An increasing amount of evidence reveals that the gut microbiota is involved in the pathogenesis and progression of various cardiovascular diseases. In patients with heart failure (HF), splanchnic hypoperfusion causes ischemia and intestinal edema, allowing bacterial translocation and bacterial metabolites to enter the blood circulation via an impaired intestinal barrier. This results in local and systemic inflammatory responses. Gut microbe-derived metabolites are implicated in the pathology of multiple diseases, including HF. These landmark findings suggest that gut microbiota influences the host’s metabolic health, either directly or indirectly by producing several metabolites. In this review, we mainly discuss a newly identified gut microbiota-dependent metabolite, trimethylamine N-oxide (TMAO), which appears to participate in the pathologic processes of HF and can serve as an early warning marker to identify individuals who are at the risk of disease progression. We also discuss the potential of the gut–TMAO–HF axis as a new target for HF treatment and highlight the current controversies and potentially new and exciting directions for future research. (Translational Research 2021; 228:109–125)

**Abbreviations:** AHF = acute HF; BNP = brain natriuretic peptide; CHF = chronic HF; CVDs = cardiovascular diseases; DASH = Dietary to Stop Hypertension; DIM = 3,3’-diindolylmethane; DMA = dimethylamine; DMB = 3,3-dimethyl-1-butanol; ER = endoplasmic reticulum; FMC = fluoromethylcholine; FMO = flavin-containing monooxygenase; FMT = fecal microbial transplantation; HF = heart failure; HFrEF = HF with reduced ejection fraction; HFrEF = HF with preserved ejection fraction; IL = interleukin; IMC = iodomethylcholine; LPS = lipopolysaccharide; LV = left ventricle; LVAD = left ventricular assist device; LVEF = LV ejection fraction; NF-κB = nuclear factor-κB; NLRP3 = nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3; NT-proBNP = N-terminal pro BNP; PKC = protein kinase C; RCT = randomized controlled trial; SCFA = short-chain fatty acid; SIRT3 = sirtuin 3; SOD2 = superoxide dismutase 2; TCA = tricarboxylic acid; TGF-β1 = transforming growth factor-β1; TMA = trimethylamine; TML = trimethyllysine; TMAO = trimethylamine N-oxide; TNF = tumor necrosis factor; VCAM = vascular cell adhesion molecule; γBB = γ-butyrobetaine
INTRODUCTION

Heart failure (HF) is the end-stage of various types of cardiovascular diseases (CVDs) and is a common cause of disability and death. Despite recent advances in new drugs and therapeutic strategies, the overall prognosis for patients with HF remains poor, which is mainly reflected in a high rate of readmission and mortality. The pathophysiological mechanisms of HF are very complicated, including abnormal hemodynamics, activation of neuroendocrine system, cardiac remodeling and inflammatory response, etc. The activation of the neuroendocrine pathways, consisted of renin-angiotensin-aldosterone system, sympathetic nervous system, and natriuretic peptide system, is traditionally believed to be the main cause for HF. It will lead to a series of pathologic myocardial remodeling processes such as myocardial hypertrophy, apoptosis, and extracellular matrix deposition and resultant fibrosis. Therefore, current treatment strategies are mainly based on neuroendocrine inhibition. However, the mechanisms underlying HF development and progression are still being explored. To reduce the disease and economic burdens that are associated with HF, it is particularly important to clarify the molecular mechanisms through which HF develops, identify crucial mediators, and further explore new potential therapeutic targets.

Recent evidence suggests the potential significance of the gut microbiota and its metabolites in mediating or modulating HF pathophysiology. The gut hypothesis of HF indicates that decreased cardiac output and alteration of systemic circulation will contribute to bowel hypoperfusion and mucosal ischemia. The impaired gut barrier, in turn, may increase gut permeability, facilitate the translocation of microorganisms, and allow the presence of microbial metabolites into the blood circulation, which ultimately leads to low-grade chronic inflammation in HF patients.

In 2013, based on an untargeted metabolomic analysis, researchers first showed that trimethylamine-N-oxide (TMAO), a molecular metabolite that is derived from the gut microbiota, predicted an increased risk of cardiovascular events in 4007 stable cardiac patients undergoing elective coronary angiography. Gut microbiota plays an obligatory role in converting dietary choline into trimethylamine (TMA), which enters the circulatory system before being subsequently oxidized to TMAO in liver. Recently, TMAO has emerged as a significant mediator, demonstrating a close relationship between gut microbiota and multiple CVDs such as atherosclerosis, hypertension, diabetes, and myocardial infarction. Remarkably, TMAO is also a powerful prognostic marker, participating in the progression of HF. Subsequent preclinical experiments show that TMAO may directly affect the heart by inducing myocardial hypertrophy and fibrosis, endothelial cell and vascular inflammation, as well as cardiac mitochondrial dysfunction, thereby aggravating the progress of HF. Here, we review current knowledge about the gut–TMAO–HF axis, and consider its potential translational value as a new therapeutic target in HF.

DISORDERED INTESTINAL METABOLISM IN HF

HF is characterized by reduced cardiac output and insufficient blood supply to meet the body’s demand. The gut is an endocrine organ that is significantly affected by this reduced blood supply. Ischemia and hyperemia caused by a decreased oxygen supply in the intestinal tract will lead to a series of metabolic disorders.

Functional dysbiosis of the gut. Generally, the gut contains a highly complex microbiota that plays a vital role in maintaining health by digesting nutrients, producing vitamins and hormones, interfering with pathogen colonization, and shaping healthy mucosal immunity.

The gut is highly abundant in blood, accounting for approximately 40% of the total human blood. In HF, it is the first organ to undergo ischemia and the last to recover, during which the intestinal villi (and microvilli) are prone to functional anoxia. In addition, visceral venous congestion caused by right HF may also lead to decreased blood flow to intestinal epithelial cells, resulting in cell hypoxia, anaerobic metabolism, and overexpression of the sodium/hydrogen exchanger 3, thereby increasing sodium transport and lowering the lumen pH. All these factors eventually contribute to a shifting composition of the gut microbiota, which is mainly manifested by a reduction in Bacteroides and Bifidobacteria and an increase in Firmicutes and Proteobacteria. Moreover, increased concentrations of enteropathogenic Candida such as Salmonella, Shigella, and Campylobacter were found in fecal samples from chronic HF (CHF) patients. Sandek et al also observed excessive bacterial growth and adhesion in the intestinal mucosa of patients with HF.

Intestinal barrier dysfunction triggers chronic inflammation. HF has been considered as a chronic systemic inflammatory disease, manifested by a significant increase in the levels of various plasma pro-inflammatory cytokines. Unresolved inflammation is a major component of CVD, but its origin remains unclear. Recent evidence suggests that disordered gut microbiota and increased intestinal permeability may be
triggers for chronic inflammation, leading to further impaired cardiac function.28,31

Structurally, systemic congestion in HF patients can cause intestinal wall edema, which results in increased intestinal permeability.28 The disrupted intestinal epithelial barrier will lead to the entry of bacteria and bacterial products into the circulation.32 Patients with bacterial DNA in peripheral blood have significantly higher levels of inflammatory markers such as hyper-sensitive C-reactive protein and interleukin (IL)-6.33 Endotoxin (lipopolysaccharide: LPS), an important toxic and immunogenic component of gram-negative bacteria, also enters the blood through the swollen intestinal wall and is a strong stimulator for activated pro-inflammatory cytokines in HF patients.34,35 LPS induces the production of various downstream inflammatory factors by acting directly on cardiomyocytes, cardiac fibroblasts, and macrophages through Toll-like receptor 4 pattern recognition receptors.36,37 Studies have found that serum levels of multiple cytokines such as IL-1, IL-6, and tumor necrosis factor (TNF) are elevated in HF patients and associated with severe clinical symptoms and worse survival rates.38-40 Meanwhile, these cytokines are also involved in the process of cardiomyocyte apoptosis, hypertrophy, and fibrosis.41

However, current attempts to treat the inflammatory response in HF patients by blocking cytokines have not achieved success.42,43 Thus, more attention has been averted to the role of microbial metabolites in HF.

**Changes in microbial metabolites: focus on TMAO.** An increasing amount of evidence suggests that gut microbiota may have systemic effects on the host by generating bioactive metabolites, such as short-chain fatty acids, bile acids, and TMAO.44 These metabolites influence intestinal health and other physiological systems, particularly the circulatory system. Although most bacterial metabolites are healthy under normal conditions, harmful metabolites will increase when the gut microbiota’s balance is disrupted, and this may be involved in HF-related cardiac pathologic processes. One of the metabolites, TMAO, was shown to be associated with the prognosis of patients with HF.

### TMAO BIOGENESIS AND METABOLISM

Gut microbiota can transform various dietary nutrients into trimethylamine (TMAO). Most of the TMA enters the circulatory system and is subsequently oxidized to TMAO by hepatic flavin-containing mono-oxygenase (FMO). The excess directly decomposes into dimethylamine (DMA) or methane.45 The host FMO family contains 5 functional enzymes, and FMO3 is the key rate-limiting enzyme that is involved in the transformation of TMA to TMAO, with the highest conversion efficiency.46 FMO3 gene mutation contributes to less TMA oxidation, and excessively accumulated TMA is excreted in the urine and sweat and by respiration, producing a strong “fishy” odor.46 Previous studies revealed that TMAO could accumulate in the heart, kidney, or other tissues, participating in various biological processes, such as activating platelet aggregation, increasing foam cell formation, inducing inflammatory responses, and decreasing reverse cholesterol transport.6,9,47 In most cases, the majority of TMAO is eliminated by the kidney, and the rest will be reduced to TMA by the enzyme TMAO reductase in the gut.48,49

Red meat, eggs, fish, and dairy products are abundant in TMA nutritional precursors, such as choline, betaine, L-carnitine, crotonobetaine, trimethyllysine, γ-butyrobetaine, phosphatidylcholine, glycerophosphocholine, and trimethylamine-N-oxide.50,51 These precursors can be converted to TMA via specific intestinal microbial enzymes. To date, 4 different microbial enzyme systems have been identified: choline-TMA lyase (cutC/D),52 carnitine monoxygenase (cntA/B),53 betaine reductase,54 and TMAO reductase.49 In addition, yeaW/X, which is homologous to cntA/B and can utilize a variety of substrates, also promotes TMA synthesis (Fig 1).55

The change in gut microbiota composition that is caused by HF can alter circulating TMAO levels.56 Researchers have discovered 9 human intestinal strains that are capable of producing TMA, such as *Firmicutes* and *Proteobacteria*.57 This is consistent with findings of an increased proportion of these gut bacterial strains in HF patients, which suggests that changes in gut microbiota may affect TMAO levels by regulating intestinal TMA synthesis.

It is worth noting that genetic factors also play an important role in the production of intestinal metabolites. Studies have revealed that host genetic directly affects the composition of the gut microbiome and regulates immune pathways and metabolic phenotypes.58,59 Goodrich et al.60 found that the susceptibility of developing diseases such as obesity may be partially due to genetics which can alter the gut microbiota. Knights et al.61 successfully identified 48 polymorphisms related to inflammatory bowel disease and suggested a complex link between host-genetics and microbial composition of this population. In a comprehensive analysis of genome-wide host—microbiota associations, genetic factors accounted for approximately 10% of the gut microbiome variation.62 Therefore, based on the important role genetics plays in the composition of intestinal flora, it is presumable that in
addition to environmental, dietary and disease factors, genetics also plays a role in the production of TMAO.

PATHOLOGICAL MECHANISMS OF TMAO IN HF

Several experimental studies have demonstrated that TMAO directly or indirectly affects the heart and exacerbates the progression of HF (Table I).

Direct effect. TMAO affects myocardial hypertrophy and fibrosis. In transverse aortic constriction-induced rats, a well-established model of pressure-overloaded HF, the circulating TMAO levels were significantly higher compared with sham-operated groups. Li et al showed that in vivo and in vitro studies, TMAO promoted myocardial hypertrophy and fibrosis via Smad3 signaling pathway. This promotion was blocked by a specific Smad3 inhibitor, SIS3. Subsequently, an inhibitor of TMA synthesis, 3,3-dimethyl-1-butanol (DMB) was found to be capable of preventing myocardial hypertrophy and fibrosis via regulating transforming growth factor-β1 (TGF-β1)/Smad3 and p65 nuclear factor-κB (NF-κB) signaling pathways, which also confirmed the role of TMAO in ventricular remodeling. Additionally, mice fed TMAO or choline showed worse pulmonary edema, left ventricular (LV) dilation, cardiac fibrosis, and elevated circulating brain natriuretic peptide (BNP) levels compared with control groups. A similar result was obtained in another study, in which TMAO aggravated myocardial interstitial and perivascular fibrosis, as well as damaged cardiac compliance and function.

TMAO induces an inflammatory response. In mice that were fed choline, TMAO directly activated inflammatory pathways such as NF-κB signaling, leading to inflammation in vascular smooth muscle cells. Moreover, it can promote the mitochondrial reactive oxygen species (mtROS) accumulation by inhibiting sirtuin 3 (SIRT3) expression and superoxide dismutase 2 (SOD2) activity, which will further activate nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasomes. When activated, the NLRP3 inflammasomes generate IL-1β and IL-18, eventually leading to endothelial cell inflammation. Additionally, Ma et al demonstrated that TMAO upregulated vascular cell adhesion molecule (VCAM)-1 expression by activating protein kinase C (PKC)/NF-κB, which directly resulted in endothelial dysfunction that was characterized by reduced self-healing capacity and increased monocyte adhesion. In a study to investigate whether inhibiting TMAO prevented myocardial inflammation, Zhang et al discovered that TMAO might induce myocardial inflammation by elevating TNF-α levels and
decreasing IL-10 levels, thereby reversing the cardioprotective effect of exercise on myocardial fibrosis.

**TMAO exacerbates mitochondrial dysfunction.** Makrecka-Kuka et al\(^\text{19}\) found that after feeding mice 120 mg/kg of TMAO for 8 weeks, the increased plasma TMAO affected cardiac energy metabolism and mitochondrial function by influencing the pyruvate and fatty acid oxidation, which finally led to the ventricular remodeling and HF development. Subsequently, Savi et al\(^\text{68}\) indicated for the first time that TMAO affected contractile function and intracellular calcium processing in cardiomyocytes, which could be attributed to reduced energy production because of TMAO-induced mitochondrial dysfunction (Fig 2).

**Indirect effect.** In addition to cardiac damage, the role of renal function in HF deterioration should not be neglected.

### Table I. Main pathophysiological mechanisms of TMAO in heart failure: directly and indirectly

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Species/cells</th>
<th>Main findings</th>
<th>Ref</th>
</tr>
</thead>
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<td>Direct effect</td>
<td></td>
<td></td>
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<tr>
<td>Li et al (2018)</td>
<td>Rats</td>
<td>TMAO promoted myocardial hypertrophy and fibrosis via Smad3 signaling pathway.</td>
<td>17</td>
</tr>
<tr>
<td>Wang et al (2020)</td>
<td>Mice</td>
<td>3,3-dimethyl-1-butanol attenuated cardiac remodeling by reducing plasma TMAO levels, which negatively regulated the TGF-β1/Smad3 signaling and p65 NF-κB signaling pathways.</td>
<td>63</td>
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<tr>
<td>Organ et al (2016)</td>
<td>Mice</td>
<td>Mice fed TMAO or choline showed pathological LV dilatation, reduced LV ejection fraction, increased circulating BNP levels, pulmonary edema and myocardial fibrosis.</td>
<td>50</td>
</tr>
<tr>
<td>Seldin et al (2016)</td>
<td>Mice</td>
<td>TMAO promoted recruitment of activated leukocytes to endothelial cells. Activation of NF-κB signaling was necessary for TMAO to induce inflammatory gene expression.</td>
<td>64</td>
</tr>
<tr>
<td>Sun et al (2016)</td>
<td>Human umbilical vein endothelial cells</td>
<td>TMAO induced inflammation and endothelial dysfunction via activating ROS-TXNIP-NLRP3 inflammasome.</td>
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<tr>
<td>Chen et al (2017)</td>
<td>Human umbilical vein endothelial cells and Mice</td>
<td>TMAO promoted vascular inflammation by activating the NLRP3 inflammasome, which was mediated through inhibition of the SIRT3-SOD2—mitochondrial ROS signaling pathway.</td>
<td>65</td>
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<tr>
<td>Ma et al (2017)</td>
<td>Human umbilical vein endothelial cells</td>
<td>Effect of TMAO on endothelial dysfunction was partly attributable to activation of PKC/NF-κB, leading to elevated expression of VCAM-1 and monocyte adhesion.</td>
<td>66</td>
</tr>
<tr>
<td>Makrecka-Kuka et al (2017)</td>
<td>Mice</td>
<td>TMAO concentration impaired pyruvate and fatty acid oxidation in cardiac mitochondria.</td>
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<tr>
<td>Savi et al (2018)</td>
<td>Rats</td>
<td>TMAO had a direct negative impact on cardiomyocyte contractile function and intracellular calcium handling.</td>
<td>68</td>
</tr>
<tr>
<td>Indirect effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tang et al (2015)</td>
<td>Mice</td>
<td>Dietary TMAO promoted renal fibrosis and dysfunction.</td>
<td>15</td>
</tr>
<tr>
<td>Zhu et al (2016)</td>
<td>Mice</td>
<td>TMAO directly increased platelet hyperreactivity and thrombosis formation.</td>
<td>70</td>
</tr>
<tr>
<td>Wang et al (2011)</td>
<td>Mice</td>
<td>Dietary supplementation of mice with TMAO promoted upregulation of multiple macrophage scavenger receptors linked to atherosclerosis.</td>
<td>9</td>
</tr>
</tbody>
</table>

Abbreviations: LV, left ventricular; NF-κB, nuclear factor-κB; NLRP3, nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3; PKC, protein kinase C; ROS, reactive oxygen species; SOD2, superoxide dismutase 2; SIRT3, sirtuin 3; TAC, transverse aortic constriction; TGF-β1, transforming growth factor-β1; TMAO, trimethylamine N-oxide; TXNIP, thioredoxin-interacting protein; VCAM, vascular cell adhesion molecule.
Because the kidney plays a vital role in the process of excreting TMAO, impaired renal function is closely associated with elevated plasma TMAO levels. Data from the Framingham heart study showed that increased TMAO directly contributed to renal interstitial fibrosis and dysfunction, promoting sodium and water retention. All these pathophysiologic mechanisms could interact in a vicious cycle that further aggravates the progression of HF.

For etiology, myocardial ischemia or infarction is the primary cause of HF. Studies have indicated that TMAO significantly induces platelet hyper-reactivity and increases the risk of thrombosis, which can potentially lead to tissue infarction. Wang et al also found that TMAO promoted foam cell formation and aggravated atherosclerosis. All the above pathologic processes are involved in the occurrence of myocardial ischemia or infarction, thus exacerbating the progression of ischemic HF.

**TMAO AS A PROGNOSTIC MARKER OF HF**

TMAO was shown to be a promising cardiovascular risk marker, representing an independent tool for predicting adverse events in patients with HF.

**AHF and CHF.** Suzuki et al first assessed the role of TMAO in acute HF (AHF) and found that circulating TMAO was a marker for predicting death and death/HF within 1 year. However, after adjusting for renal function parameters, TMAO lost the ability of independent prediction, possibly because of the significant associations between TMAO and renal function parameters (urea and estimated glomerular filtration rate).
Combining TMAO with the current clinical risk algorithm improved the risk stratification of in-hospital mortality, and adding the N-terminal pro BNP (NT-proBNP) to this model further enhanced the prediction efficiency of death/HF within 1 year. Moreover, this study also identified that patients with increased levels of both markers (TMAO and NT-proBNP) had the highest risk of death/HF. Recently, after investigating whether the associations between TMAO levels and HF outcomes were influenced by ethnicity, this team found that only elevated TMAO levels in Caucasian patients showed increased association with adverse outcomes, but not in non-Caucasian patients. However, clinical data on TMAO in AHF are very limited, and validation in a larger, multicenter study with a broader cohort is needed to gain additional information and enhance our understanding of the clinical application of TMAO in evaluating the postadmission outcomes in AHF patients.

Studies of TMAO in CHF are more in depth. In a study of 720 stable patients with CHF, the association between elevated TMAO levels and cardiovascular events was identified for the first time. Compared with age- and sex-matched subjects without HF, CHF patients had significantly higher TMAO levels that were associated with a 3.4-fold increased mortality risk. Even following adjustment for traditional risk factors and cardiorenal indexes, the elevated TMAO levels still could predict an increased risk of 5-year mortality. Another study observed that in addition to TMAO, choline and betaine both seemed to participate in the aggravation of LV diastolic dysfunction; however, only elevated TMAO levels showed an adverse prognostic value after adjusting for cardiopulmonary parameters. A similar finding was subsequently reported in a Norwegian cohort, where circulating TMAO levels in CHF patients were associated with the New York Heart Association classification, ischemic etiology, mortality, and survival rate after heart transplantation. The BIOSTAT-CHF (biological research on the personalized treatment of CHF) study first investigated the response of TMAO levels to treatments and discovered that the current guideline-based medications for CHF might not affect circulating TMAO levels. Patients who had low TMAO levels at baseline or follow-up showed higher survival; however, continuous elevated TMAO levels before and after treatment were related to a higher mortality rate. This cohort was a multicenter study involving 11 countries in Europe, and another study based on it explored the impact of geographical factors on TMAO levels. It was found that even after adjusting for confounding, TMAO levels differed by region and their relationships with HF outcomes were also different.

HFrEF and HfPEF. HF subtypes are classified as HF with preserved ejection fraction (HfPEF) and HF with reduced ejection fraction (HFrEF) based on the LV ejection fraction (LVEF). Their pathophysiological mechanisms and prognosis are quite different. To explore the role of TMAO in both HF types, a clinical study of 823 patients found that elevated TMAO levels predicted cardiovascular events in HFrEF patients, but not in HfPEF patients. Conversely, in another study focusing on HfPEF, TMAO levels contributed to risk stratification of HfPEF patients, especially when BNP levels were not high. Moreover, the author suggested that the combination of BNP and TMAO concentrations will provide more valuable prognostic information to HfPEF patients. Because the results of the 2 studies are opposite, further investigations are required to elucidate the specific value of TMAO in HfPEF patients.

Advanced HF. Patients who have been operated heart transplant may represent a subset of the population with advanced HF. In a double-blind, multicenter, open-label study, Troseid et al evaluated circulating levels of γ-butyrobetaine (γBB), TMAO, carnitine, and trimethyllysine (TML) in patients who received heart transplantation and found that the baseline levels of TMAO, carnitine and TML in heart transplant recipients were higher than controls. During the next 3 years of follow-up, plasma TMAO levels were negatively correlated with renal function; increased γBB and TML levels were associated with changes in total atrial volume within 3 years; increased γBB and carnitine levels from baseline to 1 year were associated with high incidence of acute rejection within 1 year of transplantation. Recently, a cross-sectional study explored changes in gut microbiota and inflammatory environment in advanced HF patients after left ventricular assist device (LVAD) or heart transplantation, finding that the diversity of gut microbiota decreased, while the endotoxemia and systemic inflammation increased. This result indicates that the restoration of hemodynamics is only partially responsible for improving the overall state of HF and attention to the new type of intestinal dysfunction-inflammation partnership is necessary.

GUT–TMAO–HF AXIS AS A POTENTIAL THERAPEUTIC TARGET FOR HF

Emerging evidence from various groups and clinical observations showed the relationship among gut microbiota disorder, circulating TMAO level, and HF susceptibility, suggesting that the gut–TMAO–HF axis is
a new and attractive therapeutic target for HF treatment (Table II).

**Dietary intervention.** Plasma TMAO levels are closely related to diet, and how food impacts upon the risk of CVDs has long been a question of interest in the scientific community. Because the gut microbiome composition fluctuates considerably throughout the life cycle, it is hoped to be modified by dietary interventions, which will cause rapid and substantial changes in particular nutrients. The western diet, which is rich in saturated fats, animal protein, and sugars, has been shown to contribute to gut microbiota dysbiosis, upregulated plasma TMAO levels, and an increased risk of CVDs. Moreover, overconsumption of eggs and fish also will lead to significantly higher TMAO levels in plasma and urine. However, the Mediterranean diet, which is characterized by a high intake of fruits and vegetables, nuts, whole grains, and limited quantities of meat, eggs, and sugar, is likely to promote optimal gut microbiota status and significantly reduce the incidence of HF. A systematic review and meta-analysis of randomized controlled trials (RCTs) showed that the Mediterranean diet reduced the incidence of HF by 70%. Similarly, a retrospective cohort study of 3215 postmenopausal women showed a tendency for the Mediterranean diet to be associated with reduced HF mortality, though not to a statistically significant degree. Nevertheless, Dietary to Stop Hypertension (DASH) is deeply associated with lower HF mortality rate. Two other analyses suggested that both DASH and Mediterranean diet might lead to reduced HF morbidity and possibly contribute to secondary prevention. In addition, a high-fiber diet has been reported to prevent the development of HF and effectively improve cardiac remodeling. Kerley et al found that for HF patients, a plant-based diet rich in fruits, vegetables, beans and whole wheat might be beneficial; the role of nuts, dairy products, and poultry is controversial; yet red or processed meat, eggs, and refined carbohydrates seem to be harmful.

In a subsequent postmortem cross-sectional analysis, participants who were on the Mediterranean diet showed lower urinary TMAO levels. Other studies supported this result, showing that reducing red meat and fat intake could lower plasma TMAO levels and alleviate ventricular remodeling. Recently discovered that dietary withdrawal of TMAO improved HF remodeling and cardiac function, and the use of microbial choline TMA lyase inhibitor alleviated choline diet-induced cardiac insufficiency, which suggested that strategies to reduce circulating TMAO levels might counteract the negative effects of dietary choline and TMAO on HF.

Overall, a healthy diet is the most cost-effective way to prevent and treat HF by positively affecting the gut microbiota. A promising treatment in the future is to conduct tailored monitoring of TMAO and to provide dietary advice based on individuals and their circumstances.

**Probiotics, prebiotics, and archaea.** Probiotics are live microorganisms that are beneficial to gut health. They play an essential role in altering intestinal microbiota composition, maintaining host intestinal homeostasis, and improving human health. There is an increasing amount of evidence that probiotics may be involved in regulating myocardial remodeling in HF patients. For example, *Lactobacillus plantarum* exerts cardioprotective effects on rats during ischemia–reperfusion injury by reducing the LV infarction area and improving recovery of cardiac function. Similar results were also reported in some other *Lactobacillus* species, such as *Lactobacillus rhamnosus* GR-1. In HF rats administered GR-1, the LV hypertrophy significantly decreased, and a significant improvement of systolic and diastolic hemodynamic parameters could be observed. Experiments showed that these effects were achieved by reducing serum leptin and increasing tissue taurine levels. Tang et al recently linked gut microbiota to cardiac function after myocardial infarction. In a mouse model of left anterior descending branch ligation, oral poorly absorbed antibiotics inhibited gut microbiota and significantly increased the rate of ventricular rupture and death. However, supplementing antibiotic-treated mice with probiotic Goodbelly (containing *Lactobacillus plantarum* 299v and *Bifidobacterium lactis* Bi-07) prior to myocardial infarction exerted a cardioprotective effect by shifting the host short-chain fatty acid (SCFA) balance to propionic acid. Additionally, recent studies showed that probiotics reduced TMAO levels, suggesting that their protective effects on the heart may be partly achieved by reducing circulatory TMAO. To verify whether this finding can be applied clinically, Costanza et al conducted a 3-month probiotic treatment trial in systolic chronic HF patients. Compared with the placebo groups, they observed significantly decreased inflammatory markers and increased cardiac systolic function in the experimental groups that were treated with *Saccharomyces boulardii*. Preliminary results from another ongoing RCT showed that *brady yeast* can increase LVEF by 5% in patients with systolic HF, and the final results are expected to be achieved in 2020. In conclusion, although studies of probiotics in HF patients remain scarce, this treatment strategy has a great prospect.

Another strategy for regulating gut homeostasis is the administration of prebiotics, which are
Table II. Representative research on targeting gut–TMAO–HF axis for the treatment of heart failure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intervention</th>
<th>Patients/model</th>
<th>Main findings</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>Mediterranean diet</td>
<td>AHF</td>
<td>Adherence to the Mediterranean diet was associated with decreased rate of AHF rehospitalization during the next year</td>
<td>Carbone et al (2018)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>HF</td>
<td>Higher dietary approaches to stop hypertension diet scores were associated with lower mortality in women with HF.</td>
<td>Levitan et al (2013)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>Healthy volunteers; Vegetarians; Vegans; Omnivores</td>
<td>Low adherence to the Mediterranean diet corresponded to an increase in urinary TMAO levels; High-level consumption of plant foodstuffs was associated with beneficial microbiome-related metabolomic.</td>
<td>De Filippis et al (2016)</td>
</tr>
<tr>
<td>Probiotics</td>
<td>Lactobacillus rhamnosus GR-1</td>
<td>Rats</td>
<td>Rats administered GR-1 exhibited significant attenuation of left ventricular hypertrophy and improved hemodynamic parameters.</td>
<td>Gan et al (2014)</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus plantarum 299v</td>
<td>Mice</td>
<td>Supplementing Antibiotic-treated mice with a Lactobacillus probiotic prior to MI yielded cardioprotective effects and shifted the balance of SCFAs towards propionate.</td>
<td>Tang et al (2019)</td>
</tr>
<tr>
<td></td>
<td>Saccharomyces boulardii</td>
<td>CHF patients</td>
<td>CHF patients treated with S. boulardii for 3-months presented reduced inflammatory biomarkers and improved cardiovascular function.</td>
<td>Costanza et al (2015)</td>
</tr>
<tr>
<td>Prebiotics</td>
<td>Inulin-propionate ester/inulin</td>
<td>Adults with overweight and obesity</td>
<td>Supplementing the diet with inulin-propionate ester/inulin improved glucose homeostasis and reduced inflammatory markers in humans.</td>
<td>Chambers et al (2019)</td>
</tr>
<tr>
<td>TMA-lyase inhibitors</td>
<td>FMC/IMC</td>
<td>Mice</td>
<td>FMC and IMC sustainably suppressed host TMAO levels without observed toxicity.</td>
<td>Roberts et al (2018)</td>
</tr>
<tr>
<td></td>
<td>DMB</td>
<td>Mice</td>
<td>DMB attenuated pressure overload-induced cardiac remodeling by reducing plasma TMAO levels.</td>
<td>Wang et al (2020)</td>
</tr>
<tr>
<td>Natural phytochemicals</td>
<td>Resveratrol</td>
<td>Mice</td>
<td>Resveratrol attenuated atherosclerosis by decreasing TMAO levels and increasing hepatic bile acid neosynthesis via gut microbiota remodeling.</td>
<td>Chen et al (2016)</td>
</tr>
<tr>
<td></td>
<td>Allicin</td>
<td>Mice</td>
<td>Dietary allicin might be capable of protecting the host from producing TMAO when carnitine was consumed through its impact on gut microbiota.</td>
<td>Wu et al (2015)</td>
</tr>
<tr>
<td>FMT</td>
<td>Intestinal microbiota from lean donors</td>
<td>Patients with metabolic syndrome</td>
<td>Six weeks after infusion of microbiota from lean donors, insulin sensitivity of recipients increased.</td>
<td>Vrieze et al (2012)</td>
</tr>
<tr>
<td></td>
<td>Metronidazole/ ciprofloxacin</td>
<td>Healthy adult</td>
<td>Plasma TMAO levels were markedly suppressed after administration of antibiotics and then reappeared after withdrawal of antibiotics.</td>
<td>Tang et al (2013)</td>
</tr>
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</table>

Abbreviations: AHF, acute heart failure; CHF, chronic heart failure; DMB, 3-dimethyl-1-butanol; FMC, fluoromethylcholine; FMT, fecal microbial transplantation; HF, heart failure; IMC, iodomethylcholine; MI, myocardial infarction; SCFA, short-chain fatty acid; TMAO, trimethylamine N-oxide.
nondigestible carbohydrates that may modulate the microbiota activity\textsuperscript{105} and provide health benefits to the host. In obese mice, Gibson et al\textsuperscript{106} discovered the ability of prebiotics to improve intestinal permeability, reduce metabolic endotoxemia, and decrease inflammation response. Moreover, \textit{Bifidobacterium animalis} subsp. \textit{lactis} LKM512, which is a type of prebiotics, was observed to decrease intestinal TMA levels in healthy volunteers.\textsuperscript{107} Inulin, a well-recognized prebiotic to promote the growth of beneficial bacteria, can improve diversity and function of gut microbiota to modulate the side effects of antibiotics.\textsuperscript{108} It is known that inulin can stimulate the production of SCFA.\textsuperscript{109} In a RCT study of obese patients, dietary inulin or inulin-propionate supplementation increased the release of SCFA propionate from the colon, which therefore improved insulin sensitivity and reduced systemic inflammatory markers.\textsuperscript{110} Similarly, inulin-enriched diet is able to promote weight loss in obese patients and its effect is related to the characteristics of intestinal flora.\textsuperscript{111} Several studies have also found the role of prebiotics in lowering blood pressure,\textsuperscript{92} preventing low-density lipoprotein oxidation,\textsuperscript{112} and improving visceral obesity.\textsuperscript{113} Although the current scientific evidence does not support the use of prebiotics to treat HF patients or reduce plasma TMAO levels, it can still be used in further trials for CVD.

Archaea are a group of prokaryotic microorganisms that live in extreme environments and morphologically resemble bacteria. Currently, nearly 400 archaeal genomes have been published. Studies found that archaea could reduce gut TMA by partially converting it into methane,\textsuperscript{114,115} however, it is unclear whether archaea can be developed into a therapeutic target in the future, and relevant basic and human studies have not been carried out.

Taking these observations together, we conclude that although the current data on this section are limited, most of the findings support their possible beneficial role in HF and more research will be expected in the future.

**Microbial TMA-lyase inhibitors.** Hazen’s team developed 2 potent inhibitors of cutC/D: fluoromethylcholine (FMC) and iodomethylcholine (IMC), which permanently inactivated cutC/D without affecting the viability of the symbiotic bacteria. In animal models, FMC and IMC significantly reduced the systemic TMAO levels, and reversed TMAO-induced platelet hyperactivity and thrombus formation.\textsuperscript{88} Subsequently, in mice that were fed choline, DMB was shown to reduce circulating TMAO concentrations by inhibiting TMA formation.\textsuperscript{63} DMB is a natural product that is present in extra-virgin olive oil, which is one of the main integral constituents of Mediterranean diet. Therefore, a mechanistic link may exist within the Mediterranean diet and a reduction in TMAO production.

**Natural phytochemicals.** Some chemicals from natural plants have also been shown to reduce plasma TMAO levels. For example, dietary allicin, an active antibacterial compound in garlic, can reduce TMAO formation by affecting the gut microbiota.\textsuperscript{116} Resveratrol, a polyphenolic plant antioxidant with an anti-inflammatory effect, has been reported to rescue TMAO-induced atherosclerosis by shifting the gut microbiota composition, reducing plasma TMAO levels, and accelerating liver bile acid synthesis.\textsuperscript{117}

As described above, a key regulator in the TMAO synthesis pathway is FMO3, which rapidly converts TMA to TMAO. Phytochemicals such as berberine, 3,3'-diindolylmethane (DIM) and indole-3-carbinol have shown promise in inhibiting FMO3 activity and reducing TMAO production.\textsuperscript{118,119} However, FMO3 inhibitors may lead to the occurrence of acute hepatitis and fishy syndrome,\textsuperscript{120} so their adverse effects should be considered carefully before clinical application.

**Fecal microbial transplantation.** Fecal microbial transplantation (FMT) is another approach that can be applied in diseases that are linked to gut dysbiosis, which involves the transplantation of microbes from healthy individuals into the intestines of high-risk patients. Currently, FMT has been clinically shown to be effective for treating intestinal diseases, such as 	extit{Clostridium difficile} infection and inflammatory bowel disease.\textsuperscript{121} Some parenteral diseases are also being explored, such as autism,\textsuperscript{122} obesity,\textsuperscript{123} and type 2 diabetes.\textsuperscript{124} Vrieze et al\textsuperscript{125} performed FMT treatment on patients with metabolic syndrome, and the result indicated that patients receiving gut flora from leptin donor displayed improved insulin sensitivity and increased butyrate production. This finding was validated in another RCT, suggesting that FMT might be a potential treatment strategy for metabolism-related diseases.\textsuperscript{126} In a double-blind RCT, patients with metabolic syndrome received FMT from vegan-donors; however, their ability to produce TMAO was not affected.\textsuperscript{127}

Changes in plasma TMAO level depend on the specific microbiota with TMA lyases. Romano et al\textsuperscript{57} identified nine intestinal strains that were capable of generating TMA, and they found that serum TMAO accumulated in mice that were transplanted with TMA-producing bacteria. Subsequent studies showed that transplantation of high TMA-producing microbes could transmit the potential to generate TMAO to the recipients.\textsuperscript{128,129} To date, FMT has not been used in patients with HF, it is expected that TMAO can be reduced by transplanting low-yield TMAO intestinal flora, but no such clinical studies have been conducted.
Although the initial research is encouraging, the current limitations of FMT still need to be addressed, namely the high risk for both infection and rejection. Further work is required to test the clinical significance of FMT in the field of cardiometabolic disorders. In addition to feces, transplanting specific types of bacterial strains may be an alternative to FMT.

**Antibiotic therapy.** Antibiotics can affect microbe-driven disease by altering the abundance or composition of the gut microbial community. For example, oral administration of vancomycin reduced the infarct area of the left ventricle and improved the cardiac function in a rat model of myocardial ischemia—reperfusion. Moreover, subjects on short-term, low-absorbance antibiotics showed inhibited TMAO synthesis. However, whether the use of antibiotics has a protective effect in HF patients is controversial. Although the antibiotic polymyxin B can reduce the production of pro-inflammatory cytokines and improve the endothelial function of patients with HF, it is clinically limited because of the toxicity. In addition, using antibiotics to inhibit the excessive proliferation of harmful bacteria may also lead to the presence of drug-resistant microbiota in the gut. Therefore, clinicians must weigh the potential side effects of antibiotics carefully before using this medication, and further investigations are needed to assess whether the rational use of antibiotics in certain situations will have favorable effects on heart function and improve HF patient survival.

In an ongoing randomized, open-label, controlled trial called GutHeart, which is investigating the potential relationship between gut microbiota and inflammation pathways in the cardiovascular system, 150 stable HFpEF patients were assigned, in a blinded manner, to rifaximin, brady yeast, or no treatment groups, and the primary endpoint is the LVEF that is measured by echocardiography after 3 months. This study aims to reveal whether targeting gut microbiota using antibiotics can be a new potential strategy to improve the survival rate of HF patients (Fig 3).

Fig 3. Potential therapeutic targets (green) in gut—TMAO—HF axis. Replacing the western diet with the Mediterranean diet is likely to promote optimal gut microbiota status and significantly reduce the incidence of HF. Targeting the intestinal flora, antibiotics, probiotics, prebiotics, and FMT have been shown to regulate gut disorders, as well as some natural phytochemicals such as allicin and resveratrol. DMB, FMC, and IMC, as inhibitors of TMA lyases, can reduce TMA biosynthesis. Archaea is expected to reduce TMA levels by metabolizing it into methane. Additionally, FMO3 inhibitors such as DIM, berberine, and indole-3-carbinol were shown to inhibit FMO3 activity and reduce TMAO production. Thus, the gut—TMAO—HF axis offers promising routes to address HF via these described mechanisms.
CURRENT CONTROVERSIES

Although elevated circulating TMAO is described as a risk factor for HF and is directly involved in the HF pathologic processes, some studies seem to contradict this conclusion, indicating that TMAO may be beneficial to the cardiovascular system.

Tomasz et al.\textsuperscript{135} found that chronic, low-dose TMAO significantly reduced plasma NT-proBNP and vasoressin concentrations, LV diastolic pressure, and myocardial fibrosis in hypertensive rats. Similarly, Collin et al.\textsuperscript{136} showed that in ApoE\textsuperscript{(--/--)} mice that were fed a high-dose L-carnitine, elevated TMAO levels were negatively associated with the size of the aortic lesion without altering the cholesterol content, suggesting that TMAO might delay the formation of aortic lesions and have a protective effect against the occurrence of atherosclerosis. In clinical research of ischemic encephalopathy patients, Yin et al.\textsuperscript{137} showed that CVDs were associated with significantly imbalanced gut microbiota and decreased plasma TMAO levels. However, those with asymptomatic atherosclerosis had no changes in either the gut microbiota or in TMAO levels.

All the above preclinical and clinical studies seem to indicate that a modest increase in plasma TMAO levels appears to have no adverse effect on the circulatory system. However, the limitations of these studies cannot be ignored. In Collin et al.’s study,\textsuperscript{136} the circulating TMAO levels in the L-carnitine group were far less than the estimated levels in patients, and the relationship between TMAO level and aortic lesion size was not apparent. In Yin et al.’s study,\textsuperscript{137} both the disease and the treatment might have significant effects on TMAO levels. For example, stroke patients need to control their dietary intake, which may reduce TMAO levels. Additionally, aspirin is a routine drug for stroke treatment, and it has been demonstrated to decrease plasma TMAO levels.\textsuperscript{138}

Endoplasmic reticulum (ER) stress is involved in foam cell formation and progression of atherosclerosis.\textsuperscript{139} In rodent studies, TMAO acted as a small molecular chaperone that inhibited ER responses.\textsuperscript{140} However, in mice, TMAO was shown to promote atherosclerosis by activating the overexpression of CD36 and scavenger receptor A in macrophages.\textsuperscript{56} A reasonable explanation for this paradox is that TMAO may have specific adverse effects on foam cells, which outweighs its benefits on ER stress inhibition.

Additionally, TMAO is also an osmotic substance that plays a vital role in adapting cells to osmotic and hydrostatic pressure. For example, it was observed to stabilize the structure of peptide regions, thereby protecting the protein from damage caused by hydration.\textsuperscript{141,142} An example is deep-sea fish, which can use TMAO to support protein stability from osmotic and hydrostatic pressure.\textsuperscript{143,144} Therefore, TMAO seems to function as a protein-mate for stable protein structure. Considering the beneficial effects of TMAO in cells, a hypothesis proposes that the elevated TMAO levels are the result of a compensatory response to HF, similar to BNP. When overloaded with volume and pressure, the failing heart will release BNP to alleviate overload by increasing diuresis. Therefore, it is speculated that the increase in TMAO levels during HF is a result of its beneficial role in protecting proteins from the osmotic and hydrostatic pressure.

Another argument for TMAO focuses on fish, especially deep-sea fish, which contain 1.7 g or more of TMAO per pound. A study showed that TMAO exposure from 1 pound of fish might be an order of magnitude more compared with 1 pound of red meat.\textsuperscript{145} However, epidemiological investigations suggested that high fish consumption was significantly related to a reduction in CVD risk factors.\textsuperscript{146} The plausible explanation is that omega-3 fatty acids that are contained in certain fish can protect the cardiovascular system, and the potential benefits outweigh the risks from TMAO.\textsuperscript{147,148}

Given the controversies of whether TMAO is beneficial or harmful to the organisms, some scholars believe that it is TMA, the precursor of TMAO, that has adverse biological effects on the circulatory system. Under physiological conditions, the plasma TMA level is 5–7 times higher compared with TMAO.\textsuperscript{149} As early as 1981, TMA was discovered to be a uremia toxin that had a cytotoxic effect.\textsuperscript{150} It was then shown to be elevated in patients with CVDs, and negatively correlated with eGFR.\textsuperscript{151} Therefore, some scientists consider that it is TMA, rather than TMAO, that matters in patients with a higher cardiovascular risk, and future studies should assess both TMAO and TMA.

FUTURE DIRECTIONS

Several human clinical studies have indicated the profound relationship between gut microbiota composition or its metabolites and HF. However, these studies are typical correlation studies, and it is still challenging to determine whether changes in the gut microbiota are a cause or consequence of HF. Therefore, it is necessary to further establish a prospective research cohort, combining omics data, comprehensive clinical data, and dietary factors to link the gut microbiota composition with disease progression, thereby exploring whether there is a causative relationship between gut microbiota and HF.
Generally, TMAO has 2 values in HF: (1) as a prognostic marker. TMAO is located in a new pathological pathway of HF and it represents the degree of intestinal dysbiosis. It is closely associated with the poor survival of HF patients, which helps clinicians to screen out high-risk groups and pay more attention to their follow-up monitoring as well as enhance HF-related treatments. However, the problem is that the existing clinical research subjects are mainly Caucasian patients and there is a lack of diversity. Therefore, more cohort studies enrolling patients of other races are required to show whether TMAO can be a widely used prognostic marker for HF; and (2) as a potential therapeutic target. Based on previous studies, reducing plasma TMAO levels by specific interventions is expected to reduce mortality in HF patients. However, the mechanisms of TMAO’s participation in HF are not clear, and more research is needed to reveal the underlying pathologic process. In addition, there is no evidence showing that reducing plasma TMAO levels are beneficial, so prospective intervention studies to investigate whether lowering TMAO levels improves the prognosis of HF patients are urgently needed.

Although gut microbial genome sequencing is increasingly becoming a part of research studies, there is still a long way to go before it can be used in clinical practice. Instead, measuring gut microbe-derived metabolites in blood or urine to guide targeted interventions will have a higher clinical translational value.

CONCLUSIONS

Over the past few years, multiple studies have firmly established a direct link between gut microbiota and CVDs. Now we are aware that TMAO, a metabolite produced by the gut microbiota, may provide novel insights into how the gut microbiota contributes to HF. These findings provide an excellent opportunity to develop interventions targeting the gut microbiota for treating HF, such as personalized dietary interventions, probiotics, prebiotics, and FMT. Phytochemicals targeting TMAO metabolism, such as DMB, are also expected to provide potential therapeutic value. However, a wide variety of metabolites can contribute to heart disease, and TMAO may represent the tip of the iceberg. In the future, regulating the composition of gut microbiota and targeting the gut—TMAO—HF axis are both likely to have a profound influence on HF patient survival.

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