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Gut microbiota and cardiovascular disease

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Abstract

Fecal microbial community changes are associated with numerous disease states, including cardiovascular disease (CVD). However, such data are merely associative. A causal contribution for gut microbiota in CVD has been further supported by a multitude of more direct experimental evidence. Indeed, gut microbiota transplantation studies, specific gut microbiota-dependent pathways, and down-stream metabolites have all been shown to influence host metabolism and CVD, sometimes through specific identified host receptors. Multiple metaorganismal pathways (involving both microbe and host) both impact CVD in animal models and show striking clinical associations in human studies. For example, trimethylamine N-oxide (TMAO) and more recently, phenylacetyl glutamine (PAG), are gut microbiota-dependent metabolites whose blood levels are associated with incident CVD risks in large scale clinical studies. Importantly, a causal link to CVD for these and other specific gut microbial metabolites/pathways have been shown through numerous mechanistic animal model studies. PAG, for example, was recently shown to promote adverse cardiovascular phenotypes in the host via interaction with multiple adrenergic receptors, a class of key receptors that regulate cardiovascular homeostasis. In this review, we summarize recent advances of microbiome research in CVD and related cardiometabolic phenotypes that have helped to move the field forward from associative to causative results. We focus on microbiota and metaorganismal compounds/pathways, with specific attention paid to short chain fatty acids, secondary bile acids, TMAO, and PAG. We also discuss novel therapeutic strategies for directly targeting the gut microbiome to improve cardiovascular outcomes.

Keywords

Cardiovascular Disease; Gut Microbiome; Atherosclerosis; Thrombosis; Vascular Disease; Inflammation, Metabolism

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Introduction

The collection of microbes living in the human intestinal tract (gut microbiota) and their combined genetic capacities (gut microbiome) have influence far beyond digestion. Indeed, gut microbiota generate biologically active metabolites that impact many aspects of host physiology, and collectively are widely considered the body's largest endocrine organ. While gut microbiota facilitate many necessary and beneficial physiological processes, like the digestion of macronutrients and synthesis of some vitamins, numerous lines of evidence show gut microbiota can play a role in the development of adverse phenotypes. In particular, distinct changes in the microbial community structure and function are associated with multiple disease states, including cardiovascular disease (CVD)¹.

Early work in the gut microbiome field demonstrated that alterations in fecal microbial community composition are associated with the development of obesity and insulin resistance, and that microbial transplantation could transmit heightened adiposity in the host²⁻⁴. Subsequently, it was discovered that disruptions to the microbiome early in life can promote heightened adiposity⁵. Early sequencing studies by Koren *et al.* suggested microbiota may be linked to atherosclerosis, because human atherosclerotic plaques were noted to contain bacterial DNA, though whether or not the DNA was derived from live bacteria within the artery wall was not determined⁶. The first studies revealing a potential causal link between the gut microbiome and CVD focused on trimethylamine N-oxide (TMAO), a metaorganismal metabolite formed following ingestion of dietary nutrients abundant in a Western diet (e.g. lecithin, choline, carnitine)⁷⁻⁹. The microbiome field has since rapidly expanded to involve many previously disparate areas of research, demonstrating the far-reaching effects of gut microbiota on human health and disease. Figure 1 illustrates several of the major pathways identified linking gut microbiota to CVD, numerous related phenotypes, and known molecular participants, including some of the identified host receptors. We will use Figure 1 as a template for organizing this review, first providing an overview of the field, and then focusing on several areas of more recent advancement. Finally, we will discuss therapeutic targeting of the gut microbiome as a potential treatment or prevention strategy for cardiovascular and metabolic diseases, highlighting recent advances in development of non-lethal small molecule inhibitors of specific microbial pathways as a novel approach to improve CVD outcomes.

Gut bacterial community compositional changes and association with CVD

Due to advances in culture-independent sequencing technologies and bioinformatics, the vast majority of gut microbiome related studies assess gut (often fecal) microbial community compositional changes associated with various disease states. This has led to a wealth of associative data, which, while helpful, is limited in terms of investigating causality. While it is difficult to define a truly pathogenic bacterial community, the term *dysbiosis* has been used to describe an imbalance of intestinal microbiota composition within a disease state or phenotype. Many investigators have reported an association between CVD phenotypes and changes in the relative abundance of specific microbial taxa, or gut bacterial richness or diversity. For example, in early studies bacterial DNA was detected in atherosclerotic plaques with signatures that match taxa associated with disease states⁶, and microbial

systemic circulation, and low cardiac output contribute to bowel wall edema and reduced mucosal perfusion²². Intestinal hypoperfusion in heart failure alters mucosal function, as evidenced by increased paracellular permeability and augmented intestinal bacterial biofilm formation²³. When the gut barrier is impaired, lipopolysaccharide (LPS) originating from Gram-negative bacteria can enter the host circulation, where it is mainly recognized by toll-like receptor (TLR)s on the surface of immune cells²⁴. Upon binding of bacterial ligands, TLR-signaling induces release of pro-inflammatory cytokines that orchestrate a pro-inflammatory state in the host.

Enhanced levels of LPS and other bacterial wall products, presumably derived from gut microbiota, have been mechanistically linked to modulation of inflammation, immunity, and vascular function (Figure 1). Patients with decompensated heart failure have higher endotoxin levels in the blood compared to stable patients²⁵. Translocation of LPS from the bowel in this setting is supported by higher endotoxin concentrations in the hepatic vein as compared to blood directly sampled from the ventricles²⁶. The detection of gut microbiota and metaorganismal metabolites at heightened levels in subjects with CVD, or risk for incident adverse CVD events, may in part reflect alterations of the host barrier function (Figure 1). In a recent observational study, circulating LPS concentrations were predictive of MACE in a cohort of patients with atrial fibrillation, suggesting that endotoxin translocation impacts CVD complications²⁷. It is worth noting that a Mediterranean diet was negatively associated with endotoxemia in this study, suggesting the involvement of the gut microbiota in gut barrier function of the host. Indeed, in preclinical models, gavage of western diet-fed ApoE^{-/-} mice with live *Akkermansia muciniphila* decreased intestinal permeability and lowered fecal and circulating LPS levels, which was associated with reduced aortic atherosclerosis independent of lipid metabolism²⁸. While the mechanism of *Akkermansia*-induced changes in barrier function needs to be explored, a recent proof-of-concept study in humans found that administration of pasteurized (but not live) *Akkermansia muciniphila* for 3 months led to reduction in plasma LPS in obese individuals with metabolic syndrome²⁹. Although the intervention did not change body weight, the authors observed improvement in insulin sensitivity and dyslipidemia in the *Akkermansia*-treated group. Interestingly, structural differences of LPS subtypes from different gut microbial species have been associated with altered TLR recognition and their effects on host innate immunity³⁰. For instance, gavage with the gram-negative *Bacteroides (vulgatus and dorei)* that produce penta- and tetra-acylated lipid A – in contrast to the hexa-acylated lipid A of *e. coli*, reduced colonic inflammation, endotoxemia, and atherosclerosis in ApoE^{-/-} mice³¹.

It should be noted that factors beyond gut leakiness contribute to CVD. There are many disease conditions where enhanced “gut leakiness” is present, but not all such diseases show heightened associations with CVD risks. For example, while the presence of heart failure-induced bowel wall edema has been linked to endotoxemia and CVD progression, gut barrier defects caused by colitis and inflammatory bowel diseases are not classically associated with heightened CVD risks. These observations point to a more complex relationship between bowel wall integrity, changes in gut microbial communities, and the relationship between host systemic inflammation and altered susceptibility for development of CVD. We speculate that different mechanisms of “gut leakiness” (e.g. inflammatory, bowel wall edema, etc.) may differentially impact gut microbiome and pathophysiological

processes that modulate susceptibility for development of CVD. Improved understanding of the role of gut bacterial pro-inflammatory factors in triggering systemic inflammatory cascades may help to provide novel therapeutic strategies to improve care and risk stratification among patients with CVD.

Numerous lines of evidence link multiple different facets of inflammation to heightened risks for cardiovascular disease^{32, 33}. The role of inflammatory pathways in CVD has recently been reaffirmed by the CANTOS clinical trial. Administration of canakinumab, an antibody against Interleukin(IL)-1 β , showed that inhibition of the IL-1 β pathway reduces cardiovascular event risk independent of lipid level lowering³⁴. Thus, treatment and prevention of CVD with immunomodulatory therapies seems promising. But targeting inflammatory pathways bears the risk for opportunistic infections, which may limit its use in patients with multiple comorbidities, as is often observed in CVD patients. Identification of gut microbiota that elicit host immune responses that participate in CVD pathogenesis may provide a therapeutic avenue to ameliorate inflammation-driven CVD phenotypes.

Bile acids are predominantly gut microbiota derived, and serve as modulators of host metabolism

A major function historically attributed to bile acids (BAs) has been to facilitate emulsification and adsorption of fat-soluble dietary nutrients. However, BAs are composed of a diverse array of structurally specific species whose concentrations differ by many orders of magnitude. More recent studies have shown structurally specific and distinct BAs also play additional roles, including but not limited to modulation of host lipid metabolism, glucose/insulin metabolism, and inflammation (Figure 1).

Initially synthesized from cholesterol in the host liver, primary BAs, which only represent a small fraction of the total BA pool, are then secreted into the intestinal (duodenum) lumen, where subsequent gut microbiota dependent modifications participate in the generation of a remarkably large array of BA species. The body maintains a large pool of hydrophobic BAs through reuptake in the ileum and through negative feedback. These negative feedback mechanisms are triggered by activation of the farnesoid x receptor (FXR) and cholesterol 7 α hydroxylase³⁵, or by expression of intestinal bile transporters³⁶. BAs modulate gut microbial composition via potent antimicrobial properties, immune responses³⁷ and FXR³⁸. And bile obstruction can lead to bacterial overgrowth syndromes³⁹. Gut microbiota modify primary BAs via bile salt hydrolysis and bile acid 7 α dehydroxylation, yielding secondary BAs, many of which have hormone-like functions. Following systemic adsorption, some secondary BAs in turn impact host physiology through interaction with a variety of host nuclear receptors including FXR, liver X receptor (LXR), pregnane X receptor (PXR), and specific G-protein coupled receptors like TGR5 (Figure 1)^{40–44}. Perturbations to the dynamic interactions among diet, gut microbiota, and specific BAs may thus contribute to cardiometabolic phenotypes and disease susceptibility. For example, altered levels of BAs in plasma are associated with insulin resistance in type 2 diabetes^{45, 46}, and modulation of BA signaling may contribute to metabolic improvements during some anti-diabetic treatments⁴⁷. As such, measuring systemic levels of BAs may aid in the assessment of potential gut

microbiota contributions to cardiometabolic diseases⁴⁶. A better understanding of how perturbation in bile acid profiles are associated with future development of disease states, or responses to therapy. Functional studies to determine if candidate bile acids are mechanistically linked to these processes are promising areas of future investigation.

Short chain fatty acids (SCFAs) and blood pressure in the host

SCFAs (fatty acids with 5 carbons or less) can be products of host metabolism (e.g. acetate⁴⁸). However, they are also produced in large quantities by gut microbiota through anaerobic fermentation of dietary fiber^{49, 50}. The most common SCFAs include acetate, propionate, and butyrate, which have been linked to alterations in host blood pressure homeostasis, myocardial repair and inflammation (Figure 1). The idea that circulating SCFAs are produced in large part by gut commensals is supported by studies showing that free SCFA levels are virtually undetectable in plasma recovered from germ-free animals⁵¹. Besides acting as an energy source for large intestine gut epithelial cells (e.g. colonocytes), SCFAs are absorbed into the portal blood and participate in various processes of the host, including lipid metabolism, glucose homeostasis, gut inflammation, and neurogenesis⁵². An association between SCFAs and adiposity early in life has been shown by Cho *et al*⁵. Exposure to antibiotics during weaning changed gut microbial communities with increasing metabolic capacity to produce acetate, propionate, and butyrate in C57BL/6J mice. Influx of these SCFAs to the liver resulted in substantial changes in the regulation of hepatic lipid metabolism and obese phenotype⁵. Moreover, antibiotic exposure early in life has also been associated with changes in microbiota diversity⁵³, lasting effects on the host immunity⁵⁴, and cardiometabolic diseases, such as diabetes^{55, 56}. Interestingly, transfer of antibiotic-perturbed microbiota to the next generation of mice led to loss of microbial richness and changes in metagenomic gene expression and susceptibility for colitis⁵⁷.

Initial clinical intervention studies reported that fiber intake is associated with a decrease in blood pressure⁵⁸, and support the idea that SCFAs are involved in the regulation of blood pressure. Early mechanistic studies by Pluznick and colleagues substantiated this idea by demonstrating the G-protein coupled SCFA receptors olfactory receptor 78 (Olf78) and G-protein receptor 41 (Gpr41) participate in host blood pressure regulation⁵⁹⁻⁶¹. Being expressed in vascular smooth muscle cells as well as the juxtaglomerular apparatus, Olf78 mediates renin release and changes in vascular resistance, contributing to hypertension⁶⁰. By contrast, Gpr41 is expressed in the vascular endothelium and promotes reduction in blood pressure⁶¹. Propionate administration in the absence of Gpr41 tended to increase blood pressure, while it caused a pronounced drop in blood pressure in Olf78^{-/-} mice, suggesting a differential function of both receptors in SCFA-dependent regulation of blood pressure⁶⁰. Interestingly, when the SCFA pool was depleted by antibiotics in Olf78^{-/-} mice, blood pressure went up, further corroborating a protective role of microbial SCFA generation to balance Olf78 signaling. However, the overall hypotensive effects in wildtype animals might be explained by both a decline in cardiac output and the loss of vascular resistance exhibited by SCFAs⁶². While microbiota suppression with anti-microbials (i.e. poorly-absorbed antibiotics; Figure 3) can serve as a valuable tool for demonstrating involvement of the gut microbiome in a host phenotype, it is not a viable approach for therapeutically

targeting the gut microbiome to achieve a desired long term outcome in the host, due to the selection of gut microbial communities with antibiotic resistance.

Many additional studies have supported a role for gut microbiota generation of SCFAs in modulation of blood pressure in the host. For example, fecal transfer from human hypertensive (versus normotensive) donors into germ-free mice revealed transmission of heightened blood pressure⁶³. But in another study, transplantation of feces from normotensive Dahl salt-resistant rats into hypertensive Dahl salt-sensitive rats in fact exacerbated hypertension in the recipients, suggesting that additional host genetic variables may interact with microbial factors to modulate blood pressure control⁶⁴. A role for SCFAs in hypertensive end-organ damage in angiotensin II-infused mice has also been suggested⁶⁵. Thus, numerous lines of evidence show that the gut microbial community can impact blood pressure regulation in the host, and that SCFAs represent at least one of the microbial mediators that contribute to vasomotor tone and blood pressure. Recent studies provided further evidence that SCFAs are involved in other CVD processes, such as ischemia reperfusion injury, cardiac repair following myocardial infarction, and impaired arterial compliance^{66, 67}.

SCFAs represent a readout of saccharidic metabolism by the whole microbial community, and often serve as terminal end products of (poly)microbial catabolic pathways. Therefore, their levels may reflect a convergence of multiple microbial participants and competing pathways. Further understanding of the factors that contribute to individual molecular species of SCFAs and the host receptors sensing them is an area of future investigation. Of particular interest, recent studies employing untargeted metabolomics suggest additional gut microbiota-derived plasma metabolites beyond SCFA may also contribute to host blood pressure regulation⁶⁸. These studies found multiple structurally specific compounds including some “uremic toxins” that had previously been reported to activate the renin–angiotensin–aldosterone system (RAAS), and have been linked to heightened kidney injury in model systems^{69–71}. A growing number of microbiota-dependent products, including uremic toxins like p-cresol sulfate, indoxyl sulfate, and a variety of aromatic amino acid metabolites, are thought to potentially alter host metabolism via specific receptors, including the aromatic hydrocarbon receptor (Figure 3)^{72, 73}.

Trimethylamine *N*-oxide – a metaorganismal metabolite causally linked to CVD and metabolic disease

Almost a decade ago, Wang *et al.* first causally linked gut microbiota with oral intake of nutrient precursors, TMAO production, and CVD risk (Figures 1 and 2)⁹. A combination of untargeted metabolomics and mechanistic animal model studies was used to uncover small molecules whose levels in blood associate with CVD risk in humans, and impact CVD relevant phenotypes in animal models. Several analytes linked to phosphatidylcholine metabolism, including TMAO, were identified, and TMAO was shown to both predict CVD risks in multiple clinical cohorts, and facilitate accelerated atherosclerosis (as did nutrient precursors with an intact host gut microbiome) in animal models⁹. Through these and other studies, generation of TMAO in humans and mice has been shown to occur via a multistep,

metaorganismal pathway starting with the dietary precursors choline⁹, phosphatidylcholine^{8, 9}, and carnitine⁷. These are most abundant in foods found in a Western diet, including red meat, egg yolks, and other animal products (Figure 2). Notably, plasma or serum levels of every additional TMA nutrient precursor identified (i.e. shown to generate TMA and TMAO in hosts via a gut microbiota dependent fashion), including betaine⁹, γ -butyrobetaine⁷⁴, and trimethyllysine (TML)⁷⁵ have all similarly shown associations with incident CVD risks in large scale clinical studies, and these associations appear to be mediated by TMAO (since their clinical prognostic value becomes attenuated with TMAO in the model).

Gut microbial metabolism of TMA-containing nutrient precursors begins with specific microbial TMA lyases that generate TMA, an odorous gas, as a product. The major microbial choline TMA lyase is thought to be encoded by the microbial *cutC/D* genes (choline utilization gene cluster genes C (catalytic) and D)¹². The TMA produced is then transported to liver via the portal vein and readily metabolized by host hepatic flavin monooxygenases (FMOs) (mainly FMO3⁷⁶) into TMAO⁹. In the circulation, TMA levels are typically negligible. When radiolabeled TMA or TMAO were orally administered to human volunteers, 95% of the dose label was excreted via the kidneys with the majority being TMAO⁷⁷. TMAO has been shown to enhance atherosclerosis in most, but not all, mechanistic and animal model studies^{7, 9, 78–82}. It has also been shown to promote platelet reactivity and thrombosis potential^{14, 83–85}, vascular inflammation and inflammasome activation^{86–88}, heightened heart failure^{89–91} and CKD^{92–95} related phenotypes in animal models (Figure 2).

Circulating levels of TMAO have been shown to associate with CVD and predict outcomes in the presence of multiple CVD phenotypes, including peripheral artery disease (PAD)⁹⁶, coronary artery disease (CAD)⁹⁷, acute coronary syndrome (ACS)^{97–99}, and heart failure^{100–104}. Notably, the prognostic value of TMAO withstands adjustment for traditional risk factors, highlighting its potential as a biomarker for risk stratification beyond what has been considered traditional risk. While not all studies have observed the relationship between heightened TMAO levels and incident CVD risks, examination of extant clinical studies with TMAO in multiple meta-analyses have concluded that a strong relationship indeed exists between elevated circulating TMAO levels and both CVD risk and mortality in multiple cohorts on different continents^{105–107}. In many studies, a plasma TMAO cut off value of approximately $> 6\mu\text{M}$ predicted heightened risk of adverse cardiac events¹⁰⁸. And in one recent meta-analysis comprising >25000 subjects, a 7.6% increase in all-cause mortality was noted for each $10\mu\text{mol/L}$ increment of TMAO¹⁰⁵.

Several factors impact circulating TMAO levels within subjects^{19, 108}. First, the gut microbial community composition is critical since there is an initial obligatory role of gut microbes in TMA(O) generation^{7, 9}. Second, renal functional decline leads to less efficient excretion and thus heightened levels of TMA(O)⁹⁵. But elevated levels are also frequently observed among subjects with normal kidney function¹⁰⁵. In all subjects, choline is a major and continuous nutrient precursor, since beyond diet, choline content in bile is remarkably high, and thus bathes gut microbes in omnivore and vegans/vegetarians alike (the origins of the word choline are in the Greek word *kholé*, for “bile”, since choline was first isolated by

Adolph Strecker from pig and ox bile in 1862)¹⁰⁹. However, carnitine, which is found in high levels in red meat (and some energy drinks and over the counter supplements), also serves as a nutrient precursor, and can account for significant elevation in TMAO levels, particularly in some omnivores^{7, 110, 111}. Large-scale clinical observational studies show that subjects with heightened circulating carnitine levels have higher risk for incident CVD events (heart attack, stroke, and death). The prognostic value for plasma carnitine levels, like other TMA precursors, seems to be mediated by TMAO, as inclusion of TMAO in statistical models attenuates the prognostic value of carnitine (but TMAO remains a robust predictor), and carnitine supplementation accelerates atherosclerosis development in animal models^{7, 112}.

In a recent human dietary intervention study examining protein source (red meat vs white meat vs non-meat), substantially higher levels of circulating TMAO were observed when subjects consumed a red meat diet (equivalent to 8oz of steak daily for 1 month)¹¹¹. Although there is a modest increase in choline content in omnivorous vs vegan diets, as noted above, a substantial amount of choline is introduced into the gut in the form of bile (choline in the form of phosphatidylcholine is a major component of bile). Thus, there is far more modest overall difference in choline exposure to the gut microbial community of vegan/vegetarian versus omnivore. By contrast, omnivorous diets show markedly higher carnitine content since vegan/vegetarian diets are virtually devoid of carnitine. In line with this, a major source of the observed elevation in plasma TMAO levels in subjects following 1 month of either a red meat rich diet versus white meat or non-meat diet (predominantly vegetarian protein source), was shown to arise from carnitine. In addition to having enhanced nutrient density of TMA(O) precursors (including carnitine), isotope tracer studies showed the red meat rich diet induced functional remodeling of the gut microbial community to enhance carnitine→→TMA transformation, but not choline→TMA generation¹¹¹. Interestingly, the chronic exposure to a red meat rich diet also induced a functional change in the kidneys, with reduction in the fractional renal excretion rate for TMAO, despite no change in glomerular filtration rate¹¹¹. It is also notable that numerous studies support a dose-dependent association between meat consumption and CVD risks and mortality¹¹³.

Trimethylamine N-oxide, a newly-recognized participant in atherosclerosis, thrombosis, and vascular inflammation

A causal contribution of gut microbiota to atherosclerosis susceptibility was first demonstrated with the discovery of TMAO as a gut microbiota-derived factor, and the initial functional studies demonstrating both direct provision of TMAO accelerated atherosclerosis in murine models, and that suppression of gut microbiota dependent conversion of nutrient precursors (choline) into TMA and TMAO (with antimicrobial/antibiotics) blocked choline diet dependent enhancement in atherosclerosis (Figure 2 and 3)⁹. In atherosclerosis-prone ApoE^{-/-} mice, dietary supplementation with choline led to augmented atherosclerotic lesion burden, higher aortic expression of scavenger receptors (CD36 and scavenger receptor A), increased cholesterol laden macrophage foam cell formation⁹, and impaired *in vivo* reverse cholesterol transport⁷. In addition, TMAO suppressed bile acid pool size and

therefore cholesterol clearance in the host⁷. Consistent and complementary to these findings, FMO3 knock down has been shown to impair TMA transformation into TMAO, thus reducing plasma TMAO levels and concomitantly, restoring cholesterol balance¹¹⁴. Early microbial transplantation studies of cecal microbial communities from high TMA-producing inbred C57BL/6J mice into atherosclerosis-resistant NZW/LacJ recipients demonstrated the transmissibility of dietary choline-induced TMA and TMAO generation, and atherosclerosis¹¹⁵. Not all TMAO precursor feeding studies, however, have shown similar results, supporting the notion that differences in the microbial communities present in the host can impact the final phenotypes observed¹¹⁶.

The striking association between circulating TMAO levels in subjects and thrombotic event risks, such as heart attack and stroke, has been witnessed across numerous large-scale clinical cohorts^{105, 117}. This has prompted mechanistic studies in both humans and mice to explore the role of TMAO in thrombosis. Zhu *et al.* found that TMAO alters human platelet calcium signaling, heightening their responsiveness to sub-maximal stimulation by agonists (e.g. thrombin, ADP, collagen). Consequently, heightened thrombosis potential has been observed in both whole blood and *in vivo* arterial injury models^{14, 83–85, 118}. In a subsequent human feeding study, healthy volunteers (both omnivore and vegan/vegetarian) that were orally supplemented with choline exhibited higher levels of TMAO and concomitant enhanced platelet responsiveness and aggregation^{77, 118}. Importantly, higher TMAO levels were dose-dependently associated with increased platelet aggregation responsiveness, even in subjects on low-dose aspirin. This suggests that in subjects with elevated TMAO, the anti-platelet effects of aspirin may be attenuated, highlighting the possible involvement of TMAO in on-treatment platelet reactivity and so-called “aspirin resistance”.

The mechanistic involvement of the metaorganismal TMAO pathway in platelet function and *in vivo* thrombosis potential has also been examined through both genetic gain and loss of function manipulations, including to the host gene *FMO3* (Figure 2). Multiple studies have confirmed through both genetic gain (as global *FMO3* transgene) and loss (via anti-sense oligonucleotide to *FMO3*, and via global *FMO3* knock out) of function studies in mice that manipulation of TMA and TMAO levels *in vivo* alters platelet responsiveness, rate of clot generation, and thrombosis potential^{83, 84, 119}. Moreover, cecal microbial transplantation experiments confirmed that the prothrombotic phenotype mediated by a choline-rich diet was a transmissible trait⁸³. Early studies by Craciun *et al.* first identified the choline utilization (*cut*) gene cluster in human commensals encoding the catalytic and regulatory gene products CutC and CutD¹². The presence of the microbial *cutCD* genes in human microbiota are associated with the ability to generate TMA from choline¹²⁰, and with subsequent TMAO accumulation¹²¹. Importantly, studies using germ-free mice colonized with synthetic microbial communities +/- a genetically engineered gain or loss of function *cutC* mutant human commensal confirmed that a functional microbial *cutC* gene is sufficient to transmit TMA and TMAO generation, as well as *in vivo* thrombosis potential. Microbial *cutC* may thus represent a therapeutic target for preventing thromboembolic complications¹⁴.

Recent studies have shown that beyond impacting platelet function, TMAO induces expression of tissue factor (TF), the initiator of the extrinsic clotting, in endothelial cells *in*

*vitro*¹²². Vascular TF promotes thrombosis and vascular inflammation¹²³, particularly in patients with type 2 diabetes who have higher levels of circulating TMAO^{124–126}. Animal model studies are still needed to validate a contribution of gut microbiota and TMAO generation to alterations in TF pathway *in vivo*, and to altered thrombosis potential. A recent study reported that the absence of microbiota was associated with reduced hepatic von Willebrand factor (VWF) synthesis and reduced thrombus growth after carotid artery injury in C57BL/6 germfree mice as compared to conventionally raised littermates¹²⁷. However, the role of metaorganismal metabolites, in particular TMAO, in the VWF-dependent thrombosis has yet to be determined.

Vascular inflammation is critically involved in the pathogenesis of atherosclerosis and thrombotic complications. Seldin *et al.* found that acute infusion of physiological levels of TMAO in mice heightens vascular inflammation (Figure 2), as supported by aortic endothelial cell activation (recovered by laser microdissection), including activation of mitogen-activated protein kinase (MAPK) signaling and nuclear factor (NF) κ B nuclear translocation, leading to subsequent pro-inflammatory gene expression⁸⁶. Complementary findings (TMAO stimulated vascular inflammation; Figure 2) have been observed *in vitro* using primary human aortic endothelial cells and vascular smooth muscle cells⁸⁶. After an acute injection, mouse aortas also showed increased expression of vascular adhesion molecules, such as E selectin or intercellular cell adhesion molecule (ICAM)-1, even when TMAO had been cleared from circulation, implying sustained vascular inflammation. Further, TMAO was reported to increase oxidative stress and vascular senescence, which was characterized by impaired cell proliferation and migration in human umbilical vein endothelial cells¹²⁸. In other animal model studies, gut microbiota suppression with oral poorly absorbed antibiotics was associated with decreased TMAO levels, improved endothelial function, reduced arterial stiffness, and lower oxidative stress¹²⁹. Recent investigations have suggested TMAO can impact inflammation via priming and activation of the NLRP3 inflammasome in endothelial cells, as well as the arterial vascular wall in mice, involving mitochondrial reactive oxygen species (ROS) production, thioredoxin interacting protein (TXNIP) and lysosomal destabilization^{87, 130, 131}. However, the exact mechanisms by which TMAO induces inflammasome activity have yet to be explored.

The receptor for TMA was identified long ago as a highly sensitive olfactory receptor called trace amine-associated receptor 5 (TAAR5)¹³². TAAR5 shows high specificity for TMA, and does not recognize TMAO. While TMA alone may contribute to pro-inflammatory signaling in the vasculature⁸⁶, the role of TAAR5 in CVD requires further investigation. Recently, a receptor for TMAO has been reported and shown to participate in TMAO-dependent effects on glucose and insulin metabolism¹³³. Chen *et al.* showed that TMAO directly binds to protein kinase R-like endoplasmic reticulum kinase (PERK), a main component of the unfolded protein response – a signaling pathway that adapts the cell to ER stress¹³³ (Figure 1). Dietary supplementation of TMAO in C57BL/6J wildtype animals induced hepatic PERK expression, accompanied by increased FoxO1, a key transcription factor in insulin signaling and impaired glycemic control. Genetic manipulation demonstrated that the absence of hepatic PERK blunted the TMAO-induced increases in FoxO1 expression and improved glycemic indices. Whether or not PERK plays a role in TMAO mediated effects on atherosclerosis or thrombosis remains unknown, and is an important area for

further exploration. It is also interesting to note that ER stress is implicated in the pathogenesis of many CVD phenotypes. Whether TMAO and PERK participate in these associations also remains to be explored.

Theoretical benefits of TMAO

Due to its small size and combination of hydrophilic and hydrophobic properties, TMAO behaves as a chaotropic agent, with the ability to alter protein conformation and potentially serve as an allosteric modulator to proteins¹³⁴. These features may have important physiological functions in the host (e.g. impacting the protein unfolding or ER stress response within cells). In some aquatic animals, including a subset of deep sea fish, TMAO is reported to act as an osmolyte, and to protect against pressure-induced protein destabilization^{135, 136}. Indeed, some bony fish can use large amounts of TMAO for osmoregulation, and TMAO plasma levels within the fish increase with depth of the habitat, reaching levels up to 400mmol/kg in snailfish that were caught at 7000m depth in the South Pacific Ocean¹³⁷. TMAO has been shown to stabilize proteins to elevated hydrostatic pressure, and is thought to both impact protein conformational changes, like those that occur with allosteric regulation, and modulate intracellular molecular crowding effects^{136, 138, 139}. Thus, TMAO accumulation both in deep sea creatures and in mammals may represent an adaptive mechanism to impact protein stability and intracellular signaling processes.

Other studies have reported that TMAO is involved in the adaptive freeze avoidance response to extreme cold – in other words, it acts as antifreeze – such as within Newfoundland smelt¹⁴⁰. These fish have the ability to elevate plasma osmolarity by seasonal accumulation of TMAO and other organic solutes, which depresses the freezing point of body fluids and allows them to survive at sub-freezing temperatures. It has recently been hypothesized that TMAO-induced protein stabilization may play a role to protect cardiomyocytes from hydrostatic pressure fluctuations during heart failure¹⁴¹. By contrast, high concentrations of TMAO may at times also impair function, as was reported for the activity of the actomyosin motor¹⁴².

TMAO may also be involved in tissue osmolality in vertebrates. When measuring concentrations in various mouse tissues, we observed that TMAO levels in kidney tissue largely exceeded those observed in corresponding paired plasma samples collected at the same time from the same animals (Hazen, unpublished data). In the kidneys, an osmotic gradient arising from the cortico-medullary boundary to the inner medullary tip is normally maintained as a mechanism that allows reabsorption of water and concentration of the urine, a process also called countercurrent multiplication¹⁴³. It seems plausible that high levels of TMAO in the kidneys may function analogously to urea, playing a role in osmoregulation and renal function. Understanding the molecular participants involved in TMAO secretion and cellular transport in the kidneys and other tissues is an important potential area of future investigation, as it may reveal novel targets for therapeutic intervention. For example, one could theorize a small molecule drug that targeted renal TMAO transport/secretion might functioned as a diuretic, facilitating TMAO urinary excretion and both reduced blood pressure and cardiovascular disease risks.

Many studies have shown that high TMAO levels are associated with risk for thrombotic complications. However, these same properties may theoretically confer benefits to the host during situations with high bleeding risk, such as the delivery of a baby. It is thus interesting that one study reported TMAO levels may increase during pregnancy¹⁴⁴. Studies by Bennett *et al.* found that human liver samples from female subjects showed higher *FMO1/3* expression, and therefore an increased capacity for TMAO generation, than those from males. Likewise, gonadectomized male mice treated with testosterone show reduced hepatic *FMO3* expression, while ovariectomized female mice treated with estrogen exhibit increases in hepatic *FMO3* expression⁷⁶. These results suggest that high levels of TMAO in the terminal stages of pregnancy may in theory better equip women to avoid severe blood loss during delivery. In addition, beyond *FMO3* expression, microbial community alterations are known to occur during pregnancy¹⁴⁵.

While purely speculative, evolutionary drive may have selected for metabolic changes in hosts that lead to harboring of gut commensal communities with the ability to produce TMA or other gut microbiota-generated pro-thrombotic metabolites, thus enabling hosts to better cope with environmental stressors that lead to hemorrhage (such as parturition). However, these features may have become detrimental for individuals living in modern western societies associated with a CVD-prone environment, and less need to survive traumatic injuries. Rodents show a significant sexual dimorphism with respect to *FMO3* expression, and TMAO levels, with females showing higher levels (and greater atherosclerosis capacity^{9, 76}). Although gene expression studies in humans have suggested gender differences in hepatic *FMO3* expression, plasma levels of TMAO thus far reported have failed to show sex differences, and the prognostic value of TMAO appears to be similar in both men and women¹⁰⁵. However, it should also be noted that virtually all clinical TMAO studies reported involve cohorts that are of postmenopausal age. A full exploration of sex specific differences TMAO levels in younger women warrants further investigation.

The metaorganismal metabolite phenylacetylglutamine (PAG) is both linked to CVD and acts via adrenergic receptors

The pathogenesis of type 2 diabetes goes beyond glycemic control, and traditional risk factors, including level of glucose control, poorly stratify CVD risk among diabetics. To investigate this, Nemet, Saha, Gupta, *et al.* used untargeted metabolomics to identify novel metabolites that associate with incident risk for MACE, are increased in type 2 diabetes subjects, and do not significantly correlate with glycemic control¹⁴⁶. The candidate analyte showing the strongest association with MACE (m/z 265.1188) was subsequently identified as phenylacetylglutamine (PAG), a phenylalanine-derived metabolite (Figure 4). The association of PAGln with incident risk for major adverse cardiac events like heart attack, stroke and death was further validated in an independent and non-overlapping cohort comprised of 4000 stable cardiovascular subjects, and shown to be independent of traditional CVD risk factors in both diabetics and non-diabetics alike¹⁴⁶. Additional functional studies revealed that the association of PAG with incident CVD risks likely occurs because the metabolite impacts host physiology, and fosters CVD related phenotypes. Moreover, PAG was shown to be generated via gut microbes during metabolism of phenylalanine, as

illustrated in Figure 4. Genetic engineering studies in microbes coupled with transplantation into germ free mice confirmed gut microbial (and some of the gut microbial genes) contribution to host platelet reactivity and in vivo thrombosis potential. And through multiple gain of function and loss of function genetic and pharmacological studies, PAG was shown to interact with G protein-coupled receptors (GPCRs), including the α - and β -adrenergic receptors (ADRs)¹⁴⁶. Adrenergic receptors are crucially involved in heart disease¹⁴⁷ and platelet function¹⁴⁸. However, until the discovery of PAG, ADR signaling had not yet been implicated in gut microbiota-derived factors driving CVD. The new studies by Nemet *et al.* also showed that adverse CVD related phenotypes observed with PAG administration at physiological levels was attenuated with the β -blocker carvedilol¹⁴⁶.

It is striking that gut microbiota appear to elicit adverse cardiovascular phenotypes in the host via modulation of ADRs. Such a finding may help explain some of the beneficial effects of clinical beta blocker treatment. Interestingly, ADRs may also in turn modulate microbial abundance as observed in beta-adrenergic receptor knockout mice¹⁴⁹. The novel discovery of a microbiota-ADR signaling axis is particularly interesting considering the widespread implication of ADRs in cardiovascular physiology and metabolism. There is no reason to assume that the subset of ADRs identified in this study are the only receptors modulated by PAG. It remains to be determined if other members of the ADR gene family, some of which are also known to participate in the regulation of cardiovascular homeostasis, are similarly modulated by PAG. It is intriguing to speculate that some phenotypes exhibited by microbiota are directly or indirectly mediated via ADRs. This is indirectly supported by the fact that absence of microbiota alters cardiovascular homeostasis, such as blood pressure regulation¹⁵⁰, myocardial repair following post-infarction cardiac repair⁶⁶, or thrombosis growth¹²⁷ – functions that all involve ADRs. More studies are needed to further characterize PAG-mediated functions in the host. PAG also appears to represent another potential gut microbiome pharmacological target for future efforts¹⁵¹.

Drugging the microbiome

In response to accumulating evidence that the microbiota affects susceptibility for CVD and cardiometabolic diseases, researchers have begun to develop microbiota-directed interventions to improve clinical outcomes (Figure 3). The microbiome-host axis comprises many different layers, including dietary precursors, microbial communities, and metaorganismal pathways that generate bioactive metabolites recognized by host receptors – all of which represent potential therapeutic targets to modulate community output and host phenotype. Figure 3 illustrates several different therapeutic approaches for targeting the gut microbiome to exert a beneficial effect in the host. We have already mentioned the use of anti-microbial agents such as poorly absorbed antibiotics as a valuable tool for demonstrating involvement of gut microbiota in both animal models and humans⁹, but as a poor choice as a long-term therapeutic due to the development of antibiotic resistance. While many studies have reported associations between atherosclerotic plaque and the presence of pathogens such as *cytomegalovirus*, *Chlamydia*, and *Helicobacter pylori*^{152–155}, multiple prospective randomized trials with antibiotics have thus far failed to demonstrate clinical benefit^{156–158}. Further, the impact of antibiotic treatment on microbial communities is hard to predict, often because the microbial community that recolonizes after cessation of

antibiotics can be variable, depending upon many factors, including the microbes one is exposed to as the antibiotics are metabolized and excreted. Currently, there is no clear evidence that antibiotics have efficacy in the treatment of CVD in humans. Use of antibiotics thus seems better suited to eradicating true pathogens, than as a chronic long-term preventive intervention.

In a recent study that screened over 1000 commonly prescribed non-antibiotic drugs for their impact on a broad selection of human gut commensals, nearly a quarter of the tested medications demonstrated antibiotic-like activity, significantly inhibiting the growth of at least one human commensal¹⁵⁹. These results suggest that commonly prescribed medications may impact human gut microbial communities, thereby impacting host phenotypes through indirect effects mediated by changes in the gut microbiome. One well studied example of this is the widely-used anti-diabetic drug metformin. Recent studies by Bäckhed and colleagues show that some of the anti-diabetic effects of metformin therapy appear to be mediated by alterations in gut microbiota composition, since microbial transplantation studies reveal transmission of anti-diabetic effects in recipient germ free mice following fecal transfer from metformin treated donors¹⁶⁰. In addition to the potential for common medications to impact gut microbial composition and function, thus potentially contributing to drug effects in the host, it is becoming increasingly clear that there is broad diversity in gut microbial metabolism of medications, leading to potential altered responsiveness to drugs. The diverse drug-microbiome interactions that vary between individuals are another area where further studies are needed, and shows promise for possible use in drug development efforts and personalized medicine¹⁶¹.

Defined microbial compositions (probiotics) and non-microbial substances that may alter microbial community structure (prebiotics) have also been proposed to improve CVD (Figure 3). Indeed, there are many preclinical and some clinical intervention studies using either prebiotics or probiotics that have shown promising results. However, these studies tend to be relatively small in size, and their adoption in clinical practice would require further study. The use of probiotics and prebiotics has recently been extensively reviewed, including discussions of the many promises and potential challenges of their development and use^{162–167}. While a complete review of this topic is beyond the scope of the present review, below we discuss several preclinical studies involving probiotics, particularly where a mechanistic role for alteration in gut microbiome related processes to improved host phenotype were reported. One example includes a recent rodent hypertension study in which either a high-fiber diet (a prebiotic) or acetic acid alone (a gut microbial product) resulted in reduced blood pressure and adverse cardiac remodeling¹⁶⁸. In a different mouse model involving Apoe E^{-/-} mice on a n-3 polyunsaturated fatty acid (PUFA)-depleted diet for 12 weeks, supplementation of dietary inulin-type fructans were shown to reverse endothelial dysfunction in carotid arteries via activation of the nitric oxide (NO) synthase/NO pathway¹⁶⁹. Beyond changes in vascular function, probiotic use in animal studies of heart failure have also shown promise. For example, administration of *Lactobacillus rhamnosus* GR-1 in rodents improved systolic and diastolic left ventricular function following coronary artery ligation¹⁷⁰. This study was of interest because it was shown that while the GR-1 strain did not colonize to distal intestines where most anaerobes reside, beneficial cardiac remodeling was none-the-less observed. In another recent study, provision of *Akkermansia*

muciniphila was associated with reduced aortic atherosclerosis in the hypercholesterolemic Apoe^{-/-} mouse model²⁸. This probiotic is of interest because of its use in human clinical investigations. For example, provision of *Akkermansia muciniphila* in a randomized placebo controlled double blinded interventional exploratory study was reported to promote a reduction in plasma LPS in obese individuals with metabolic syndrome²⁹.

While prebiotic and probiotic interventions have shown ability to favorably alter metabolic profiles in some human studies, results are highly variable, and animal model findings have not yet been translated to evidence of clinical efficacy. It is still unclear whether most of the used microbes survive the acidity of the stomach, if they colonize the colon where most of the gut microbiota reside, and whether the beneficial effects are mediated by the ingested microbes, caused by shifts in the community structure, or possibly even caused by secondary effects on host immune education and function. One difficulty with probiotic studies where secondary effects on microbial communities likely plays a role in beneficial effects if observed is the vastness of the gut microbial community and the inter-individual differences in community structure. These factors are thought to give rise to variable responses to probiotics or prebiotics in general¹⁷¹. Thus, results of probiotic or prebiotic administration have been difficult to predict. Moreover, it is worth noting that the current selection of probiotics seems to be primarily driven by abundance-based analyses of microbiota composition, wherein microbial community members whose proportions are highly associated with beneficial phenotypes are the focus of interest. However, the keystone commensal organizing a community architecture, or providing a key gain of function as discussed above, can be a very low abundance component, and is often not easily detected by current conventional sequencing depths of analyses.

Perhaps the most obvious potential therapeutic intervention for targeting the gut microbiome is diet (Figure 3). The TMAO pathway is an excellent example of this, given the nutrient precursors are more abundant in a Western diet, and diets rich in phosphatidylcholine and carnitine are associated with heightened levels of TMAO, whereas vegetarian or vegan diets have reduced nutrient precursors^{111, 118}. Notably, however, TMAO levels appear to be driven more so by the gut microbial community composition than by the dietary intervention, and significant variation in TMAO production among individuals on a given diet is observed^{14, 111}. While a low choline or carnitine containing diet can be generated using a primarily vegetarian or vegan selection of food, and are rational recommendations for subjects with high TMAO levels, such dietary recommendations are harder to envision with other gut microbiota generated metabolites, where either the nutrient precursors are numerous (e.g. SCFA) or an essential amino acid (e.g. phenylalanine and PAG), so cannot be easily avoided. However, even with phenylalanine, there are dietary choices that can be made to reduce intake. For example, sufferers of the inborn error of metabolism called phenylketonuria (PKU) have a host enzyme deficiency that makes eating foods abundant in phenylalanine harmful (i.e. proteins). Consequently, throughout life, a phenylketonuric diet is highly recommended, which has an overall low phenylalanine content¹⁷². The impact of adopting a PKU diet in subjects with high PAG levels (e.g. subjects with diabetes, renal disease, etc) has yet to be examined, but is of considerable interest.

Recent work has examined the selective non-lethal targeting of gut microbial enzymes for TMA generation as a therapeutic approach for the treatment or prevention of CVD (Figure 3). Key to this approach is the development of a small molecule inhibitor that is “non-lethal” to the microbe, and thus does not trigger as great a selective pressure as an antibiotic for development of resistance. The first study of this type targeting the gut microbiome for the treatment of CVD used a choline structural analogue, 1,3 dimethylbutanol (DMB). Through a series of studies, DMB was shown to serve as a non-lethal microbial enzyme inhibitor of choline→TMA transformation, and to reduce TMAO production in vivo without affecting microbial fitness⁷⁸. When fed to animals, DMB inhibited choline diet-dependent TMAO generation, reduced macrophage foam cell formation, and inhibited aortic atherosclerotic plaque development⁷⁸. Next-generation choline TMA lyase inhibitors have since been developed that selectively target and accumulate in microbiota, thereby limiting systemic exposure in the host. The choline TMA lyase suicide substrate inhibitor fluoromethylcholine (FMC) was shown to be over 10,000 fold more potent an inhibitor of *cutC* than DMB, and markedly blocks microbial choline catabolism⁸⁵. Interestingly, owing to their highly polar nature, FMC and related halomethylcholines were shown to be poorly absorbed into the host, limiting systemic exposure and thus chances of side effects. In addition, *cutC* inhibition by FMC and iodomethylcholine (IMC) was shown to result in microbial cytosolic choline elevation, which appears to be sensed as an abundant fuel source by the microbe. This then induces expression of the entire choline utilization gene cluster, including both *cutC* and the microbial choline transporter. This leads to active microbial uptake of FMC and the substrate of *cutC*, choline. A positive feedback loop is thus created, whereby the greater microbial catabolism of choline into TMA is inhibited, the greater the elevation in cytosolic choline within the gut microbe (sensed as an abundant fuel source), and the greater the sequestering of choline from the intestinal lumen into the inhibited gut microbe. As intestinal luminal choline is depleted, microbial community TMA production is globally inhibited, even from neighboring community members who might otherwise not be potently inhibited⁸⁵.

Although human clinical studies with choline TMA lyase inhibitors have not yet been reported, numerous efforts are ongoing in this area. Like any drug, microbiota targeting non-lethal small molecule inhibitors will require the same sort of safety testing as any other drug. But with compounds that have reduced systemic absorption, there is a theoretical potential benefit of limiting adverse side effects from off target inhibitory activities in the host. Thus, pharmacological interventions aimed at “drugging the microbiome” with non-lethal small molecule inhibitors represents a novel therapeutic approach for both the treatment and prevention of cardiometabolic diseases that will need to be validated in clinical intervention studies.

Conclusions

Although our knowledge about how microbiota impact CVD is still rudimentary, the rate at which new discoveries are emerging is impressive. As outlined, there is overwhelming evidence that gut microbiota-derived processes in general are linked to numerous CVD relevant phenotypes, including but not limited to atherosclerosis, platelet reactivity and thrombosis potential, blood pressure, lipid metabolism, adiposity, glucose homeostasis, and vascular inflammation. Investigative approaches have included a wide array of microbiota

transplantation studies, both animal and human dietary interventions in colonized versus antibiotic suppressed states (or germ-free mice), direct provision (dietary or via infusion) of specific gut microbiota metabolites, and both genetic and pharmacological studies that have targeted multiple components of metaorganismal pathways (including gut microbial genes, and both host transformational enzymes and end organ receptors). New therapeutic approaches that target gut microbes for the treatment and prevention of cardiovascular diseases represent exciting areas of investigation. The development of nonlethal microbial inhibitors that target specific pathways, yet show limited systemic exposure in the host, are just one of the new and potentially promising therapeutic approaches. Yet others include but are not limited to dietary interventions, probiotics, and/or prebiotics that hopefully can be used to someday “terraform” the microbial community to alter its functional output to the betterment of the host. As with any therapeutic, large prospective interventional studies will be needed to validate novel gut microbiome targeted therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Non-standard Abbreviations and Acronyms

| | |
|--------------|-----------------------------|
| ACS | acute coronary syndrome |
| ADP | adenosine diphosphate |
| ADR | adrenergic receptor |
| BA | bile acids |
| CAD | coronary artery disease |
| CKD | chronic kidney disease |
| CVD | cardiovascular disease |
| cut | choline utilization cluster |
| DMB | dimethylbutanol |
| FMC | fluoromethylcholine |
| FMO | flavin monooxygenases |
| FXR | farnesoid x receptor |
| GPCR | G protein-coupled receptors |
| GRP41 | G-protein receptor 41 |

| | |
|--------------|--|
| ICAM | intercellular cell adhesion molecule |
| IMC | iodomethylcholine |
| LPS | lipopolysaccharide |
| LXR | liver X receptor |
| MACE | major adverse cardiac events |
| MAPK | mitogen-activated protein kinase |
| NFkB | nuclear factor NFkB |
| NO | nitric oxide |
| Olf78 | olfactory receptor 78 |
| PAD | peripheral artery disease |
| PAG | phenylacetylglutamine |
| PERK | protein kinase R-like endoplasmic reticulum kinase |
| PKU | phenylketonuria |
| PXR | pregnane X receptor |
| RAAS | renin–angiotensin–aldosterone system |
| ROS | reactive oxygen species |
| SCFA | short chain fatty acids |
| TAAR5 | trace amine-associated receptor 5 |
| TF | tissue factor |
| TLR | toll-like receptor |
| TMA | trimethylamine |
| TMAO | trimethylamine N-oxide |
| TML | trimethyllysine |
| VWF | von Willebrand factor |

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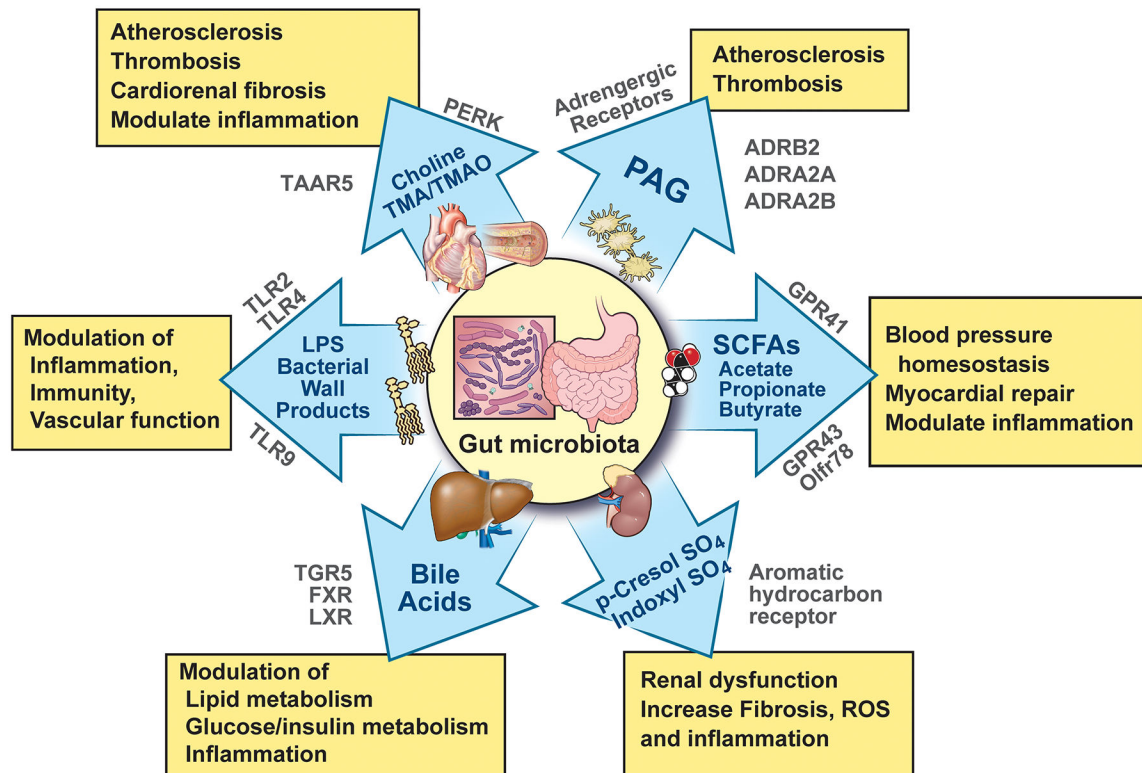


Figure 1:

Molecular pathways and host receptors that link gut microbiota-derived products and metabolites with cardiovascular and cardiometabolic disease phenotypes. ADRA, adrenergic receptor alpha; ADRB, adrenergic receptor beta; GPR, G-protein–coupled receptor; FXR, farnesol X receptor; LPS, lipopolysaccharide; LXR, liver X receptor; Olfr, olfactory receptor; PAG, phenylacetylglutamine; PERK, protein kinase R-like endoplasmic reticulum kinase; ROS, reactive oxygen species; SCFA, short chain fatty acid; TGR, takeda G protein-coupled receptor; TLR, toll-like receptor; TMA, trimethylamine; TMAO, trimethylamine N-oxide; and TAAR, trace amine-associated receptor.

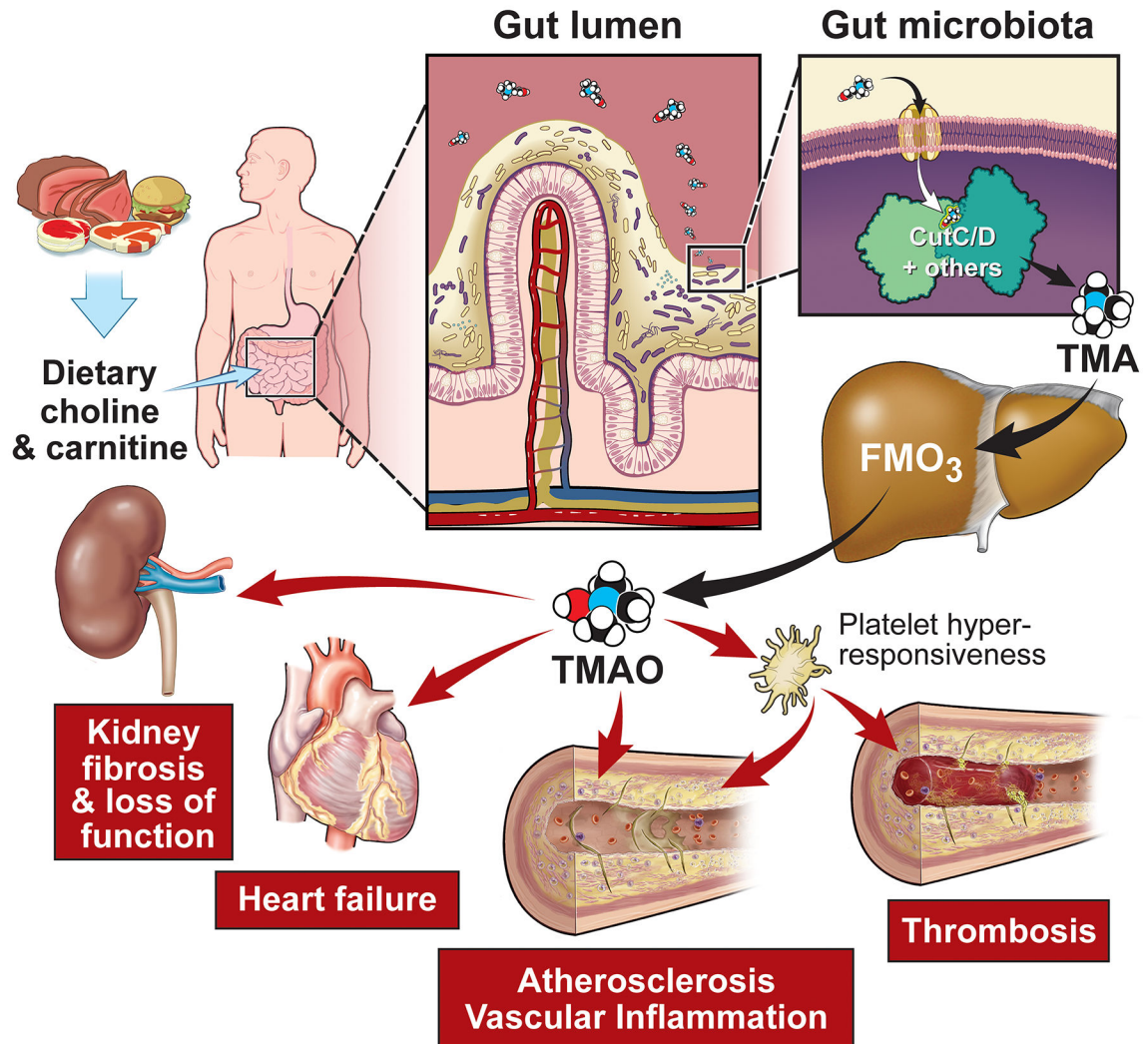


Figure 2: Dietary precursors, such as choline and carnitine, are metabolized into trimethylamine (TMA) by gut microbiota via specific genes, including members of the choline utilization gene cluster (Cut)C/D. Host hepatic flavin monooxygenases (FMOs) oxidize TMA into TMAO, which promotes metabolic and functional changes in the host including cardiovascular and renal end-organ damage.

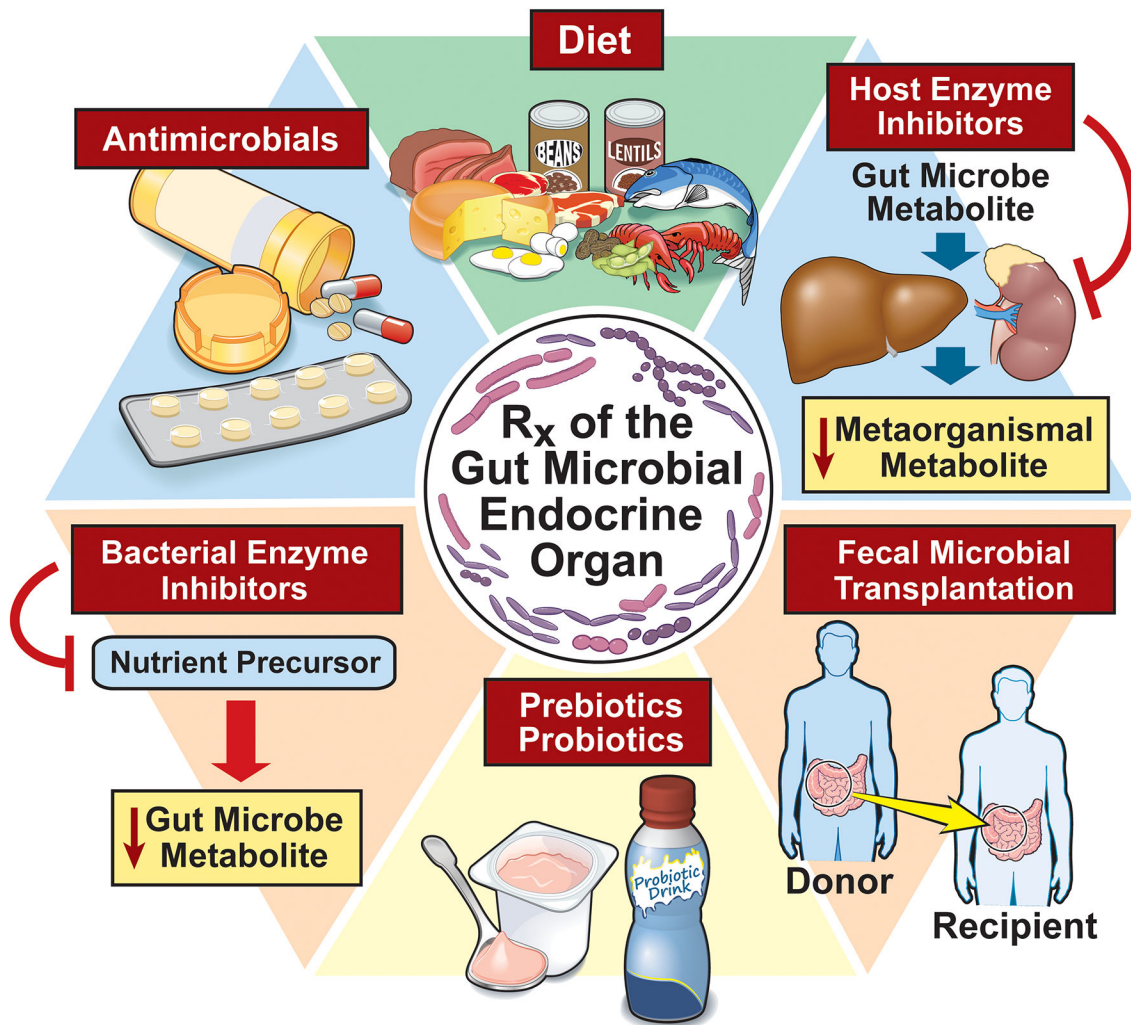


Figure 3: Illustration of current strategies to improve cardiovascular disease by manipulating gut microbiota include dietary interventions, targeting host enzymes involved in generation of metaorganismal metabolites, fecal microbial transplantation, pre/probiotics, bacterial enzyme inhibitors, and antimicrobials.

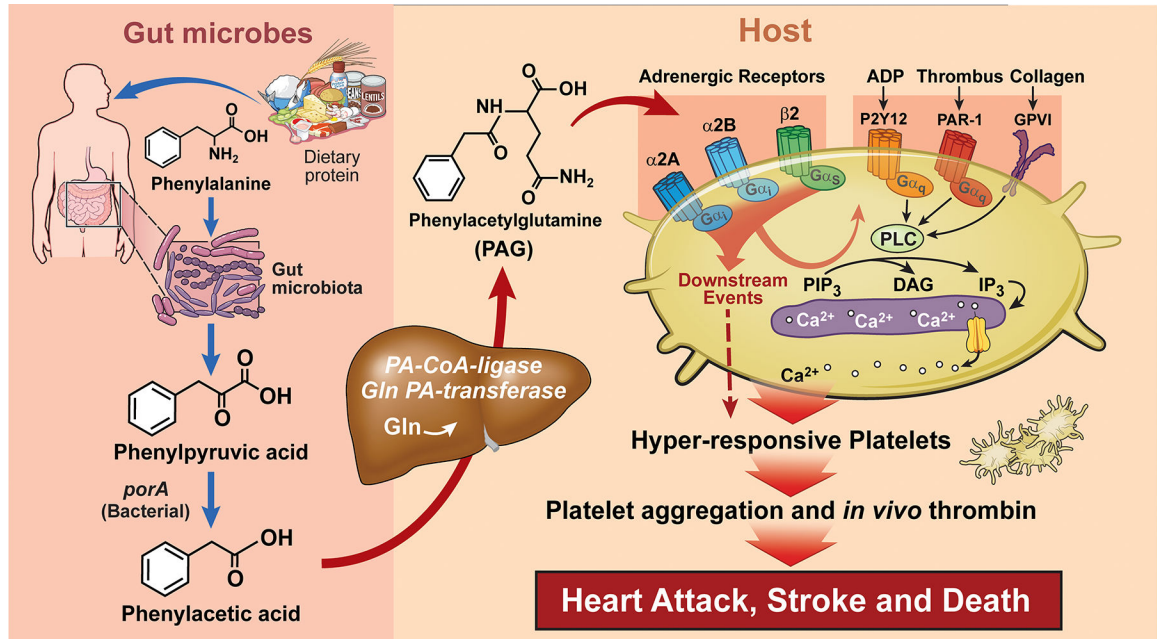


Figure 4:

The metaorganismal metabolite phenylacetylglutamine (PAG) is derived from microbial metabolism of phenylalanine, and is involved in enhancement of platelet thrombotic potential via adrenergic receptors. ADP, adenosine diphosphate; $\alpha 2A$, $\alpha 2A$ adrenergic receptor; $\alpha 2B$, $\alpha 2B$ adrenergic receptor; $\beta 2$, $\beta 2$ adrenergic receptor; GPVI, Glycoprotein VI; IP₃, inositol 1,4,5-triphosphate; P2Y12, purinergic receptor P2Y12; PAR-1, Protease-activated receptor 1; PIP₃, phosphatidylinositol trisphosphate, and PLC, phospholipase C.