [37]. As a result, the low intake of dietary fiber and increased levels of fat and sugar in our food, which are typical of a westernized lifestyle and diet, may contribute to the depletion of specific bacterial taxa, at least in part [38]. Fiber, fruit, legume and vegetable consumption is linked to an increased microbial richness in the gut microbiota [39, 40], and several recent epidemiological studies have found an inverse relationship between dietary fiber consumption and CVD risk variables [41–45]. Non -digestible carbohydrates present in dietary fiber are converted by intestinal bacteria into Short-Chain fatty acids(SCFAs) like acetate, propionate and butyrate [46, 47]. SCFAs have been shown to have a direct effect on renin release and vasomotor function, resulting in lower blood pressure [48–50]. Butyrate has been shown to have a potential adjuvant effect in the lowering of diastolic blood pressure by reducing inflammation in a recent controlled experiment [51]. Moreover, in early pregnancy, the presence of butyrate-producing bacteria was found to be inversely related to blood pressure and plasminogen activator inhibitor-1 levels [52].

2.1 Gut microbiota dysbiosis and implications in CVD risk

Most microbiome-related diseases have skyrocketed in the last century, implying that a change in lifestyle could disturb gut microbiota symbiosis by removing helpful, protective bacteria [53]. Patients with a variety of CVD risk factors, such as hypertension, dyslipidemia, insulin resistance, and other metabolic abnormalities, have been found to have variations in microbial composition [36, 37]. Dysbiosis of the gut microbiota can lead to chronic inflammation, which is a major contributor to obesity, cardiovascular disease, and notably atherosclerosis [38, 39]. In symptomatic atherosclerosis patients, metagenome research indicated a higher concentration of triglycerides and a lower level of high-density lipoprotein in the circulation, as well as an increased abundance of *Collinsella* and a decreased abundance of *Roseburia* and *Eubacterium* [54]. Jie et al. [55] discovered an elevated relative abundance of *Enterobacteriaceae* and *Streptococcus* spp. taxa in atherosclerotic CVD patients. In coronary artery disease patients, Emoto et al. discovered a distinct alteration in microbial composition, with a large increase in *Lactobacillales* (Firmicutes) and a decrease in Bacteroidetes [56]. In another study, patients with type 2 diabetes had a lower number of Firmicutes and a non-significant rise in Bacteroidetes and Proteobacteria [57]. Some cross-sectional studies have found evidence that high-protein and high-fat diets (associated with Western lifestyles) are linked to gut microbial populations characterized by the Bacteroides enterotype, while diets heavy in carbohydrates and simple sugars are linked to the Prevotella enterotype [58].

The metabolism-independent pathway and the metabolism-dependent pathway are two key pathways via which gut dysbiosis can contribute to the development and progression of atherosclerosis [59]. In the metabolism -independent pathways, bacterial components located on the outer membrane of Gram-negative bacteria, such as lipopolysaccharides (LPS), can encourage the production of foam cells, which are a primary component of atherosclerotic plaque [60]. To prevent the accumulation of excess cholesterol in peripheral tissues, the body has internal homeostatic systems in place, such as reverse cholesterol transport (RCT). Excess cholesterol is transported to the liver and transformed into bile acids through the RCT process [61–63]. By producing metabolic endotoxemia, gut dysbiosis can overload systems like RCT and encourage the development of foam cells [64–66]. Metabolic endotoxemia is a condition marked by a high level of LPS in the bloodstream [67]. The presence of *Bifidobacteria*, which typically enhance intestinal barrier function and inhibit bacterial translocation, is reduced in high-fat (HF) diet-induced dysbiosis [60].

3. Synthesis and metabolism of gut microbial metabolite TMA and TMAO

3.1 Production of trimethylamine by gut bacteria

Trimethylamine (TMA) is the source of TMAO in humans. TMA is derived either directly from meals high in TMA, such seafood, [68, 69] or indirectly from the bacterial metabolism of dietary choline and choline-containing substances in the colon, such as phosphatidylcholine [16], betaine [70], and dietary L-carnitine [33, 71]. The ability of different gut microbes to produce TMA from food precursors varies. This is because it is produced in the gut via a variety of microbial mechanisms (**Figure 1**). As a result, the composition of an individual's microbiota influences the magnitude of TMA production. It's worth noting that the genes essential for TMA formation are found in just a small percentage of the microorganisms in the intestine (less than 1%) [72]. TMA formation appears to be possible even at extremely low concentrations of these microorganisms, highlighting the importance of the gut microbiota in this context [73]. TMA and TMAO levels have been linked to increased activity in bacteria belonging to the phylum Firmicutes and Proteobacteria, which are known producers of this metabolite. Furthermore, because Bacteroidetes are unable to make TMA [74], TMA and TMAO levels have been connected to an enhanced Firmicutes/Bacteroidetes ratio, with higher levels of Firmicutes and lower levels of Bacteroidetes [57, 58]. The genes coding for the glycyl radical enzyme choline TMA-lyase (CutC) and its related radical S-adenosyl-L-methionine (SAM) (CutD) activating protein were discovered in the Choline Utilization (cut) gene cluster in gut bacteria, which is responsible for the anaerobic breakdown of choline into TMA [75]. A two-component CntA/CntB oxygenase/reductase system capable of cleaving L-carnitine into TMA and malic semialdehyde is another microbial metabolic route that generates TMA from L-carnitine [76]. The yeaW/X gene products (YeaW/X TMA lyase) are a closely similar bacterial lyase. Choline, betaine, L-carnitine, and -butyrobetaine can all be converted to TMA by this promiscuous lyase [74, 77]. Aside from these TMA-generating processes from dietary trimethylamines, some gut microbes like *E.coli* have also been found to have another pathway that converts TMAO to TMA via the activity of a torA-like gene product which acts as a reductase [78, 79].

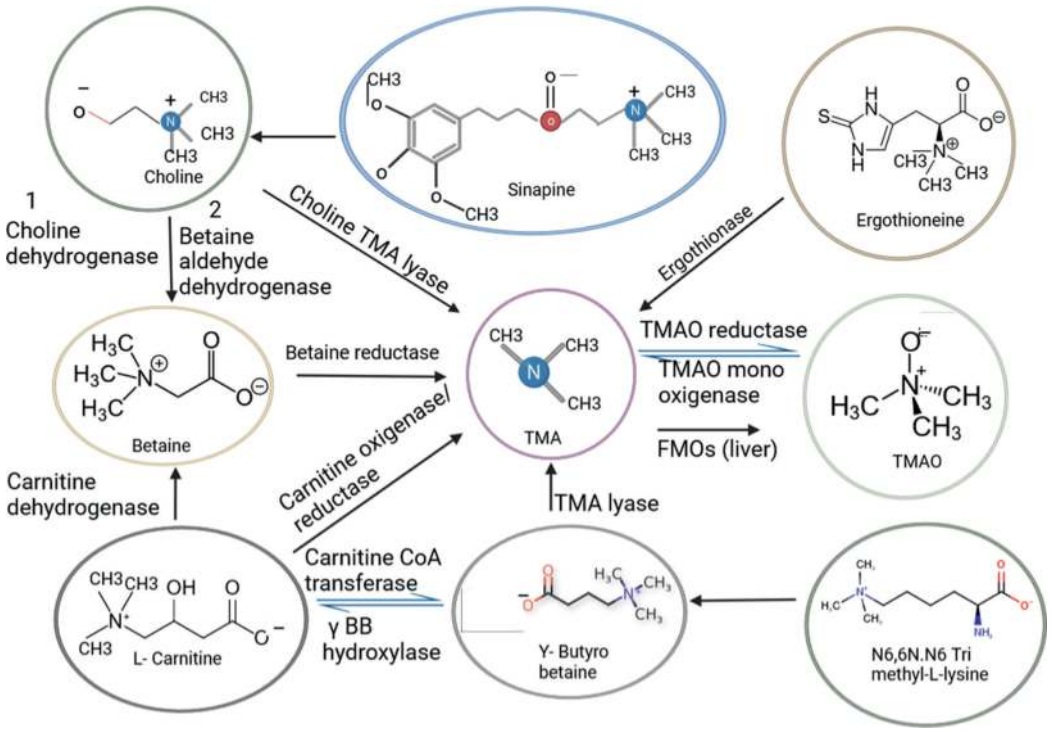

Figure 1.
Chemical formulae of TMA and TMAO's principal dietary precursors. The key metabolic pathways for the synthesis of TMA by the gut microbiota and endogenous enzymes, as well as the conversion of TMA to TMAO by hepatic FMOs, are depicted in this diagram.

3.2 Conversion of TMA into TMAO and its regulation

TMA generated from a choline -rich diet through various metabolic pathways is absorbed from the gut into the hepatic portal circulation and oxidized by the enzymes flavin-dependent monooxygenase isoforms 1 and 3 (FMO1 and FMO3) in the liver to create Trimethylamine-N-oxide(TMAO) (**Figure 2**) [80]. TMAO is excreted out of the body, usually through urine [81]. Sweat, feces (4%), exhaled air (less than 1%), and other body secretions are some of the other ways TMAO is excreted [82]. TMAO can be metabolized to DMA(Dimehtylamine), formaldehyde, ammonia, and methane by methanogenic bacteria that carry the TMAO demethylase enzyme [83]. Furthermore, it has been demonstrated that TMAO derived from food can be absorbed directly in the gut [84]. As a result, plasma TMAO levels are regulated by TMA synthesis and degradation, as well as the rate at which TMA, and TMAO are secreted [85].

3.3 Dietary precursors of TMAO and the relationship between TMAO levels and dietary habits

As discussed in Section 3.1, seafood is a rich source of dietary TMA/TMAO and various dietary precursors like L-carnitine, choline, ergothioneine and betaine (**Figure 1**) equally contribute to the generation of TMAO in the body. Free TMAO present in seafood is not metabolized by gut microbiota and is directly absorbed into the systemic circulation [86]. L-carnitine is present in high concentrations in meals derived from animals (meat and dairy products), and in smaller amounts in grains

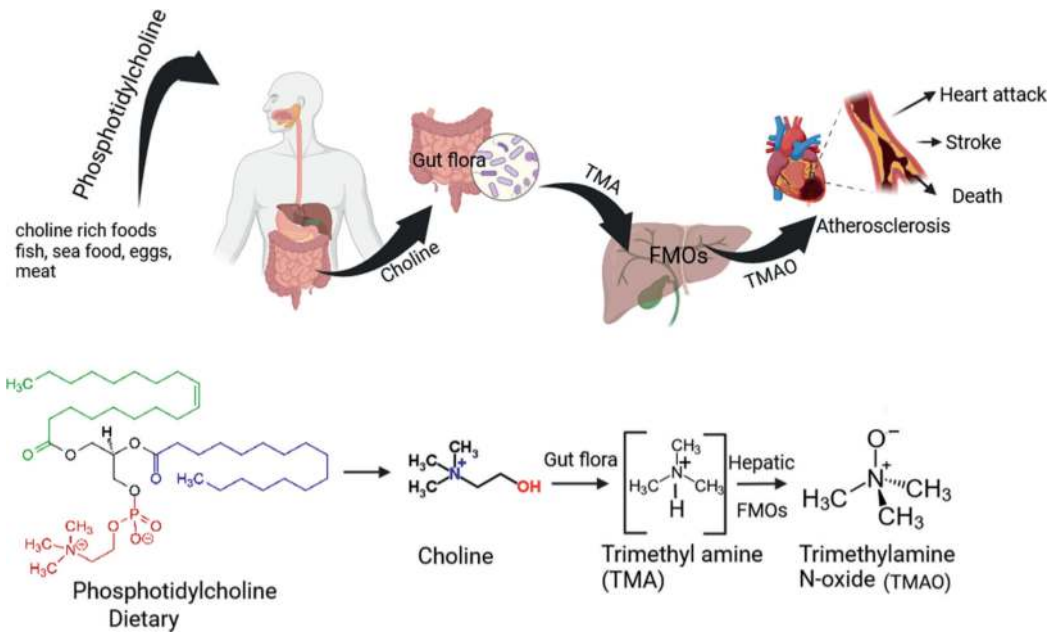

Figure 2.
Gut flora mediated synthesis of TMA and hepatic conversion to TMAO.

and vegetables [87]. The most common sources of choline in the diet are eggs and liver, followed by meats and fish, whole grain, cereal, vegetables, fruits, milk, fats, and oils [88]. One of the most important sources of betaine is cereal-based foods [89]. Betaine can also be found in spinach, beets, crabs, and finfish [90]. Dietary sources are the only way to get ergothioneine. Ergothioneine is found in only a few foods, with the largest quantities found in boletus and oyster mushrooms, as well as to a lesser level in chicken and pork liver and kidney, oat bran, and black and red beans [91]. As discussed in Section 2, a westernized lifestyle and diet full of junk fatty foods and refined sugar, devoid of fiber and important nutrients, predisposes one to increased CVD risk and other chronic diseases. Plasma TMAO levels have been observed to rise when people eat Western-style or high-fat diets [92–94]. However, conversely, epidemiological studies have linked the Mediterranean diet to a lower risk of cardiovascular disease (CVD) [95]. A typical Mediterranean diet is defined by plant based foods (vegetables, fruits, nuts,), olive oil based fats and moderate to low amounts of seafood, eggs and meats [96, 97]. This makes this type of diet high in fiber and low in choline -rich food. The importance of fiber- rich foods has already been mentioned in Section 2. High dietary fiber consumption, followed by gut microbiota-mediated fermentation, appears to reduce TMAO levels in experiments on animal models and clinical medicine [98].

4. Role of TMAO in increasing cardiovascular disease risk

A choline-rich diet puts a person at risk of increased TMAO levels [16], which is directly correlated to an increased CVD risk [99]. Angiographic markers of coronary artery atherosclerotic burden and cardiac risks have strong relationships with systemic TMAO levels, and higher levels of TMAO in the blood are linked to an increased risk of incident cardiovascular events such as myocardial infarction, recurrent stroke,

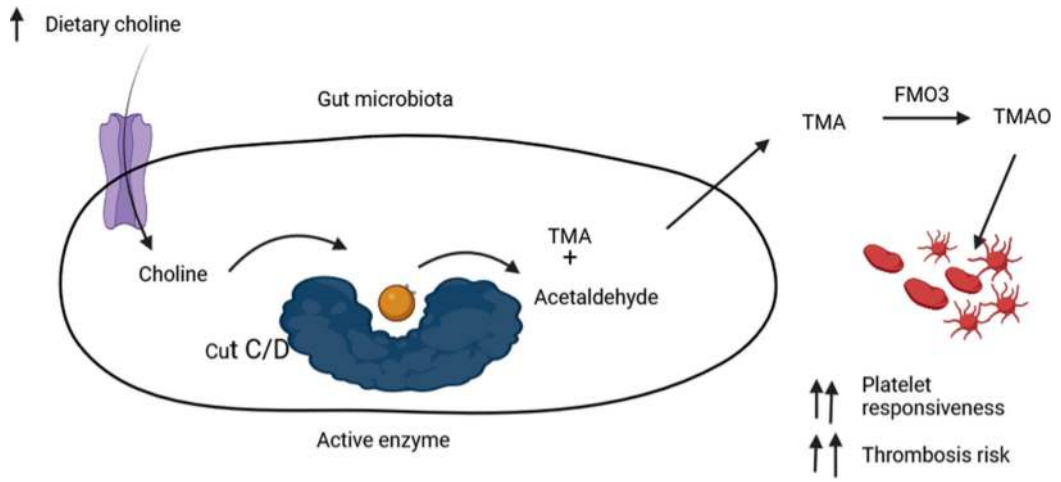

Figure 3.
TMAO- mediated platelet hyper-responsiveness and increased thrombosis risk.

and even cardiovascular death [55, 100]. Gut microbes play a role in modifying platelet reactivity and generating a pro-thrombotic phenotype in vivo by producing TMAO (**Figure 3**) [101]. Zhu *et al.,* has shown that direct exposure of platelets to TMAO, caused activation of the platelets by the release of intracellular calcium. This modulates the platelet hyper-responsiveness and the potential of thrombosis and causes thrombosis and atherosclerosis [101]. Rebecca *et al*., states that knockdown of FMOs can protect mice from obesity, which is a major cause for cardiovascular diseases [102, 103]. Increased amount of TMAO, obtained from the diet, causes monocytes to enter the subendothelial space and differentiate into colony -stimulating factors when they encounter the growth factors. These form large cells known as dendritic cells and macrophages which possess high expression of SR-A1 and CD36 [104]. These cells take up oxidized, low- density lipid particles to create foam cells that are irregular in the uptake of cholesterol with fatty acids and ester bonds, thus stimulating atherosclerosis [105]. It is suggested that CD36/MAPK/JNK pathways play a vital role in the formation of foam cells [106]. Research studies show that apoe−/− mice fed with choline diet for 8 weeks, gradually exhibited an increase in TMAO, which further recruited macrophages and pro – inflammatory cytokines [107]. Another study by Boini et al. indicates the link between TMAO and inflammation, where TMAO induces NLRP3 inflammasome formation and causes other immune responses [108]. An imbalance of cholesterol transport is observed in individuals with high TMAO, and studies show that mice with administered TMAO inhibited the synthesis of hepatic bile acid by downregulating the expression of Cyp7a1, which promoted atherosclerosis [109]. The activation of oxidative stress pathways following exposure to TMAO, which triggers inflammatory cytokines, is the molecular basis for increasing cardiovascular illnesses. It can also activate the p38 MAPK and NF-kappa beta signaling pathways, which enhances NLRP3 production in the inflammasome and promotes vascular calcification and endothelial cell damage [110]. High administration of TMAO causes oxidative stress, inflammation and suppressed cellular functions, while low levels exhibit a contrary response [111]. A recent study proved that patients with aortic stenosis, had their TMAO levels as 5.5 μM, when the control was 3.6 μM. TMA is also associated with cardiovascular diseases as the levels of TMA in these patients were 59.5 μM and the control was 23.2 μM [112]. Thus, TMAO is considered to be an independent risk factor for cardiovascular diseases.

5. Targeting the TMA/TMAO pathway as a therapeutic strategy to combat CVD risk-current research and future directions

The gut microbiome is a growing area of research in metabolic health and its link to CVD risk. The development of high-throughput metagenomic tools has aided a new understanding of the gut microbiome's role in CVD risk [113]. The gut microbiome can be targeted to modify TMAO synthesis, according to recent fecal microbial transplant research [114] and as a result TMA/TMAO levels can be regulated. Based on research by Maisto et al., in healthy subjects, grape pomace polyphenolic extract has been found to lower serum levels of TMAO [115]. Resveratrol (RSV) reduces TMAO- induced atherosclerosis by lowering TMAO levels and enhancing hepatic bile acid synthesis through gut microbiota remodeling [116]. Antimicrobial phytochemicals, such as allicin, a dietary dosage derived from garlic, effectively neutralize the metabolic ability of gut microbiota to produce TMAO- induced by L-carnitine intake [117]. Luhong granules, a complex blend of herbs, flowers, animal parts, seeds, and roots, prolong ventricular remodeling after myocardial infarction by lowering TMAO and LPS levels in the bloodstream by increasing the gut microflora and intestinal barrier function [118]. A single oral dosage of a cutC/D inhibitor lowers plasma TMAO levels for up to three days and reverses diet-induced platelet reactivity and thrombus formation as studied in animal models, with no toxicity or increased bleeding risk [119]. In experiments with mice models, *Lactobacillus plantarum* ZDY04 significantly reduced serum TMAO and cecal TMA levels in mice by modulating the relative abundance of specific bacterial species, including *Bacteroids* and significantly inhibiting the development of TMAO-induced atherosclerosis in choline fed mice [120]. In high-fat diet-induced obese mice, capsanthin extract prevents obesity, lowers serum TMAO levels, and modifies the gut microbiota composition by decreasing serum triglycerides, total cholesterol, and TMAO levels and markedly increasing microbial diversity [121]. The ability of several oral probiotics to modify circulating TMAO levels in different cohorts, including healthy participants and patients with a CVD-related disease, has been investigated [122–125]. None of them, however, appeared to have a significant effect on TMAO levels in the treatment groups as compared to the placebo groups. In another study, TMA-degrading microorganisms were used by Qiu et al. (2017) to investigate another promising technique for lowering TMA levels in the gut where oral administration of a TMA-metabolizing strain (*Enterobacter aerogenes* ZDY01) reduced TMA in the cecum and TMAO in the serum, as well as changing the microbial community composition in mice, according to their findings [126]. In human studies, changes in urine TMAO levels have been discovered in untargeted metabolomics investigations following supplementation with *Origanum dictamus* tea and *Curcuma longa* extract [127, 128].

6. Conclusions

Diet has been shown to have an important role in the formation of TMAO because it offers the nutritional precursors needed to create TMA and TMAO. There is a positive correlation between circulating TMAO levels and the consumption of food rich in dietary precursors of TMAO like seafood, meat, eggs etc. Targeting the TMA/TMAO metabolism has emerged as a promising tool for cardiovascular disease prevention and treatment in recent years. Targeting the microbiota and host metabolic systems implicated in TMA and TMAO production shows potential for future intervention.

Animal models have largely established the capacity of specific diets, food ingredients, and phytochemicals found in herbs to reduce circulation of TMAO levels. The link between changes in TMAO levels and gut microbiota has only been shown in a few cases, and the exact processes behind the impacts of the dietary items under investigation are yet unknown. More importantly, there are few studies that suggest that lowering circulating TMAO levels has a favorable effect in humans. Because the majority of the studies have been conducted on animal models, the results are difficult to apply to humans. Future research in this area should address conventional microbial research obstacles as well as those more specific to the study of TMA/TMAO metabolism, such as the substantial intra-individual variability of plasma TMAO levels observed in some humans. With the advancement and availability of next-generation sequencing and other omics technologies, a change from studies focusing on defining microbial community composition to more function-oriented research on the gut microbiota is envisaged. Bioinformatic approaches, shotgun metagenomics, meta-transcriptomics, meta-proteomics, and metabolomics, are all expected to be crucial in unraveling the intricate relationships between nutrition, microbial metabolism, and host health.

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Conflict of interest

The authors declare no conflict of interest.

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