HHS Public Access

Author manuscript

Transl Res. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

Transl Res. 2017 January ; 179: 108–115. doi:10.1016/j.trsl.2016.07.007.

Microbiome, Trimethylamine *N*-Oxide (TMAO), and Cardiometabolic Disease

W. H. Wilson Tang, MD[1,2,3] and **Stanley L. Hazen, MD PhD**[1,2]

[1]Department of Cellular and Molecular Medicine, Lerner Research Institute, Cleveland Clinic, Cleveland OH 44195

[2]Department of Cardiovascular Medicine, Heart and Vascular Institute, Cleveland Clinic, Cleveland OH 44195

[3]Center for Clinical Genomics, Cleveland Clinic, Cleveland OH 44195

Abstract

There is increasing appreciation that changes in microbiome composition and function can promote long-term susceptibility for cardiometabolic risk. Gut microbe-derived metabolites that are biologically active, such as trimethylamine N-oxide (TMAO), are now recognized as contributors to atherogenesis. This review summarizes our current understanding of the role of TMAO in the pathogenesis of cardiometabolic diseases, and will discuss current findings, controversies, and further perspectives in this new area of investigation. Better appreciation of the interactions between dietary nutrient intake with gut microbiota-mediated metabolism may provide clinical insights into defining individuals at risk for disease progression in cardiometabolic diseases, as well as additional potential therapeutic targets for reducing risks for cardiometabolic disease progression.

Cardiometabolic diseases include hypertension, type 2 diabetes, dyslipidemia, and excess body fat—individually and collectively, risk factors for cardiovascular disease. While factors such as stress, lack of exercise, obesity, smoking and poor diet are all known to contribute to cardiometabolic disease, the fact remains that in the majority of cases of cardiometabolic disease, no identifiable cause is observed.

Our greatest environmental exposure comes from dietary intake, which in turn exerts important influences on the metabolism and composition of intestinal microbiota. In recent years, there is a mounting body of evidence that suggests that the intestinal microbiome – the bacteria that live in the intestines and are responsible for aiding in the digestion of food – plays an active role in inflammation and metabolism, processes which are strongly linked

Address for Correspondence: W. H. Wilson Tang, MD, 9500 Euclid Avenue, Desk J3-4, Cleveland, OH 44195. Phone: (216) 444-2121, Fax: (216) 445-6165, tangw@ccf.org.

CONFLICTS OF INTEREST

Dr. Tang has no relationships relevant to the contents of this paper to disclose.

with myriad human conditions ranging from hypertension and diabetes to heart and kidney diseases.[1–3] Based on our evolving understanding of how dietary intake is modified by intestinal microbiota, we now consider the intestinal microbiota as perhaps one of the largest "endocrine" systems of the human body, capable of producing metabolites that influence their hosts' (humans') health and disease.

Investigations into the role of microbiome in the pathophysiology of cardiometabolic diseases have traditionally focused on comparing intestinal microbial compositions between disease states. More recently, direct associations between dietary nutrients, intestinal microbiota, and cardiometabolic disease came from recent discovery of trimethylamine *N*-oxide (TMAO) and its mechanistic links to the pathogenesis of atherosclerosis and cardiometabolic diseases.[2, 3] Intestinal microbiota play an obligatory role in the generation of TMAO via specific microbial choline trimethylamine (TMA) lyases. Specifically, microbial conversion of dietary nutrients that possess a TMA moiety (such as choline, phosphatidylcholine and L-carnitine) are converted to TMA by specific microbial enzymes (TMA lyase) via a wide variety of metabolic pathways.[3] TMA is then absorbed by the host and converted into TMAO by hepatic flavin monooxygenase 3 (FMO3) and excreted by the kidneys.[3] Direct demonstration of an obligatory role for gut microbes in TMAO generation in humans has been shown with studies employing poorly orally absorbed antibiotics coupled with the use of dietary intake of isotope-labelled phosphatidylcholine.[4] This review summarizes our current understanding of the role of TMAO in the pathogenesis of cardiometabolic diseases, and will discuss current findings, controversies, and further perspectives in this new area of investigation.

Proatherogenic Properties of TMAO in Atherosclerotic Coronary Artery Disease

Using an untargeted metabolomics approach to generate unbiased small-molecule metabolomic profiles in human plasma in stable cardiac patients undergoing elective coronary angiography, the original experiments were conducted to identify potential circulating metabolites that can be indicative of an underlying pathophysiologic process leading to cardiovascular diseases. Three metabolites of the dietary lipid phosphatidylcholine family (choline, TMAO and betaine) were identified as highly correlated and tracked with adverse cardiovascular event risk over an iterative process.[5]

To directly demonstrate the proatherogenic contribution of microbial-host TMA/TMAO generation from metabolism of dietary nutrients, we leveraged the opportunity of eliminating intestinal microbiota via short-term antibiotic administration or the use of gnobiotic (germ-free) mice. We observed that *ApoE*$^{-/-}$ C57BL/6J mice fed with a choline-rich diet led to increase in plasma TMAO levels and foam cells formation, resulting in more aortic atherosclerotic plaque being developed that were attenuated by antibiotics.[5] Conversely, germ-free mice (lacking gut microbes) and short-term broad-spectrum antibiotic suppression of gut microbiota eliminated TMAO generating capacity, and the latter reduced atherosclerotic burden.[5] These effects were not unique to choline or phosphatidylcholine, but have similarly been observed with other dietary nutrients that can generate TMAO

downstream, including L-carnitine[6] and gamma butyrobetaine.[7] Together, these studies provide key insights into the important role intestinal microbiota play in modulating cardiometabolic diseases in the presence of specific dietary nutrient exposures.

Translating these findings to humans requires demonstration of the association between elevated TMAO levels and adverse clinical consequences in independent cohorts. For example, the original human studies of more than 1,800 stable cardiac patients undergoing elective coronary angiography demonstrated that all TMAO-associated metabolites - choline, betaine, and L-carnitine - had a positive association with prevalent cardiovascular diseases and incident cardiovascular events.[5] Of these three compounds, circulating TMAO levels exhibited a positive correlation with the atherosclerotic plaque size, whereas triglyceride, lipoproteins, fasting glucose, and hepatic triglycerides did not.[5] In a subsequent study of over 4,000 subjects undergoing elective coronary angiography, elevated TMAO levels predicted major adverse cardiac events (MACE, including death, myocardial infarction, and stroke) over a 3-year follow-up period.[4] Specifically, patients in the upper quartile were observed to have a 2.5-fold increased risk of having a major adverse cardiac event compared to the lowest quartile.[4] Such prognostic value was independent of traditional cardiac risk factors, lipid parameters, C-reactive protein, and even renal function.[4] While elevated circulating carnitine and choline/betaine levels (all nutrient precursors for TMAO production) were also associated with future risk of MACE, their prognostic values were primarily restricted to those with concomitant elevated TMAO levels.[8] Of note, the apparent rate limiting step in the TMA/TMAO metaorganismal pathway in subjects appears to be in large part the intestinal microbial step, with vast excess FMO3 activity in most subjects. It thus is perhaps not surprising that genomewide association studies have so far failed to identify significant genetic determinants of TMAO levels in either humans or mice.[9]

Taking advantage of the fact that dietary L-carnitine is primarily found in red meat, a “natural experiment” in humans was to explore the impact of this pathway on host physiology by comparing omnivores versus vegetarians or vegans. Comparison of both intestinal microbiota composition and function between omnivores and vegans/vegetarians revealed stark differences in gut microbial capacity to produce TMA and TMAO from dietary L-carnitine, with vegetarians and vegans having minimal capacity to form TMA from carntine.[6]

Meanwhile, subsequent animal studies have shown that gut microbial formation of TMA from carnitine can also involve initial generation of gamma butyrobetaine, which is then metabolized to TMA[7]. These observations highlight the complexity of multiple metabolic pathways in the gut microbiota ecosystem.

The demonstration of a role of gut microbes in TMA/TMAO formation and atherosclerosis development suggests that one can transmit susceptibility for atherosclerosis by transplanting microbes from donor to recipient. This was experimentally tested in animals by selecting stable microbial communities (cecal microbes) with different TMA-generating capacities to serve as donor, and then transplanting them into recipient atherosclerosis-prone mice, C57BL/6J *ApoE*$^{-/-}$ mice. Different inbred strains of mice were screened, and the proatherogenic C57BL/6J mouse was found to have high TMA and TMAO levels, and the

atherosclerosis resistant NZW/LacJ mouse found to be a low TMAO-producing in-bred strain.[10] Fulfilling the Koch's postulate, use of the atherosclerosis-prone (and high TMA/TMAO producing) C57BL/6J cecal microbes as donor (compared with atherosclerosis-resistant NZW/LacJ-donor microbes) resulted in transmission of both enhanced TMA and TMAO production, and corresponding enhancement in atherosclerotic plaque burden in antibiotic-treated recipients.[10] In fecal/cecal transplant studies with donor microbial community transferring into germ-free recipients, microbial DNA analyses revealed corresponding taxa proportions from donor fecal microbial strains in recipients. Such transmissibility of donor microbial communities directly correlated with plasma TMAO levels and the propensity to develop atherosclerotic lesions in recipients.[10]

Prothrombotic Properties of TMAO

Recently, TMAO has been shown to both associate with incident thrombosis risk in stable cardiac patients undergoing elective coronary angiography, and to directly act on platelets from apparently healthy human subjects, rendering them more hyperresponsive, fostering a prothrombotic phenotype *in vivo*.[11] The mechanism involves TMAO directly acting on the platelets, altering stimulus-dependent calcium release, with resultant augmentation in platelet activation with a submaximal level of agonist like thrombin, adenosine diphosphate, or collagen.[11]

Demonstration of a role for the gut microbial TMAO pathway in fostering a pro-thrombotic phenotype suggested that one might similarly be able to show thrombosis potential was a transmissible trait with microbial transplantation in animal studies. Thus, in an analogous experimental design to that employed for atherosclerosis, cecal microbes from either C57BL/6J (high TMA-producing) versus NZW/LacJ (low TMA-producing) microbes were used as donors, and germ-free recipient mice colonized and placed on choline supplemented vs normal chow diet. Remarkably, microbial transplantation with the high TMA/TMAO producing microbes enhanced TMA/TMAO production, and thrombosis potential in vivo, demonstrating platelet hyperresponsiveness is transmissible from donor to recipient.[11]

TMAO in Diabetes and Insulin Resistance

The role of intestinal microbiota in contributing to obesity and insulin resistance has been established for over a decade when it was first observed that germ-free mice had less body weight compared to their conventional counterparts even with increased caloric intake.[12] Furthermore, germ-free mice were protected from the development of glucose intolerance and insulin resistance from high-fat diet.[13, 14] Meanwhile, obesity has also been shown to be transmissible through microbiome transplantation in studies with germ-free mice. The mechanism is thought to be via increased efficiency of energy harvest in the obesity-related microbiome in part via the production of short-chain fatty acids.[15, 16] These landmark observations have since established the connection between intestinal microbiota, diabetes, and insulin resistance.

Whether intestinal microbiota-generated metabolites such as TMA/TMAO can directly modulate metabolic pathways that are central to the development of obesity and insulin

resistance remains to be explored.[12] Several groups have observed that patients with diabetes tend to have higher TMAO and betaine levels when compared to healthy controls.[17–19] Nevertheless, betaine levels may not directly convert to TMAO, and in the absence of elevated TMAO levels have yet to demonstrate any detrimental adverse consequences.[8]

Animal models have also suggested that elevated TMAO levels can be found in mice with selective hepatic insulin resistance (Liver Insulin Receptor Knockout [LIRKO mice]) via upregulation of the TMAO-producing enzyme FMO3 in the liver.[20] Interestingly, post-prandial insulin can suppress hepatic expression of FMO3 while stimulated by glucagon while being regulated with the insulin-responsive transcription factor FoxO1.[20] Meanwhile, dietary supplementation of TMAO exacerbates glucose intolerance in high fat fed mice,[21] which can be attenuated by fish oil.[22] Consistent with these observations, knockdown of FMO3 (the liver enzymes that converts TMA into TMAO) in cholesterol-fed mice alters biliary lipid secretion, blunts intestinal cholesterol absorption, and limits the production of hepatic oxysterols and cholesteryl esters.[23, 24] Nevertheless, it is important to note that FMO3 is a promiscuous xenobiotic metabolizing enzyme with many substrates and products other than TMA/TMAO, and FMO3 expression can be downregulated by androgens and induced by dietary bile acids in animals.[24] Interestingly, FMO3 knockdown promotes hepatic endoplasmic reticulum stress and inflammation by dampening liver X receptors activation despite lower TMAO levels.[23] Moreover, in the LIRKO mouse model of diabetes on the low density lipoprotein receptor knockout background, antisense oligonucleotide based suppression of FMO3 reduced TMAO and suppressed the hyperglycemia, hypercholesterolemia, and atherosclerosis noted in that model. Interestingly, dietary supplementation of TMAO in mice with antisense oligonucleotide induced FMO3 suppression did not rescue effects on hepatic lipid metabolism or inflammation.[23]

TMAO in Heart Failure and Hypertension

Heart failure (HF) is considered as a final common syndrome that results from a variety of initial cardiac insults (including hypertension as the most common cause) and subsequent imbalances in pathogenic and compensatory mechanisms. It has long been recognized that chronic HF is associated with altered intestinal function. While the "gut hypothesis" in HF has prevailed over the years, emphasis has focused on enhanced gut bacterial translocation and resultant heightened inflammatory responses and oxidative stress as a consequence of HF induced ischemia and congestion within the intestines.[25] Few studies have definitively linked dietary associations with HF outcomes (with the exception of salt intake). Further, the studies supporting the “gut hypothesis” thus far are associative and have not demonstrated a causal link between gut microbial processes and HF pathogenesis.[26]

Several groups have reported the consistent association between higher TMAO, choline, and betaine levels in patients with HF compared with healthy controls.[17, 27, 28] Specifically, elevated plasma TMAO were more likely to be observed in diastolic rather than systolic dysfunction in one study,[27] and in multiple studies, higher TMAO levels are associated with poorer long-term survival, even after adjusting for cardio-renal parameters, and regardless of underlying etiology.[28, 29] In the acute setting where patients were admitted to the hospital

for decompensated heart failure, elevated TMAO levels also have been demonstrated to be an independent predictor for poor survival.[30]

In studies designed to directly test for a dietary induced susceptibility of HF related to the choline/phosphatidylcholine-TMAO pathway, choline- and TMAO-fed C57BL/6J mice were observed more susceptible (vs. control diet) to adverse cardiac remodeling following cardiac insult by transverse aortic constriction, an established animal model of pressure overload (hypertensive) HF.[31] Specifically, increases in plasma TMAO levels in both dietary choline-fed and TMAO-fed mice (compared to chow-fed mice) were associated with increases in natriuretic peptide levels, lung edema, and cardiac structure and function changes indicative of adverse ventricular remodeling (including fibrosis, chamber dilatation, LV wall thinning and reduction in shortening fraction).[31] Interestingly, the degree of myocardial fibrosis was attenuated when TMAO production and gut microbiota were suppressed with a cocktail of poorly-absorbed oral antibiotics.[31] These observations directly demonstrated that dietary choline and TMAO can induce metabolic alterations that foster accelerated development and progression of adverse cardiac remodeling and HF *in vivo*.

Recent studies have suggested a gut microbial contribution to elevated blood pressure via interactions between short-chain fatty acids (especially propionate) and various newly discovered sensory receptors (Olfactory receptor 78 [Olfr78] and G protein couple receptor 41 [Gpr41]).[32] Although hypertension itself is not associated with elevated TMAO levels in the absence of other cardio-renal functional impairments, there have been intriguing animal data to suggest the propensity of elevated TMAO levels to influence the susceptibility of elevated blood pressure. In a rat model with infusion of angiotensin II (AngII) and TMAO (each individually, or combined), elevated blood pressure was only found to be elevated during the infusion period for AngII-treated animals.[33] However, the subgroup receiving both AngII/TMAO infusion demonstrated blood pressure increase well beyond the cessation of infusion, suggesting metabolic alterations by TMAO that influenced the susceptibility for hypertension.[33] These observations are intriguing, as distinct microbiome profiles have been associated as a factor that may possibly explain the race and geographic differences.[34]

TMAO in Chronic Kidney Disease

Since TMAO has a relatively low molecular weight of 75.1 Da, it has long been recognized that the kidneys play a major role in the excretion of both TMA and TMAO,[35] and diminished renal function may impair the ability for the kidneys to eliminate TMAO. In a chicken model, infusion of ^{14}C labeled TMA into the renal portal circulation was almost entirely metabolized in vivo to urinary TMAO, which can be inhibited by either the cationic (quinine) anionic (probenecid) transport blocker in chicken.[35] In contrast, direct infusion of ^{14}C-TMAO did not demonstrate an active excretory transport.[35] Since Urinary TMAO levels rose with episodes of kidney graft dysfunction in renal transplant recipients,[36, 37] an intrinsic accumulation of TMAO (presumably as an osmolyte like urea) that is released during damage of the renal medullary cells may contribute to urinary excretion beyond filtration function. Interestingly, TMAO have one half of the volume of distribution compared to urea, hence resulting in a lower dialytic clearance and greater accumulation in patients with end-stage kidney disease.[38–41] Understanding this delicate relationship

between circulating and urinary TMAO may reveal the dynamic homeostatic imbalance of TMAO formation and elimination and its adverse cardiovascular and renal consequences.

In humans, TMAO is easily filtered and can be effectively removed by hemodialysis. TMAO levels have been observed to be elevated in small cohorts of subjects with end-stage renal disease and chronic kidney disease (CKD), and correlate with both serum urea and creatinine levels.[42, 43] In recent studies with larger patient cohorts of stable patients undergoing elective coronary angiography[29, 41] or hemodialysis,[38–40] TMAO levels are elevated in subjects with CKD (estimated glomerular filtration rate [eGFR] <60mL/min/1.73m^2) compared to non-CKD subjects. Moreover, within the non CKD cohort, higher levels of TMAO were observed in the highest tertile of cystatin C, a marker of renal glomerular filtration function. Interestingly, the prognostic value for TMAO in predicting future MACE in this cohort remained robust even after adjustment for traditional risk factors.[29, 40, 41] These observations highlight the potential association between elevated TMAO and poor outcomes in the setting of CKD – a vulnerable population commonly recognized to be impacted by accumulation of uremic toxins.[44]

Since treatment strategies to reverse CKD are limited, the ability to prevent the development of CKD will be important. A recent untargeted metabolomic study from the Framingham Heart Study in patients without CKD investigated the role of several urinary and circulating metabolites that were associated with the incident development of renal insufficiency[45]. Among the analytes examined, urinary TMAO was detected in relatively high abundance with renal functional impairment and elevated choline and TMAO levels were associated with an increased in future risk of developing CKD.[45] These clinical observations imply a potential association between elevated TMAO levels and the propensity for cardio-renal insult.

Pathogenic Mechanism and Therapeutic Implications of TMAO in Cardiometabolic Diseases

There have been emerging reports from various groups regarding the potential pathophysiologic mechanisms that may contribute to the TMAO pathway and cardiovascular disease pathogenesis. The overall metaorganismal pathway involves a complex interplay between dietary nutrients, intestinal microbiota metabolism, and host pathogenic pathways. Several mechanisms have been postulated based on animal experiments (some with human validation) to date. First, there appears to be an alteration in the host sterol and lipid metabolic pathways based on investigations across intestinal tissues in animal models following exposure to dietary choline or TMAO. Specifically, there was an increase in forward cholesterol transport and a decrease in reverse cholesterol transport, as well as decrease in bile acid pool size and altered composition.[6] Furthermore, inhibition of the TMAO producing enzyme FMO3 protects mice from atherosclerosis, in part by stimulating an intestinal pathway of reverse cholesterol transport called trans-intestinal cholesterol excretion.[23, 46] As described above, another mechanism that has recently been discovered is the effects of TMAO on platelet responsiveness. Submaximal stimulus-dependent platelet activation from multiple agonists can be enhanced through augmented Ca2+ release from

intracellular stores, which may explain the ability for TMAO to modulate platelet hyperresponsiveness and thrombosis potential in both animal models and in humans.[11]

Direct pathogenic effects of TMAO may occur also at the level of the kidneys. In keeping with the human studies showing elevated TMAO levels predict risk for incident development of CKD,[45] animal studies reveal that chronic exposure to a high choline- or TMAO-supplemented diet (compared to chow-fed mice) resulted in both significant TMAO elevations and corresponding increases in renal tubulointerstitial fibrosis and collagen deposition.[29] Meanwhile, dietary supplementation with either choline or TMAO both resulted in increases in renal fibrosis and renal phospho-Smad3 levels, an important regulator of the profibrotic transforming growth factor-β (TGFβ)/Smad3 signaling pathway during fibrotic kidney disease.[29] Using mouse embryonic fibroblasts, phosphorylation of ERK1/2 and Smad3 (all members of TGFβ-associated pathways) in cell lysates can be associated with enhanced collagen 1 generation with TMAO exposure,[47] further implicating the activation of TGFβ-associated profibrotic pathways as a mechanism stimulated by TMAO.

Finally, recent studies indicate acute administration of TMAO can activate the mitogen-activated protein kinase, extracellular signal-related kinase, and nuclear factor-kappa B signaling cascade in both endothelial cells and vascular smooth muscle cells, as well as promote endothelial cell adhesion of leukocytes in vivo.[47] Taken together, there is growing data to indicate TMAO triggers vascular inflammation, injury, and pro-thrombotic and fibrotic processes that may contribute to the development of cardiometabolic diseases.

Inter-individual variability in capacity to produce TMAO is widely observed.[48, 49] Current data supports the conclusion that the major origin of inter-individual differences is due to the large diversity of gut microbiota and community structure among individuals. Recent observations indicate that overall gut microbial community structure is modulated by long-term dietary exposure.[50] Indeed, oral supplementation of L-carnitine has been shown to raise microbial capacity for TMAO production in the setting of impaired excretion in patients undergoing hemodialysis.[51] In fact, generation of TMAO is intricately related to the presence of the substrate (nutrient) itself rather than the food groups being consumed – as in the case of persistent TMAO production with oral L-carnitine supplementation despite dietary meat restrictions.[52] These observations directly support the potential therapeutic implications for targeting and monitoring TMAO levels for dietary interventions.

Various microbial enzymes have been identified that are capable of generating TMA from different dietary nutrients (microbial TMA lyases).[6, 53–55] Microbial TMA lyases are expressed in diverse array of intestinal microbial taxa, and has been shown to generate systemic TMAO upon choline exposure.[10, 56] In recent studies, it has been shown that a small molecule antagonists can be used to inhibit microbial TMA formation, attenuating TMA and TMAO levels *in vivo*, with coordinate reduction in atherosclerosis. A natural product, 3,3-dimethyl-1-butanol (DMB), was shown to inhibit distinct microbial TMA lyases, and to both inhibit TMA production from physiologic polymicrobial cultures and reduce TMAO levels in mice fed a high-choline/L-carnitine diet.[57] Furthermore, DMB inhibited choline diet-enhanced endogenous macrophage foam cell formation and

atherosclerotic lesion development in *ApoE*$^{-/-}$ C57BL/6J mice, all without alterations in circulating cholesterol levels.[57] These promising findings suggest that specific targeting of intestinal microbial TMA production by specific inhibitors is feasible and can serve as a potential therapeutic approach for the treatment of cardiometabolic diseases.

Future Perspectives

A rapidly growing body of literature supports the role of gut microbiota and TMAO pathway as important contributors to the atherosclerosis process. Moreover, it is now clear that the gut microbiome generates biologically active compounds that circulate in the blood stream and act at distant sites. Thus, the gut microbiome functions as an endocrine organ, and participates in generating signals to the host that can promote obesity, insulin resistance and atherosclerosis susceptibility. However, many questions remain and required future investigations. For example, it is still unclear whether individual health conditions and their beneficial treatment could modify the underlying microbiome and their metabolites in humans, especially in conditions with end-organ dysfunction (heart failure, chronic kidney disease) that have been well recognized to generate great metabolic perturbations. Serial samples in human subjects with different conditions before and after treatment would allow further investigations into biological variability and treatment responses as detected by these metabolites. As no genetic influences to TMAO have yet been found and yet there can be multiple metaorganismal sites that may influence TMAO production and elimination, investigations using novel mouse genetic techniques or human rare variants may provide further clarifications. More detailed studies looking into the role of dietary and lifestyle modifications would also be insightful.

Given the strong associations of the gut microbial TMAO pathway with human disease, this work has broad implications in drug discovery efforts targeting gut microbes themselves instead of the human host they reside in. Further studies are warranted to explore potential druggable interventions aiming to target the gut microbiome, including identification and development of effective nutritional interventions and supplements, medications and possibly probiotics/prebiotics that are protective and safe for human consumption. Although mechanistic data support a direct role for TMA/TMAO-specific signaling pathways in the host, addition work is needed to understand the most therapeutically tractable targets in the entire meta-organismal pathway.

Acknowledgments

All authors have read the journal’s authorship agreement and that the manuscript has been reviewed by and approved by all named authors.

FUNDING

Drs. Tang and Hazen are supported by grants from the National Institutes of Health (NIH) and the Office of Dietary Supplements (R01HL103866, P20HL113452, R01DK106000) related to the content of this paper. Dr. Hazen was partially supported by a gift from the Leonard Krieger endowment.

Dr. Hazen is named as inventor on pending patents held by the Cleveland Clinic relating to cardiovascular diagnostics and therapeutics. Dr. Hazen is a paid consultant for Esperion and P&G. Dr. Hazen has received research funds from P&G, Pfizer Inc., Roche Diagnostics, and Takeda. Dr. Hazen has received royalty payments for

inventions or discoveries related to cardiovascular diagnostics or therapeutics from Cleveland HeartLab, Siemens, Esperion, and Frantz Biomarkers, LLC.

REFERENCES

1. Aron-Wisnewsky J, Clement K. The gut microbiome, diet, and links to cardiometabolic and chronic disorders. Nat Rev Nephrol. 2016; 12:169–181. [PubMed: 26616538]
2. Brown JM, Hazen SL. The gut microbial endocrine organ: Bacterially derived signals driving cardiometabolic diseases. Annu Rev Med. 2015; 66:343–359. [PubMed: 25587655]
3. Tang WH, Hazen SL. The contributory role of gut microbiota in cardiovascular disease. J Clin Invest. 2014; 124:4204–4211. [PubMed: 25271725]
4. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med. 2013; 368:1575–1584. [PubMed: 23614584]
5. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, Hazen SL. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011; 472:57–63. [PubMed: 21475195]
6. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, Smith JD, DiDonato JA, Chen J, Li H, Wu GD, Lewis JD, Warrier M, Brown JM, Krauss RM, Tang WH, Bushman FD, Lusis AJ, Hazen SL. Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med. 2013; 19:576–585. [PubMed: 23563705]
7. Koeth RA, Levison BS, Culley MK, Buffa JA, Wang Z, Gregory JC, Org E, Wu Y, Li L, Smith JD, Tang WH, DiDonato JA, Lusis AJ, Hazen SL. Gamma-butyrobetaine is a proatherogenic intermediate in gut microbial metabolism of l-carnitine to tmao. Cell Metab. 2014; 20:799–812. [PubMed: 25440057]
8. Wang Z, Tang WH, Buffa JA, Fu X, Britt EB, Koeth RA, Levison BS, Fan Y, Wu Y, Hazen SL. Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-n-oxide. Eur Heart J. 2014; 35:904–910. [PubMed: 24497336]
9. Hartiala J, Bennett BJ, Tang WH, Wang Z, Stewart AF, Roberts R, McPherson R, Lusis AJ, Hazen SL, Allayee H. Comparative genome-wide association studies in mice and humans for trimethylamine n-oxide, a proatherogenic metabolite of choline and l-carnitine. Arterioscler Thromb Vasc Biol. 2014; 34:1307–1313. [PubMed: 24675659]
10. Gregory JC, Buffa JA, Org E, Wang Z, Levison BS, Zhu W, Wagner MA, Bennett BJ, Li L, DiDonato JA, Lusis AJ, Hazen SL. Transmission of atherosclerosis susceptibility with gut microbial transplantation. J Biol Chem. 2015; 290:5647–5660. [PubMed: 25550161]
11. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, Li L, Fu X, Wu Y, Mehrabian M, Sartor RB, McIntyre TM, Silverstein RL, Tang WH, DiDonato JA, Brown JM, Lusis AJ, Hazen SL. Gut microbial metabolite tmao enhances platelet hyperreactivity and thrombosis risk. Cell. 2016; 165:111–124. [PubMed: 26972052]
12. Li D, Kirsop J, Tang WH. Listening to our gut: Contribution of gut microbiota and cardiovascular risk in diabetes pathogenesis. Curr Diab Rep. 2015; 15:63. [PubMed: 26208694]
13. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:15718–15723. [PubMed: 15505215]
14. Backhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:979–984. [PubMed: 17210919]
15. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. Nature. 2009; 457:480–484. [PubMed: 19043404]
16. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444:1027–1031. [PubMed: 17183312]

17. Tang WH, Wang Z, Fan Y, Levison B, Hazen JE, Donahue LM, Wu Y, Hazen SL. 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; 64:1908–1914. [PubMed: 25444145]
18. Lever M, George PM, Slow S, Bellamy D, Young JM, Ho M, McEntyre CJ, Elmslie JL, Atkinson W, Molyneux SL, Troughton RW, Frampton CM, Richards AM, Chambers ST. Betaine and trimethylamine-n-oxide as predictors of cardiovascular outcomes show different patterns in diabetes mellitus: An observational study. PLoS One. 2014; 9:e114969. [PubMed: 25493436]
19. Dambrova M, Latkovskis G, Kuka J, Strele I, Konrade I, Grinberga S, Hartmane D, Pugovics O, Erglis A, Liepinsh E. Diabetes is associated with higher trimethylamine n-oxide plasma levels. Exp Clin Endocrinol Diabetes. 2016; 124:251–256. [PubMed: 27123785]
20. Miao J, Ling AV, Manthena PV, Gearing ME, Graham MJ, Crooke RM, Croce KJ, Esquejo RM, Clish CB, Vicent D, Biddinger SB. Flavin-containing monooxygenase 3 as a potential player in diabetes-associated atherosclerosis. Nat Commun. 2015; 6:6498. [PubMed: 25849138]
21. Gao X, Liu X, Xu J, Xue C, Xue Y, Wang Y. Dietary trimethylamine n-oxide exacerbates impaired glucose tolerance in mice fed a high fat diet. Journal of bioscience and bioengineering. 2014; 118:476–481. [PubMed: 24721123]
22. Gao X, Xu J, Jiang C, Zhang Y, Xue Y, Li Z, Wang J, Xue C, Wang Y. Fish oil ameliorates trimethylamine n-oxide-exacerbated glucose intolerance in high-fat diet-fed mice. Food Funct. 2015; 6:1117–1125. [PubMed: 25686243]
23. Warrier M, Shih DM, Burrows AC, Ferguson D, Gromovsky AD, Brown AL, Marshall S, McDaniel A, Schugar RC, Wang Z, Sacks J, Rong X, Vallim TA, Chou J, Ivanova PT, Myers DS, Brown HA, Lee RG, Crooke RM, Graham MJ, Liu X, Parini P, Tontonoz P, Lusis AJ, Hazen SL, Temel RE, Brown JM. The tmao-generating enzyme flavin monooxygenase 3 is a central regulator of cholesterol balance. Cell Rep. 2015
24. Bennett BJ, de Aguiar Vallim TQ, Wang Z, Shih DM, Meng Y, Gregory J, Allayee H, Lee R, Graham M, Crooke R, Edwards PA, Hazen SL, Lusis AJ. Trimethylamine-n-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. Cell Metab. 2013; 17:49–60. [PubMed: 23312283]
25. Verbrugge FH, Dupont M, Steels P, Grieten L, Malbrain M, Tang WH, Mullens W. Abdominal contributions to cardiorenal dysfunction in congestive heart failure. J Am Coll Cardiol. 2013; 62:485–495. [PubMed: 23747781]
26. Nagatomo Y, Tang WH. Intersections between microbiome and heart failure: Revisiting the gut hypothesis. J Card Fail. 2015; 21:973–980. [PubMed: 26435097]
27. Tang WH, Wang Z, Shrestha K, Borowski AG, Wu Y, Troughton RW, Klein AL, Hazen SL. Intestinal microbiota-dependent phosphatidylcholine metabolites, diastolic dysfunction, and adverse clinical outcomes in chronic systolic heart failure. J Card Fail. 2015; 21:91–96. [PubMed: 25459686]
28. Troseid M, Ueland T, Hov JR, Svardal A, Gregersen I, Dahl CP, Aakhus S, Gude E, Bjorndal B, Halvorsen B, Karlsen TH, Aukrust P, Gullestad L, Berge RK, Yndestad A. Microbiota-dependent metabolite trimethylamine-n-oxide is associated with disease severity and survival of patients with chronic heart failure. J Intern Med. 2015; 277:717–726. [PubMed: 25382824]
29. Tang WH, Wang Z, Kennedy DJ, Wu Y, Buffa JA, Agatisa-Boyle B, Li XS, Levison BS, Hazen SL. Gut microbiota-dependent trimethylamine n-oxide (tmao) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. Circ Res. 2015; 116:448–455. [PubMed: 25599331]
30. Suzuki T, Heaney LM, Bhandari SS, Jones DJ, Ng LL. Trimethylamine n-oxide and prognosis in acute heart failure. Heart. 2016
31. Organ CL, Otsuka H, Bhushan S, Wang Z, Bradley J, Trivedi R, Polhemus DJ, Tang WH, Wu Y, Hazen SL, Lefer DJ. Choline diet and its gut microbe-derived metabolite, trimethylamine n-oxide, exacerbate pressure overload-induced heart failure. Circ Heart Fail. 2016; 9:e002314. [PubMed: 26699388]
32. Pluznick J. A novel scfa receptor, the microbiota, and blood pressure regulation. Gut Microbes. 2014; 5:202–207. [PubMed: 24429443]

33. Ufnal M, Jazwiec R, Dadlez M, Drapala A, Sikora M, Skrzypecki J. Trimethylamine-n-oxide: A carnitine-derived metabolite that prolongs the hypertensive effect of angiotensin ii in rats. Can J Cardiol. 2014; 30:1700–1705. [PubMed: 25475471]
34. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI. Human gut microbiome viewed across age and geography. Nature. 2012; 486:222–227. [PubMed: 22699611]
35. Acara M, Camiolo S, Rennick B. Renal n-oxidation of trimethylamine in the chicken during tubular excretion. Drug Metab Dispos. 1977; 5:82–90. [PubMed: 13980]
36. Foxall PJ, Mellotte GJ, Bending MR, Lindon JC, Nicholson JK. Nmr spectroscopy as a novel approach to the monitoring of renal transplant function. Kidney Int. 1993; 43:234–245. [PubMed: 8433564]
37. Le Moyec L, Pruna A, Eugene M, Bedrossian J, Idatte JM, Huneau JF, Tome D. Proton nuclear magnetic resonance spectroscopy of urine and plasma in renal transplantation follow-up. Nephron. 1993; 65:433–439. [PubMed: 8289995]
38. Hai X, Landeras V, Dobre MA, DeOreo P, Meyer TW, Hostetter TH. Mechanism of prominent trimethylamine oxide (tmao) accumulation in hemodialysis patients. PLoS One. 2015; 10:e0143731. [PubMed: 26650937]
39. Kaysen GA, Johansen KL, Chertow GM, Dalrymple LS, Kornak J, Grimes B, Dwyer T, Chassy AW, Fiehn O. Associations of trimethylamine n-oxide with nutritional and inflammatory biomarkers and cardiovascular outcomes in patients new to dialysis. J Ren Nutr. 2015; 25:351–356. [PubMed: 25802017]
40. Missailidis C, Hallqvist J, Qureshi AR, Barany P, Heimburger O, Lindholm B, Stenvinkel P, Bergman P. Serum trimethylamine-n-oxide is strongly related to renal function and predicts outcome in chronic kidney disease. PLoS One. 2016; 11:e0141738. [PubMed: 26751065]
41. Stubbs JR, House JA, Ocque AJ, Zhang S, Johnson C, Kimber C, Schmidt K, Gupta A, Wetmore JB, Nolin TD, Spertus JA, Yu AS. Serum trimethylamine-n-oxide is elevated in ckd and correlates with coronary atherosclerosis burden. J Am Soc Nephrol. 2016; 27:305–313. [PubMed: 26229137]
42. Bell JD, Lee JA, Lee HA, Sadler PJ, Wilkie DR, Woodham RH. Nuclear magnetic resonance studies of blood plasma and urine from subjects with chronic renal failure: Identification of trimethylamine-n-oxide. Biochim Biophys Acta. 1991; 1096:101–107. [PubMed: 2001424]
43. Bain MA, Faull R, Fornasini G, Milne RW, Evans AM. Accumulation of trimethylamine and trimethylamine-n-oxide in end-stage renal disease patients undergoing haemodialysis. Nephrol Dial Transplant. 2006; 21:1300–1304. [PubMed: 16401621]
44. Lekawanvijit S, Kompa AR, Wang BH, Kelly DJ, Krum H. Cardiorenal syndrome: The emerging role of protein-bound uremic toxins. Circ Res. 2012; 111:1470–1483. [PubMed: 23139286]
45. Rhee EP, Clish CB, Ghorbani A, Larson MG, Elmariah S, McCabe E, Yang Q, Cheng S, Pierce K, Deik A, Souza AL, Farrell L, Domos C, Yeh RW, Palacios I, Rosenfield K, Vasan RS, Florez JC, Wang TJ, Fox CS, Gerszten RE. A combined epidemiologic and metabolomic approach improves ckd prediction. J Am Soc Nephrol. 2013; 24:1330–1338. [PubMed: 23687356]
46. Shih DM, Wang Z, Lee R, Meng Y, Che N, Charugundla S, Qi H, Wu J, Pan C, Brown JM, Vallim T, Bennett BJ, Graham M, Hazen SL, Lusis AJ. Flavin containing monooxygenase 3 exerts broad effects on glucose and lipid metabolism and atherosclerosis. J Lipid Res. 2015; 56:22–37. [PubMed: 25378658]
47. Seldin MM, Meng Y, Qi H, Zhu W, Wang Z, Hazen SL, Lusis AJ, Shih DM. Trimethylamine n-oxide promotes vascular inflammation through signaling of mitogen-activated protein kinase and nuclear factor-kappab. J Am Heart Assoc. 2016; 5
48. McEntyre CJ, Lever M, Chambers ST, George PM, Slow S, Elmslie JL, Florkowski CM, Lunt H, Krebs JD. Variation of betaine, n, n-dimethylglycine, choline, glycerophosphorylcholine, taurine and trimethylamine-n-oxide in the plasma and urine of overweight people with type 2 diabetes over a two-year period. Ann Clin Biochem. 2015; 52:352–360. [PubMed: 25013088]
49. Mi Park E, Lee E, Jin Joo H, Oh E, Lee J, Lee JS. Inter- and intra-individual variations of urinary endogenous metabolites in healthy male college students using (1)h nmr spectroscopy. Clinical chemistry and laboratory medicine. 2009; 47:188–194. [PubMed: 19191725]

50. Albenberg LG, Wu GD. Diet and the intestinal microbiome: Associations, functions, and implications for health and disease. Gastroenterology. 2014; 146:1564–1572. [PubMed: 24503132]
51. Bain MA, Faull R, Milne RW, Evans AM. Oral l-carnitine: Metabolite formation and hemodialysis. Curr Drug Metab. 2006; 7:811–816. [PubMed: 17073580]
52. Miller MJ, Bostwick BL, Kennedy AD, Donti TR, Sun Q, Sutton VR, Elsea SH. Chronic oral l-carnitine supplementation drives marked plasma tmao elevations in patients with organic acidemias despite dietary meat restrictions. JIMD Rep. 2016
53. Craciun S, Balskus EP. Microbial conversion of choline to trimethylamine requires a glycyl radical enzyme. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109:21307–21312. [PubMed: 23151509]
54. Craciun S, Marks JA, Balskus EP. Characterization of choline trimethylamine-lyase expands the chemistry of glycyl radical enzymes. ACS chemical biology. 2014; 9:1408–1413. [PubMed: 24854437]
55. Zhu Y, Jameson E, Crosatti M, Schafer H, Rajakumar K, Bugg TD, Chen Y. Carnitine metabolism to trimethylamine by an unusual rieske-type oxygenase from human microbiota. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111:4268–4273. [PubMed: 24591617]
56. 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. [PubMed: 25784704]
57. Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, Gu X, Huang Y, Zamanian-Daryoush M, Culley MK, DiDonato AJ, Fu X, Hazen JE, Krajcik D, DiDonato JA, Lusis AJ, Hazen SL. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. Cell. 2015; 163:1585–1595. [PubMed: 26687352]