

Review Article

Trimethylamine N-oxide, The Important Therapeutic Target for Heart Failure

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Abstract

Heart Failure (HF) is a common cause of morbidity and mortality and is characterized by high morbidity and mortality. The pathophysiology of HF is complex, and it is important to elucidate the molecular mechanism of HF, identify key agents, and conduct further research to discover new therapeutic targets to decrease the incidence and economic burden associated with HF. In 2011, a metabolomic-based study identified that Trimethylamine N-Oxide (TMAO) could predict Cardiovascular Diseases (CVDs) risk. In subsequent studies, TMAO has been found to influence the pathological processes of various CVDs significantly. This review clarifies the effect of TMAO on heart failure from three aspects: the production of Trimethylamine (TMA) and TMAO, TMAO in the pathological process of HF and TMAO can be the therapeutic target for HF, hoping to provide reference for clinical treatment of HF and research on HF.

Keywords: TMAO; Heart failure; cardiovascular diseases

Abbreviations: HF: Heart Failure; TMAO: Trimethylamine N-Oxide; CVDs: Cardiovascular Diseases; TMA: Trimethylamine; FMOs: Flavin-Containing Monooxygenases; FMO3: FMO Isoform 3; DMB: 3,3-Dimethyl-1-Butanol; NF- κ B: Nuclear Factor-kappa-B; TGF- β 1: Transforming Growth Factor beta-1; VECs: Vascular Endothelial Cells; NLRP3: Leucine-Rich Repeat Protein 3; FMD: Fasting-Mimicking Diet; CKD: Chronic Kidney Disease; TCM: Traditional Chinese Medicine; BBR: Berberine; B - GB: Ginkgolide B

Introduction

As the end stage of various Cardiovascular Diseases (CVDs), Heart Failure (HF) is a group of clinical syndromes caused by abnormal cardiac structural and functional changes due to various factors, leading to ventricular systolic and diastolic dysfunctions [1]. It is a common cause of morbidity and mortality. Although an increasing number of medical scientists and researchers have focused on therapeutic targets for HF and great progress has been made in the treatment strategy and new drug research, the incidence of HF is still very high, and the number of patients with HF is increasing. According to statistics, there are 5.7 million people over the age of 20 years with HF in the United States, and the number of adults with HF is expected to rise to 8 million by 2030 [2]. Overall, the prognosis of patients with HF remains poor, and readmission and mortality rates remain high. In a study, 83% of the patients were admitted to the

hospital at least once a year, and 43% were admitted at least four times a year [3]. All-cause mortality ranged from 21.6% to 36.5% and 6.9% to 15.6% in patients with acute HF and chronic HF, respectively [4]. The pathophysiology of HF is complex, including hemodynamic abnormalities, neuroendocrine system activation, cardiac remodeling, and inflammatory response [5]. The investigation of the mechanisms underlying the pathological progression of HF is still ongoing. It is important to elucidate the molecular mechanism of HF, identify key agents, and conduct further research to discover new therapeutic targets to decrease the incidence and economic burden associated with HF.

Recent studies have identified the importance of gut microbiota in the pathophysiology of HF [6-13]. Reduced cardiac output and altered systemic circulation due to HF can cause intestinal hypoperfusion, intestinal mucosal ischemia, and edema

[12]. This increases the likelihood of intestinal barrier damage and permeability, promotes microbial translocation, leads to microbial metabolites entering the blood circulation, increases proinflammatory mediators, and finally leads to chronic inflammation in patients with HF [7,13]. Increased intestinal permeability leads to bacterial translocation across the intestinal barrier, increasing endotoxin and other bacterial wall compounds, which may exacerbate the pathophysiological progression of HF [9,14]. Higher levels of enteropathogenic candida, including *Campylobacter* spp, *Shigella* spp, and *Yersinia enterocolitica*, have been observed in patients with CHF [15].

In 2011, a metabolomic-based study identified metabolites of the dietary lipid phosphatidylcholine, including choline, Trimethylamine N-Oxide (TMAO), and betaine, in an independent large clinical cohort. This research also demonstrated that they could predict CVD risk. The germ-free mice in these studies confirmed that dietary choline and gut microbiota significantly affect TMAO production, macrophage cholesterol accumulation, and foam cell formation. This discovery that gut microbiota-dependent metabolism of dietary phosphatidylcholine contributes to the pathogenesis of CVD opened another way for diagnosing and treating CVDs [16]. In subsequent studies, researchers found that TMAO is associated with a variety of CVDs, such as atherosclerosis [17], hypertension [18], type 2 diabetes [19], myocardial infarction [20], and HF [21]. TMAO has been found to influence the pathological processes of various CVDs significantly. This review aims to discuss the generation of Trimethylamine (TMA) and TMAO, the effect of TMAO in the course of HF, and the potential of TMAO as an important therapeutic target for HF, to have significant references for clinical work.

Production of TMA and TMAO

TMA nutritional precursors, including choline, betaine, croton betaine, trimethyl lysine, G-butyl betaine, phosphatidylcholine, glycerol phosphate choline, and TMAO [22], can be transformed to TMA by particular gut microbial enzymes. Red meat, fish, eggs and products made from cow's milk are rich in these components. Most TMA enter the circulatory system and are subsequently oxidized to TMAO by flavin-containing monooxygenases (FMOs) [23-25]. In the five functional enzymes in the host, FMO isoform 3 (FMO₃) is the crux in this process, with the highest conversion efficiency [26]. In most cases, the kidneys clear TMAO, and the remaining TMAO is reduced to TMA. This process is finished by TMAO reductase in the intestines [27].

It has been shown that changes in the gut microbiota caused by HF can result in changes in TMAO. Nine human gut strains, including Firmicutes and Proteobacteria, were able to produce TMA [28]. Corresponding to these findings, in patients with HF, the increased proportion of gut strains is consistent with the nine human gut strains capable of producing TMA, suggesting that, changes in the gut microbiota can affect TMAO levels. This process is achieved by adjusting the intestinal TMA synthesis.

However, the inherent causes may also significantly affect TMAO levels in humans. Human genes directly influence gut microbiota and can affect immune pathways and metabolic phenotypes [29,30]. In some chronic diseases, such as type 2 diabetes, disease susceptibility can be partly because of genetics that changes the gut microbiome [31]. In an overall analysis of genome-wide gut microbial hosts, microbiota and genetic factors accounted for about 10% of the variation in gut microbiota [32]. On the basis of the significant role of genetics in the gut microbiota, genetics could be thought to play a role in TMAO production.

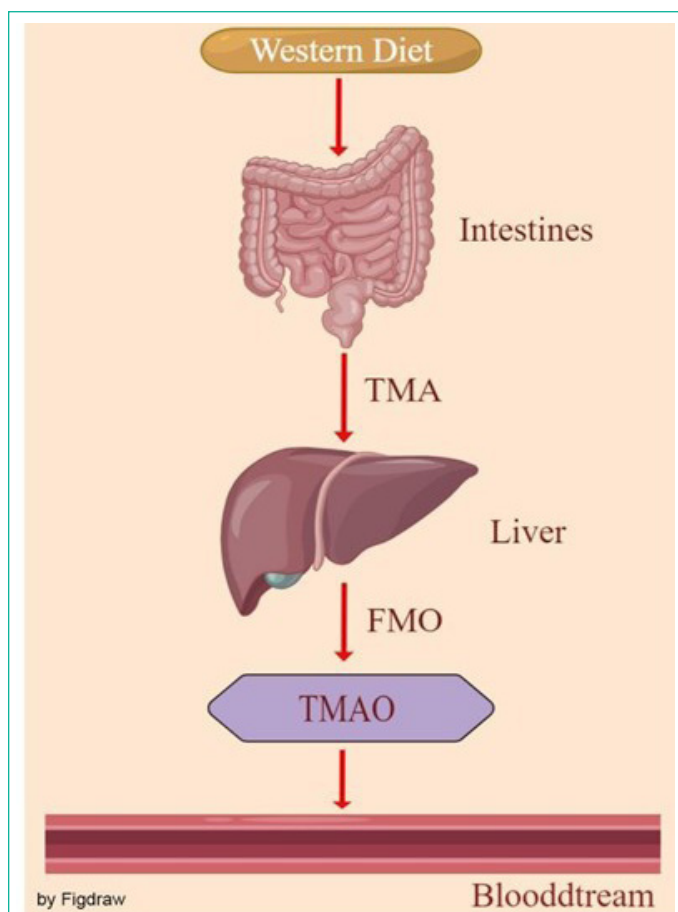


Figure 1: Western diets are abundant in precursors of TMA, which are converted into TMA by specific intestinal microbial enzymes. Most TMA is oxidized to TMAO via FMO. Among the FMOs, FMO3 is key in this process, with the highest conversion efficiency.

TMAO in the Pathological Process of HF

Plasma TMAO level is significantly higher in patients with HF with a preserved ejection fraction [33]. Rats induced by cross-sectional aortic coarctation are excellent animal models of HF. In this model, circulating TMAO levels were significantly higher than in the sham-operated group [34]. In an animal study, mice fed with TMAO or choline exhibited more severe left ventricular dilatation, pulmonary edema, and myocardial fibrosis; the circulating brain natriuretic peptide levels were higher than those in control mice. In patients with HF, higher plasma TMAO levels are associated with poorer prognosis [35]. The effect of TMAO on HF progression is significant [36].

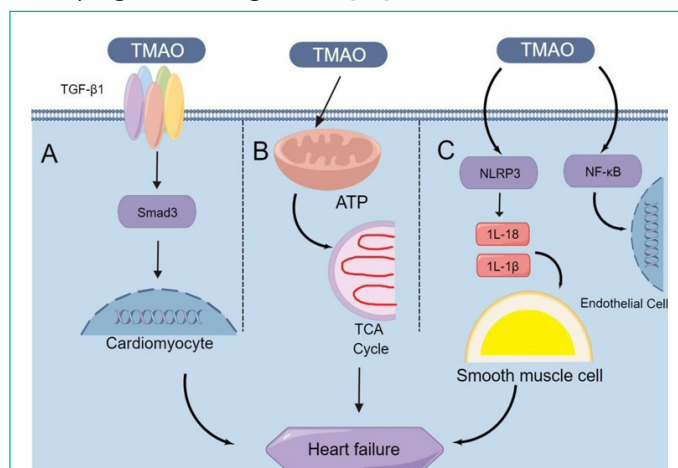


Figure 2: TMAO mainly induces HF by (a) aggravating myocardial hypertrophy and fibrosis, (b) aggravating mitochondrial dysfunction, and (c) inducing inflammatory responses.

TMAO Exacerbates Myocardial Hypertrophy and Fibrosis

In an animal study, TMAO induced cardiac hypertrophy and fibrosis via Smad3 signaling, and a specific inhibitor of Smad3 blocked this effect [37]. 3,3-Dimethyl-1-Butanol (DMB), an inhibitor of TMAO biosynthesis, can prevent myocardial hypertrophy, fibrosis, and inflammation by negatively influencing the p65 Nuclear Factor- κ B (NF- κ B) and Transforming Growth Factor Beta-1 (TGF- β 1)/Smad3 signaling pathways [38]. TMAO can also reprogram skin fibroblasts, adipocyte progenitors, and Vascular Endothelial Cells (VECs) into myofibroblasts via TMAO receptor protein R-like endoplasmic reticulum kinase. Under TGF- β 1 stimulation, FMO3 expression can be observed in skin fibroblasts [39].

TMAO Exacerbated Mitochondrial Dysfunction

Plasma TMAO increased after feeding mice with 120 mg/kg of TMAO for 8 weeks. Impaired pyruvate and fatty acid oxidation disrupted cardiac energy metabolism and mitochondrial dysfunction, leading to ventricular remodeling and HF [40]. Elevated TMAO levels hurt cardiomyocyte mechanics and intracellular calcium handling, and the cardiomyocytes exhibited glycogen accumulation, a higher number of mitochondria, and lipofuscin-like pigment deposition after TMAO exposure. Urolithin B- glucuronide caused cellular contractility and calcium dynamics to recover completely and reduced glycogen accumulation morphologically [41]. There is a potential relationship between TMA metabolism and mitochondrial dynamics, indicating a new direction for future research [42].

TMAO Induced an Inflammatory Response

Inflammation is a vital factor in HF progression. TMAO can stimulate hepatocytes to produce exosomes widely distributed throughout the body and promote vascular inflammation. TMAO can also affect VECs by promoting the production of exosomes that carry important genetic information [43,44]. After a choline diet feeding, the aortas of low-density lipoprotein receptor-deficient mice showed increased inflammatory gene expression than those of controls. TMAO injection can induce the same inflammatory factors and activate mitogen-activated protein kinase, extracellular signal-related kinase, and NF- κ B signaling cascades, leading to inflammation of vascular smooth muscle cells [45]. TMAO activated the Leucine-Rich Repeat Protein 3 (NLRP3) inflammasome to induce vascular inflammation, and NLRP3 inflammasome activation is mediated via the inhibition of the sirtuin 3-superoxide dismutase 2- mitochondrial reactive oxygen species signaling pathway. When NLRP3 inflammasome is activated, it releases inflammatory cytokines, including interleukin (IL)-1 β and IL-18, ultimately leading to endothelial cell inflammation [46,47]. Meanwhile, TMAO levels decreased in NLRP3-deficient mice [48]. TMAO boosted the expression of vascular cell adhesion molecule-1, promoted monocyte adherence, and activated protein kinase C and p-NF- κ B [49]. Molecular studies have shown that in sedentary mice fed a Western diet, plasma TMAO levels were elevated, myocardial inflammation and fibrosis were increased, and voluntary exercise could inhibit all of these [5].

TMAO can be the Therapeutic Target for HF

With increasing research on the important role of TMAO in the pathological process of HF, much progress has been made in reducing TMAO levels in various ways to slow down the symptoms of HF.

Changes in Lifestyle Habits

The TMAO levels are closely related to dietary habits [51]. Chronic exposure to a high-fat diet alters intestinal epithelial physiology and enhances *E. coli* cholinergic metabolism. In addition, cholinergic metabolism in *E. coli* increases the circulating TMAO [52]. Fasting-Mimicking Diet (FMD) is a type of intermittent fasting that restricts calories and limits protein sources of animal origin, and it is applied in daily cycles for 5 days. In one study, FMD decreased plasma TMAO levels by 2-fold, but it was not evident in volunteers eating a vegetarian diet. This suggests that FMD is a viable strategy for decreasing plasma TMAO levels by restricting the intake of calories and animal-derived proteins [53]. TMAO levels are reduced by either dietary TMAO or gut microbial blockade of TMAO generation. Furthermore, these therapeutic strategies alleviate the TMAO levels [54]. The metabolism of dietary L-carnitine and TMA in gut microbiota produces TMAO and aggravates atherosclerosis. Omnivorous volunteers consuming L-carnitine via microbiota-dependent mechanisms produced significantly higher TMAO levels than vegetarians [55]. Eating eggs and fish in bulk also causes markedly higher levels of plasma TMAO [56-60], and the impact of saltwater fish on TMAO is even greater [61]. A vegan diet can significantly reduce plasma TMAO levels [62]. The Mediterranean diet, mainly with fruits and vegetables, nuts, and whole grains, supplemented with small amounts of meat, eggs, and sugar, can optimize the gut microbiota, thus markedly reducing the incidence of HF [63]. Urinary TMAO levels were lower in participants on the Mediterranean diet than in those on the omnivorous diet [64]. Concentrations of TMAO are lower in the presence of a Mediterranean diet, even with a certain intake of red meat [65]. Regular consumption of cocoa and coffee may be an effective strategy for decreasing TMAO levels [66]. *Ligustrum robustum* is rich in flavonoids. It can reduce TMAO levels by modulating gut microbiota to attenuate diet-induced atherosclerosis [67]. In addition, consuming a vitamin D-rich diet can also reduce TMAO levels. A clinical study showed that leptin and TMAO levels were significantly reduced in vitamin D-deficient patients supplemented with 25-hydroxyvitamin D3 [68]. Vitamin D supplementation can modulate intestinal flora in mice fed with high-choline diets to reduce plasma TMAO levels [69]. Physical activity is strongly associated with cardiovascular health. Moderate to vigorous physical activity is associated with lower TMAO levels [70].

Probiotic Flora

Probiotics are live microorganisms that benefit intestinal health [71]. They have a significant effect in adjusting the gut microbiota and maintaining the intestinal environment. A growing body of research suggests that probiotics may be associated with the modulation of myocardial recovery in patients with HF. For instance, in rats, *Lactobacillus plantarum* protects the heart during myocardial ischemia and attenuates reperfusion injury by reducing the left ventricular infarct size and improving cardiac recovery [72]. Both *Enterobacter aerogenes* ZDY01 and *Lactobacillus plantarum* ZDY04 can reduce TMAO levels, showing that the positive effects of them on the heart may be partially realized by reducing TMAO levels [73,74]. *Enterobacter aerogenes* ZDY01 utilized cecal TMA as a nutrient to reduce cecal TMA and serum TMAO levels, but not via altering hepatic FMO3 expression and gut microbiota composition [75]. In an animal study, the researchers chose three lactobacilli with strong adherence capability, and mice with high choline were fed the Multistrain Formula (MF). On days 7, 14, and 28, researchers

observed that, in the MF, *L. amylovorus* LAM1345, *Lpb. plantarum* LP1145, and *Lim. fermentum* LF33 significantly decreased serum TMAO levels [76]. *A. muciniphila* can reduce the level of TMAO by adjusting the microbial enzymes associated with TMA synthesis. In cold environments, these probes are destroyed [77].

Microbial TMA Inhibitors

In Lu'an GuePian tea, the Kaempferol 3-O-rutinoside, quercetin 3-O-rhamnosylgalactoside, kaempferol 3-O-rhamnosylgalactoside, and myricetin 3-O-galactoside bind well with TMA-lyase [78]. Aiming at a major TMA-generating enzyme pair, CutC and CutD (CutC/D), researchers have developed potent, time-dependent, and irreversible inhibitors that do not affect commensal viability. In animal models, the Tookcccc CutC/D inhibitor significantly decreased plasma TMAO levels. This process lasted for 3 days [79]. DMB is a substance present in extra-virgin olive oil. It is a kind of the main components of the Mediterranean diet. DMB reduced circulating TMAO levels in choline-fed mice by inhibiting its formation [38]. Gallic acid and chlorogenic acid also exhibited strong TMA inhibitory activities. Up to 80–90% of TMA was inhibited, with a half-maximal inhibitory concentration of approximately 5 mM [80].

Natural Phytochemicals and Chinese Medicine

Some chemicals in plants can reduce plasma TMAO levels. In human volunteers with high TMAO levels, TMAO formation was reduced after a week of raw garlic juice intake [81]. In a rat model of impaired cardiac function established by intraperitoneal injection of TMAO for 50 days, after administering *Ganoderma lucidum* spores extract, a decrease in serum TMAO level was observed [82]. Resveratrol is a polyphenolic plant anti-toxin. It has anti-inflammatory effects; according to the report, it can alleviate atherosclerosis caused by TMAO by changing intestinal microbiota composition and accelerating the synthesis of hepatobiliary acid [83]. Perinatal resveratrol treatment protects adult male offspring from maternal Chronic Kidney Disease (CKD) and prenatal exposure to asymmetric dimethylarginine-related nitric oxide deficiency and TMAO-induced hypertension, mainly by regulating intestinal flora and its metabolites [84,85]. In resveratrol-fed tilapia, the TMAO level was significantly lower than that in the high-fat diet group at 3, 6, and 9 weeks [86]. Resveratrol and β -sitosterol can also inhibit the production of TMA [87]. The black raspberry extract decreased serum low-density lipoprotein cholesterol and TMAO in rats by affecting the gut bacterial community and microbial metabolites [88]. Traditional Chinese Medicine (TCM) and its extract could also effectively reduce TMAO levels [89]. Berberine (BBR), an extract of *Coptis chinensis*, effectively reduces plasma TMAO levels [90,91]. BBR inhibited FMO3 and generated plasma TMA/TMAO levels in hypertensive mice [92]. Ginkgolide B (GB) is a natural product extracted from Ginkgo leaves. GB could inhibit the mRNA and protein expression of FMO3 and then reduce TMA/TMAO concentrations [93]. In Xuefu Zhuyu decoction, naringin, paeoniflorin, β -ecdysterone, 18 β -glycyrrhizic acid, amygdalin, albiflorin, and saikosaponin A reduced FMO3 activity, and TMAO synthesis [94]. Gypenosides can remodel the microbiota, reduce plasma TMAO levels, and affect TMA-lyase, which is required to convert choline to TMA in the gut microbiota. They can also negatively affect tricarboxylic acid and lipid metabolism enzymes, affecting TMAO and lipid metabolism [95]. Danlou tablet combined with *Salvia miltiorrhiza* ligustrazine injection decreased TMAO levels in patients with central sleep apnea [96]. *Alisma orientalis* is a TCM combined with various medicinal plants. In an animal

study, it decreased the serum TMAO and hepatic FMO₃ levels [97]. In the treatment of acute ischemic stroke, Sanhuang Xiexin Decoction can also decrease the levels of TMAO [98].

Antibiotic

After the use of antibiotics, the plasma levels of TMAO were significantly decreased. Nevertheless, a month after antibiotic withdrawal, the levels of TMAO increased again [99]. TMAO may lead to the failure of anti-infectious therapy. TMAO increased the minimum inhibitory concentration of quinolones, aminoglycosides, and β -lactams as the concentration of TMAO increased and increased the lethal dose of antibiotics against *E. coli* [100]. Nevertheless, it is controversial whether antibiotics have a protective effect in patients with HF. However, not all antibiotics are effective against TMAO. A randomized placebo-controlled trial showed that short-term rifaximin treatment could not reduce gut-derived cardiovascular toxins and inflammatory cytokines in patients with CKD [101]. In patients with HF, although polymyxin B can reduce proinflammatory cytokine production, its application is limited owing to its toxicity [102]. This suggests that, before prescribing antibiotics, doctors should carefully consider safety implications and the potential for antibiotic resistance, necessitating the cautious use of antibiotics.

Other Drugs

Statins have lipid-lowering and many CVD-reducing effects, providing new possibilities for clinicians to choose treatments [103]. Statins are also associated with TMAO. In clinical studies, the plasma TMAO level of patients treated with statins was significantly reduced, indicating that the decrease of TMAO mediated by statins can benefit the cardiovascular system besides improving lipid status and alleviating inflammation [104,105]. Metformin has a therapeutic effect by adjusting the intestinal flora structure and metabolic function. In choline-fed C57BL/6J mice, oral metformin significantly decreased the serum TMAO levels. Metformin can inhibit serum TMAO levels by remodeling the intestinal flora of choline-producing TMA [106,107]. A clinical trial has shown that the cardioprotective drug meldonium can increase the excretion of TMAO in urine and reduce the concentration of TMAO in plasma [108]. Enalapril also decreased the plasma concentration of TMAO in an animal experiment [109].

Conclusion

Although HF is still a serious disease threatening human health, research on its diagnosis and treatment is ongoing. Many studies have shown a direct association between CVD and TMAO. TMAO, a metabolite of the intestinal flora, provides an important reference for analyzing the pathological process of HF and optimizing its treatment. It has many effects on the pathological mechanisms of HF, such as aggravating myocardial hypertrophy, fibrosis, and mitochondrial dysfunction and inducing inflammatory reactions. There has also been much progress in the study of TMAO's impact on HF. It has been found that changing living habits, the use of probiotics, microbial TMA-lyase inhibitors, natural phytochemicals, TCM, and antibiotics can all affect HF by reducing TMAO levels. Despite many achievements, research on TMAO has only recently begun. With the advancement of TMAO research, it is believed that TMAO can profoundly affect the treatment of patients with HF.

References

- Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. 2016; 37: 2129-200.
- Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, et al. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation*. 2015; 131: e29-322.
- Dunlay SM, Redfield MM, Weston SA, Therneau TM, Hall Long K, et al. Hospitalizations after heart failure diagnosis a community perspective. *J Am Coll Cardiol*. 2009; 54: 1695-702.
- Crespo-Leiro MG, Anker SD, Maggioni AP, Coats AJ, Filippatos G, et al. European Society of Cardiology Heart Failure Long-Term Registry (ESC-HF-LT): 1-year follow-up outcomes and differences across regions. *Eur J Heart Fail*. 2016; 18: 613-25.
- Shah AM, Mann DL. In search of new therapeutic targets and strategies for heart failure: recent advances in basic science. *Lancet*. 2011; 378: 704-12.
- Tang WHW, Backhed F, Landmesser U, Hazen SL. Intestinal Microbiota in Cardiovascular Health and Disease: JACC State-of-the-Art Review. *J Am Coll Cardiol*. 2019; 73: 2089-105.
- Sandek A, Bauditz J, Swidsinski A, Buhner S, Weber-Eibel J, et al. Altered intestinal function in patients with chronic heart failure. *J Am Coll Cardiol*. 2007; 50: 1561-9.
- Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, et al. Population-level analysis of gut microbiome variation. *Science*. 2016; 352: 560-4.
- Peschel T, Schonauer M, Thiele H, Anker SD, Schuler G, et al. Invasive assessment of bacterial endotoxin and inflammatory cytokines in patients with acute heart failure. *Eur J Heart Fail*. 2003; 5: 609-14.
- Tang WH, Wang Z, Fan Y, Levison B, Hazen JE, et al. Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: refining the gut hypothesis. *J Am Coll Cardiol*. 2014; 64: 1908-14.
- Tang WH, Kitai T, Hazen SL. Gut Microbiota in Cardiovascular Health and Disease. *Circ Res*. 2017; 120: 1183-96.
- Verbrugge FH, Dupont M, Steels P, Grieten L, Malbrain M, et al. Abdominal contributions to cardiorenal dysfunction in congestive heart failure. *J Am Coll Cardiol*. 2013; 62: 485-95.
- Krack A, Richartz BM, Gastmann A, Greim K, Lotze U, et al. Studies on intragastric PCO₂ at rest and during exercise as a marker of intestinal perfusion in patients with chronic heart failure. *Eur J Heart Fail*. 2004; 6: 403-7.
- Sharma R, Bolger AP, Rauchhaus M, von Haehling S, Doehner W, et al. Cellular endotoxin desensitization in patients with severe chronic heart failure. *Eur J Heart Fail*. 2005; 7: 865-8.
- Pasini E, Aquilani R, Testa C, Baiardi P, Angioletti S, et al. Pathogenic Gut Flora in Patients With Chronic Heart Failure. *JACC Heart Fail*. 2016; 4: 220-7.
- Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011; 472: 57-63.
- Ivashkin VT, Kashukh YA. Impact of L-carnitine and phosphatidylcholine containing products on the proatherogenic metabolite TMAO production and gut microbiome changes in patients with coronary artery disease. *Vopr Pitan*. 2019; 88: 25-33.
- Ge X, Zheng L, Zhuang R, Yu P, Xu Z, et al. The Gut Microbial Metabolite Trimethylamine N-Oxide and Hypertension Risk: A Systematic Review and Dose-Response Meta-analysis. *Adv Nutr*. 2020; 11: 66-76.
- Tang WH, Wang Z, Li XS, Fan Y, Li DS, et al. Increased Trimethylamine N-Oxide Portends High Mortality Risk Independent of Glycemic Control in Patients with Type 2 Diabetes Mellitus. *Clin Chem*. 2017; 63: 297-306.
- Tan Y, Sheng Z, Zhou P, Liu C, Zhao H, et al. Plasma Trimethylamine N-Oxide as a Novel Biomarker for Plaque Rupture in Patients With ST-Segment-Elevation Myocardial Infarction. *Circ Cardiovasc Interv*. 2019; 12: e007281.
- Anderson KM, Ferranti EP, Alagha EC, Mykityshyn E, French CE, et al. The heart and gut relationship: a systematic review of the evaluation of the microbiome and trimethylamine-N-oxide (TMAO) in heart failure. *Heart Fail Rev*. 2022.
- Wang Z, Zhao Y. Gut microbiota derived metabolites in cardiovascular health and disease. *Protein Cell*. 2018; 9: 416-31.
- Day-Walsh P, Shehata E, Saha S, Savva GM, Nemeckova B, et al. The use of an in-vitro batch fermentation (human colon) model for investigating mechanisms of TMA production from choline, L-carnitine and related precursors by the human gut microbiota. *Eur J Nutr*. 2021; 60: 3987-99.
- Goris M, Puntervoll P, Rojo D, Claussen J, Larsen O, et al. Use of Flavin-Containing Monooxygenases for Conversion of Trimethylamine in Salmon Protein Hydrolysates. *Appl Environ Microbiol*. 2020; 86: e2105-20.
- Bennett BJ, de Aguiar Vallim TQ, Wang Z, Shih DM, Meng Y, et al. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metab*. 2013; 17: 49-60.
- Hernandez D, Janmohamed A, Chandan P, Phillips IR, Shephard EA. Organization and evolution of the flavin-containing monooxygenase genes of human and mouse: identification of novel gene and pseudogene clusters. *Pharmacogenetics*. 2004; 14: 117-30.
- Pascal MC, Burini JF, Chippaux M. Regulation of the trimethylamine N-oxide (TMAO) reductase in *Escherichia coli*: analysis of tor::Mud1 operon fusion. *Mol Gen Genet*. 1984; 195: 351-5.
- Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide. *mBio*. 2015; 6: e02481.
- Jacobs J, Braun J. Host genes and their effect on the intestinal microbiome garden. *Genome Med*. 2014; 6: 119.
- Hall AB, Tolonen AC, Xavier RJ. Human genetic variation and the gut microbiome in disease. *Nat Rev Genet*. 2017; 18: 690-9.
- Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, et al. Human genetics shape the gut microbiome. *Cell*. 2014; 159: 789-99.
- Wang J, Thingholm LB, Skieceviciene J, Rausch P, Kummel M, et al. Genome-wide association analysis identifies variation in vitamin D receptor and other host factors influencing the gut microbiota. *Nat Genet*. 2016; 48: 1396-406.
- Dong Z, Zheng S, Shen Z, Luo Y, Hai X. Trimethylamine N-Oxide is Associated with Heart Failure Risk in Patients with Preserved Ejection Fraction. *Lab Med*. 2021; 52: 346-51.

34. Organ CL, Otsuka H, Bhushan S, Wang Z, Bradley J, et al. Choline Diet and Its Gut Microbe-Derived Metabolite, Trimethylamine N-Oxide, Exacerbate Pressure Overload-Induced Heart Failure. *Circ Heart Fail*. 2016; 9: e002314.
35. Li W, Huang A, Zhu H, Liu X, Huang X, et al. Gut microbiota-derived trimethylamine N-oxide is associated with poor prognosis in patients with heart failure. *Med J Aust*. 2020; 213: 374-9.
36. Zhang Y, Wang Y, Ke B, Du J. TMAO: how gut microbiota contributes to heart failure. *Transl Res*. 2021; 228: 109-25.
37. Li Z, Wu Z, Yan J, Liu H, Liu Q, et al. Gut microbe-derived metabolite trimethylamine N-oxide induces cardiac hypertrophy and fibrosis. *Lab Invest*. 2019; 99: 346-57.
38. Wang G, Kong B, Shuai W, Fu H, Jiang X, et al. 3,3-Dimethyl-1-butanol attenuates cardiac remodeling in pressure-overload-induced heart failure mice. *J Nutr Biochem*. 2020; 78: 108341.
39. Kim SJ, Bale S, Verma P, Wan Q, Ma F, et al. Gut microbe-derived metabolite trimethylamine N-oxide activates PERK to drive fibrogenic mesenchymal differentiation. *iScience*. 2022; 25: 104669.
40. Makrecka-Kuka M, Volska K, Antone U, Vilskersts R, Grinberga S, et al. Trimethylamine N-oxide impairs pyruvate and fatty acid oxidation in cardiac mitochondria. *Toxicol Lett*. 2017; 267: 32-8.
41. Savi M, Bocchi L, Bresciani L, Falco A, Quaini F, et al. Trimethylamine-N-Oxide (TMAO)-Induced Impairment of Cardiomyocyte Function and the Protective Role of Urolithin B- Glucuronide. *Molecules*. 2018; 23: 549.
42. Bordoni L, Malinowska AM, Petracci I, Szwengiel A, Gabbianelli R, et al. Diet, Trimethylamine Metabolism, and Mitochondrial DNA: An Observational Study. *Mol Nutr Food Res*. 2022; 66: e2200003.
43. Liu X, Tu J, Zhou Z, Huang B, Zhou J, et al. TMAO-Activated Hepatocyte-Derived Exosomes Are Widely Distributed in Mice with Different Patterns and Promote Vascular Inflammation. *Cardiol Res Pract*. 2022; 2022: 5166302.
44. Liu X, Shao Y, Tu J, Sun J, Li L, et al. Trimethylamine-N-oxide-stimulated hepatocyte-derived exosomes promote inflammation and endothelial dysfunction through nuclear factor-kappa B signaling. *Ann Transl Med*. 2021; 9: 1670.
45. Seldin MM, Meng Y, Qi H, Zhu W, Wang Z, et al. Trimethylamine N-Oxide Promotes Vascular Inflammation Through Signaling of Mitogen-Activated Protein Kinase and Nuclear Factor- kappaB. *J Am Heart Assoc*. 2016; 5: e002767.
46. Sun X, Jiao X, Ma Y, Liu Y, Zhang L, et al. Trimethylamine N-oxide induces inflammation and endothelial dysfunction in human umbilical vein endothelial cells via activating ROS-TXNIP- NLRP3 inflammasome. *Biochem Biophys Res Commun*. 2016; 481: 63-70.
47. Chen ML, Zhu XH, Ran L, Lang HD, Yi L, et al. Trimethylamine-N-Oxide Induces Vascular Inflammation by Activating the NLRP3 Inflammasome Through the SIRT3-SOD2-mtROS Signaling Pathway. *J Am Heart Assoc*. 2017; 6: e006347.
48. Sokolova M, Yang K, Hansen SH, Louwe MC, Kummen M, et al. NLRP3 inflammasome deficiency attenuates metabolic disturbances involving alterations in the gut microbial profile in mice-exposed to high fat diet. *Sci Rep*. 2020; 10: 21006.
49. Ma G, Pan B, Chen Y, Guo C, Zhao M, et al. Trimethylamine N-oxide in atherogenesis: impairing endothelial self-repair capacity and enhancing monocyte adhesion. *Biosci Rep*. 2017; 37: BSR20160244.
50. Zhang H, Meng J, Yu H. Trimethylamine N-oxide Supplementation Abolishes the Cardioprotective Effects of Voluntary Exercise in Mice Fed a Western Diet. *Front Physiol*. 2017; 8: 944.
51. Li J, Li Y, Ivey KL, Wang DD, Wilkinson JE, Franke A, et al. Interplay between diet and gut microbiome, and circulating concentrations of trimethylamine N-oxide: findings from a longitudinal cohort of US men. *Gut*. 2022; 71: 724-33.
52. Yoo W, Zieba JK, Foegeding NJ, Torres TP, Shelton CD, et al. High-fat diet-induced colonocyte dysfunction escalates microbiota-derived trimethylamine N-oxide. *Science*. 2021; 373: 813-8.
53. Videja M, Sevostjanovs E, Upmale-Engela S, Liepinsh E, Konrade I, et al. Fasting- Mimicking Diet Reduces Trimethylamine N-Oxide Levels and Improves Serum Biochemical Parameters in Healthy Volunteers. *Nutrients*. 2022; 14: 1093.
54. Organ CL, Li Z, Sharp TE, 3rd, Polhemus DJ, Gupta N, et al. Non-lethal Inhibition of Gut Microbial Trimethylamine N-oxide Production Improves Cardiac Function and Remodeling in a Murine Model of Heart Failure. *J Am Heart Assoc*. 2020; 9: e016223.
55. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med*. 2013; 19: 576- 85.
56. Miller CA, Corbin KD, da Costa KA, Zhang S, Zhao X, et al. Effect of egg ingestion on trimethylamine-N-oxide production in humans: a randomized, controlled, dose-response study. *Am J Clin Nutr*. 2014; 100: 778-86.
57. Svensson BG, Akesson B, Nilsson A, Paulsson K. Urinary excretion of methylamines in men with varying intake of fish from the Baltic Sea. *J Toxicol Environ Health*. 1994; 41: 411-20.
58. Wang Z, Tang WHW, O'Connell T, Garcia E, Jeyarajah EJ, et al. Circulating trimethylamine N-oxide levels following fish or seafood consumption. *Eur J Nutr*. 2022; 61: 2357-64.
59. Liu X, Shao Y, Sun J, Tu J, Wang Z, et al. Egg consumption improves vascular and gut microbiota function without increasing inflammatory, metabolic, and oxidative stress markers. *Food Sci Nutr*. 2022; 10: 295-304.
60. Kang JW, Zivkovic AM. Are eggs good again? A precision nutrition perspective on the effects of eggs on cardiovascular risk, taking into account plasma lipid profiles and TMAO. *J Nutr Biochem*. 2022; 100: 108906.
61. Lombardo M, Aulisa G, Marcon D, Rizzo G, Tarsisano MG, et al. Association of Urinary and Plasma Levels of Trimethylamine N-Oxide (TMAO) with Foods. *Nutrients*. 2021; 13: 1426.
62. Argyridou S, Davies MJ, Biddle GJH, Bernieh D, Suzuki T, et al. Evaluation of an 8- Week Vegan Diet on Plasma Trimethylamine-N-Oxide and Postchallenge Glucose in Adults with Dysglycemia or Obesity. *J Nutr*. 2021; 151: 1844-53.
63. Carbone S, Billingsley HE, Abbate A. The Mediterranean Diet to Treat Heart Failure: A Potentially Powerful Tool in the Hands of Providers. *JACC Heart Fail*. 2018; 6: 264.
64. De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Stora A, et al. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut*. 2016; 65: 1812-21.
65. Krishnan S, O'Connor LE, Wang Y, Gertz ER, Campbell WW, et al. Adopting a Mediterranean-style eating pattern with low, but not moderate, unprocessed, lean red meat intake reduces fasting serum trimethylamine N-oxide (TMAO) in adults who are overweight or obese. *Br J Nutr*. 2021; 128: 1- 21.
66. Iglesias-Carres L, Racine KC, Neilson AP. Phenolic-rich beverages reduce bacterial TMA formation in an ex vivo-in vitro colonic fer-

- mentation model. *Food Funct.* 2022; 13: 8022-37.
67. Liu S, He F, Zheng T, Wan S, Chen J, et al. *Ligustrum robustum* Alleviates Atherosclerosis by Decreasing Serum TMAO, Modulating Gut Microbiota, and Decreasing Bile Acid and Cholesterol Absorption in Mice. *Mol Nutr Food Res.* 2021; 65: e2100014.
 68. Ozorowski M, Wicinski M, Wrobel L, Fajkiel-Madajczyk A. Cholecalciferol supplementation lowers leptin and TMAO but increases NO and VEGF-A levels in obese vitamin D deficient patients: Is it one of the potential cardioprotective mechanisms of vitamin D? *Nutr Metab (Lond).* 2022; 19: 31.
 69. Wang X, Li X, Dong Y. Vitamin D Decreases Plasma Trimethylamine-N-oxide Level in Mice by Regulating Gut Microbiota. *Biomed Res Int.* 2020; 2020: 9896743.
 70. Argyridou S, Bernieh D, Henson J, Edwardson CL, Davies MJ, et al. Associations between physical activity and trimethylamine N-oxide in those at risk of type 2 diabetes. *BMJ Open Diabetes Res Care.* 2020; 8: e001359.
 71. Fuller R. Probiotics. *Soc Appl Bacteriol Symp Ser.* 1986; 15: 1S-7S.
 72. Lam V, Su J, Koprowski S, Hsu A, Tweddell JS, et al. Intestinal microbiota determine severity of myocardial infarction in rats. *FASEB J.* 2012; 26: 1727-35.
 73. Qiu L, Yang D, Tao X, Yu J, Xiong H, Wei H. *Enterobacter aerogenes* ZDY01 Attenuates Choline- Induced Trimethylamine N-Oxide Levels by Remodeling Gut Microbiota in Mice. *J Microbiol Biotechnol.* 2017; 27: 1491-9.
 74. Qiu L, Tao X, Xiong H, Yu J, Wei H. *Lactobacillus plantarum* ZDY04 exhibits a strain-specific property of lowering TMAO via the modulation of gut microbiota in mice. *Food Funct.* 2018; 9: 4299- 309.
 75. Tang J, Qin M, Tang L, Shan D, Zhang C, et al. *Enterobacter aerogenes* ZDY01 inhibits choline-induced atherosclerosis through CDCA-FXR-FGF15 axis. *Food Funct.* 2021; 12: 9932-46.
 76. Ramireddy L, Tsen HY, Chiang YC, Hung CY, Wu SR, et al. Molecular Identification and Selection of Probiotic Strains Able to Reduce the Serum TMAO Level in Mice Challenged with Choline. *Foods.* 2021; 10: 2931.
 77. Luo Y, Zhang Y, Han X, Yuan Y, Zhou Y, et al. *Akkermansia muciniphila* prevents cold- related atrial fibrillation in rats by modulation of TMAO induced cardiac pyroptosis. *EBioMedicine.* 2022; 82: 104087.
 78. Hua F, Zhou P, Bao GH, Ling TJ. Flavonoids in Lu'an GuaPian tea as potential inhibitors of TMA- lyase in acute myocardial infarction. *J Food Biochem.* 2022; 46: e14110.
 79. Roberts AB, Gu X, Buffa JA, Hurd AG, Wang Z, Zhu W, et al. Development of a gut microbe- targeted nonlethal therapeutic to inhibit thrombosis potential. *Nat Med.* 2018; 24: 1407-17.
 80. Iglesias-Carres L, Essenmacher LA, Racine KC, Neilson AP. Development of a High-Throughput Method to Study the Inhibitory Effect of Phytochemicals on Trimethylamine Formation. *Nutrients.* 2021; 13: 1466.
 81. Panyod S, Wu WK, Chen PC, Chong KV, Yang YT, et al. Atherosclerosis amelioration by allicin in raw garlic through gut microbiota and trimethylamine-N-oxide modulation. *NPJ Biofilms Microbiomes.* 2022; 8: 4.
 82. Liu Y, Lai G, Guo Y, Tang X, Shuai O, et al. Protective effect of *Ganoderma lucidum* spore extract in trimethylamine-N-oxide-induced cardiac dysfunction in rats. *J Food Sci.* 2021; 86: 546-62.
 83. Chen ML, Yi L, Zhang Y, Zhou X, Ran L, et al. Resveratrol Attenuates Trimethylamine-N- Oxide (TMAO)-Induced Atherosclerosis by Regulating TMAO Synthesis and Bile Acid Metabolism via Remodeling of the Gut Microbiota. *mBio.* 2016; 7: e02210-15.
 84. Hsu CN, Hou CY, Chang-Chien GP, Lin S, Yang HW, et al. Perinatal Resveratrol Therapy Prevents Hypertension Programmed by Maternal Chronic Kidney Disease in Adult Male Offspring: Implications of the Gut Microbiome and Their Metabolites. *Biomedicines.* 2020; 8: 567.
 85. Hsu CN, Hou CY, Chang-Chien GP, Lin S, Chan JYH, et al. Maternal resveratrol therapy protected adult rat offspring against hypertension programmed by combined exposures to asymmetric dimethylarginine and trimethylamine-N-oxide. *J Nutr Biochem.* 2021; 93: 108630.
 86. Zheng Y, Shi Y, Yang X, Gao J, Nie Z, et al. Effects of resveratrol on lipid metabolism in liver of red tilapia *Oreochromis niloticus*. *Comp Biochem Physiol C Toxicol Pharmacol.* 2022; 261: 109408.
 87. Heng X, Liu W, Chu W. Identification of choline-degrading bacteria from healthy human feces and used for screening of trimethylamine (TMA)-lyase inhibitors. *Microb Pathog.* 2021; 152: 104658.
 88. Lim T, Lee K, Kim RH, Cha KH, Koo SY, et al. Black raspberry extract can lower serum LDL cholesterol via modulation of gut microbial composition and serum bile acid profile in rats fed trimethylamine-N-oxide with a high-fat diet. *Food Sci Biotechnol.* 2022; 31: 1041-51.
 89. Li Y, Ji X, Wu H, Li X, Zhang H, Tang D. Mechanisms of traditional Chinese medicine in modulating gut microbiota metabolites-mediated lipid metabolism. *J Ethnopharmacol.* 2021; 278: 114207.
 90. Ma SR, Tong Q, Lin Y, Pan LB, Fu J, et al. Berberine treats atherosclerosis via a vitamin- like effect down-regulating Choline-TMA-TMAO production pathway in gut microbiota. *Signal Transduct Target Ther.* 2022; 7: 207.
 91. Li X, Su C, Jiang Z, Yang Y, Zhang Y, et al. Berberine attenuates choline-induced atherosclerosis by inhibiting trimethylamine and trimethylamine-N-oxide production via manipulating the gut microbiome. *NPJ Biofilms Microbiomes.* 2021; 7: 36.
 92. Wang Z, Wu F, Zhou Q, Qiu Y, Zhang J, et al. Berberine Improves Vascular Dysfunction by Inhibiting Trimethylamine-N-oxide via Regulating the Gut Microbiota in Angiotensin II-Induced Hypertensive Mice. *Front Microbiol.* 2022; 13: 814855.
 93. Lv Z, Shan X, Tu Q, Wang J, Chen J, Yang Y. Ginkgolide B treatment regulated intestinal flora to improve high-fat diet induced atherosclerosis in ApoE(-/-) mice. *Biomed Pharmacother.* 2021; 134: 111100.
 94. Yu H, Chai X, Geng WC, Zhang L, Ding F, et al. Facile and label-free fluorescence strategy for evaluating the influence of bioactive ingredients on FMO3 activity via supramolecular host-guest reporter pair. *Biosens Bioelectron.* 2021; 192: 113488.
 95. Zhang Q, Guo X, Xie C, Cao Z, Wang X, et al. Unraveling the metabolic pathway of choline- TMA-TMAO: Effects of gypenosides and implications for the therapy of TMAO related diseases. *Pharmacol Res.* 2021; 173: 105884.
 96. Zhao X, Chen Y, Li L, Zhai J, Yu B, et al. Effect of DLT-SML on Chronic Stable Angina Through Ameliorating Inflammation, Correcting Dyslipidemia, and Regulating Gut Microbiota. *J Cardiovasc Pharmacol.* 2021; 77: 458-69.
 97. Zhu B, Zhai Y, Ji M, Wei Y, Wu J, et al. *Alisma orientalis* Beverage Treats Atherosclerosis by Regulating Gut Microbiota in ApoE(-/-) Mice. *Front Pharmacol.* 2020; 11: 570555.

98. Song J, Chen X, Lyu Y, Zhuang W, Zhang J, et al. Sanhuang Xiexin decoction promotes good functional outcome in acute ischemic stroke. *Brain Behav.* 2019; 9: e01185.
99. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med.* 2013; 368: 1575-84.
100. Qiao J, Liang Y, Wang Y, Morigen. Trimethylamine N-Oxide Reduces the Susceptibility of *Escherichia coli* to Multiple Antibiotics. *Front Microbiol.* 2022; 13: 956673.
101. Kimber C, Zhang S, Johnson C, West RE, 3rd, Prokopienko AJ, Mahnken JD, et al. Randomized, Placebo-Controlled Trial of Rifaximin Therapy for Lowering Gut-Derived Cardiovascular Toxins and Inflammation in CKD. *Kidney360.* 2020; 1: 1206-16.
102. Conraads VM, Jorens PG, De Clerck LS, Van Saene HK, Ieven MM, et al. Selective intestinal decontamination in advanced chronic heart failure: a pilot trial. *Eur J Heart Fail.* 2004; 6: 483- 91.
103. Lawler PR, Bhatt DL, Godoy LC, Luscher TF, Bonow RO, et al. Targeting cardiovascular inflammation: next steps in clinical translation. *Eur Heart J.* 2021; 42:113-31.
104. Li DY, Li XS, Chaikijurajai T, Li L, Wang Z, et al. Relation of Statin Use to Gut Microbial Trimethylamine N-Oxide and Cardiovascular Risk. *Am J Cardiol.* 2022 ;178: 26-34.
105. Xiong X, Zhou J, Fu Q, Xu X, Wei S, et al. The associations between TMAO-related metabolites and blood lipids and the potential impact of rosuvastatin therapy. *Lipids Health Dis.* 2022; 21: 60.
106. Su C, Li X, Yang Y, Du Y, Zhang X, et al. Metformin alleviates choline diet-induced TMAO elevation in C57BL/6J mice by influencing gut-microbiota composition and functionality. *Nutr Diabetes.* 2021; 11: 27.
107. Kuka J, Videja M, Makrecka-Kuka M, Liepins J, Grinberga S, et al. Metformin decreases bacterial trimethylamine production and trimethylamine N-oxide levels in db/db mice. *Sci Rep.* 2020; 10: 14555.
108. Dambrova M, Skapare-Makarova E, Konrade I, Pugovics O, Grinberga S, et al. Meldonium decreases the diet-increased plasma levels of trimethylamine N-oxide, a metabolite associated with atherosclerosis. *J Clin Pharmacol.* 2013; 53: 1095-8.
109. Konop M, Radkowski M, Grochowska M, Perlejewski K, Samborowska E, et al. Enalapril decreases rat plasma concentration of TMAO, a gut bacteria-derived cardiovascular marker. *Biomarkers.* 2018; 23: 380-5.