The Gut Microbial Endocrine Organ: Bacterially Derived Signals Driving Cardiometabolic Diseases

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Abstract

The human gastrointestinal tract is home to trillions of bacteria, which vastly outnumber host cells in the body. Although generally overlooked in the field of endocrinology, gut microbial symbionts organize to form a key endocrine organ that converts nutritional cues from the environment into hormone-like signals that impact both normal physiology and chronic disease in the human host. Recent evidence suggests that several gut microbial-derived products are sensed by dedicated host receptor systems to alter cardiovascular disease (CVD) progression. In fact, gut microbial metabolism of dietary components results in the production of proatherogenic circulating factors that act through a meta-organismal endocrine axis to impact CVD risk. Whether pharmacological interventions at the level of the gut microbial endocrine organ will reduce CVD risk is a key new question in the field of cardiovascular medicine. Here we discuss the opportunities and challenges that lie ahead in targeting meta-organismal endocrinology for CVD prevention.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in most developed countries, and despite the widespread use of statin drugs, one in six deaths in the United States is still attributable to CVD (1–3). Given this unmet need for effective therapies, there is increasing interest in targeting novel pathways that underlie CVD pathogenesis. Previous genetic mapping studies and recent genome-wide association studies have provided clear evidence that genetic variants can predispose to CVD development (4–6), and these studies have identified several new attractive therapeutic targets. However, even the largest of genetic studies thus far have only been able to account for a relatively small portion of attributable cardiovascular risks. For example, in a recent genome-wide association study of nearly 200,000 subjects (63,746 coronary artery disease cases and 130,681 controls), the genetic variants identified cumulatively only explained ~10.6% of the coronary artery disease heritability (7). Because disease susceptibility is due to factors arising from either genetic or environmental sources, the contribution of environmental factors to the development of CVD is enormous. Understanding how environmental cues drive CVD pathogenesis will be central in drug discovery. Although many environmental factors converge to promote CVD, here we focus on how diverse components within our diet (macronutrients, micronutrients, symbionts, pathogens, etc.) participate in meta-organismal (microbe to host) signaling pathways to promote CVD risk. Until recently, dietary constituents were assumed to be simply absorbed or metabolized by our cells primarily for energy needs and general cell health. However, we now understand that microbial communities resident in the human gastrointestinal tract not only enable us to efficiently harvest energy from our food (8) but also function, essentially, as a key endocrine organ by secreting metabolites that act as hormone-like factors that are sensed by dedicated receptor systems in the human host. Gut microbes can also signal to the host to regulate innate immunity through metabolism-independent pathways, where constituents of the microbial cell wall are sensed by host cells through pattern recognition receptors to further impact CVD progression. Collectively, through both nutrient metabolism-dependent and metabolism-independent mechanisms, the gut microbiome forms a largely overlooked plastic endocrine organ that integrates input cues from the diet and interfaces with the host to play a role in the pathogenesis of CVD and metabolic disorders (**Figure 1**).

The concept of the meta-organism was first proposed by an insightful German zoologist, Karl Möbius, when he discovered that the health of European oyster populations was dependent on the presence of other species in the surrounding ecosystem (9). This meta-organismal theory can also be applied to human health and disease. It is increasingly accepted that gut microbial communities act as an endocrine organ, bioactivating vitamins and producing other factors from dietary nutrients that can impact normal physiological processes, educate mucosal immune systems, and/or induce pathological responses (10). Within the last decade, there have been numerous reports linking meta-organismal pathways to human diseases, including obesity (8, 10), diabetes (11, 12), nonalcoholic fatty liver disease (13), osteoporosis (14), cancer (15–17), and disorders of innate/adaptive immune responses (18, 19). However, the purpose of this review is to highlight recent discoveries linking the gut microbial endocrine organ to CVD risk (20–27a).

THE GUT MICROBIAL ENDOCRINE ORGAN

The human body consists of much more than just human cells. In fact, it has been estimated that >100 trillion (10^{14}) microbial cells reside in different compartments within the human body, vastly outnumbering human cells (28, 29). Amazingly, it has been estimated that <10% of DNA found in the human meta-organism derives from *Homo sapiens* origin (29). Fortuitously, the majority

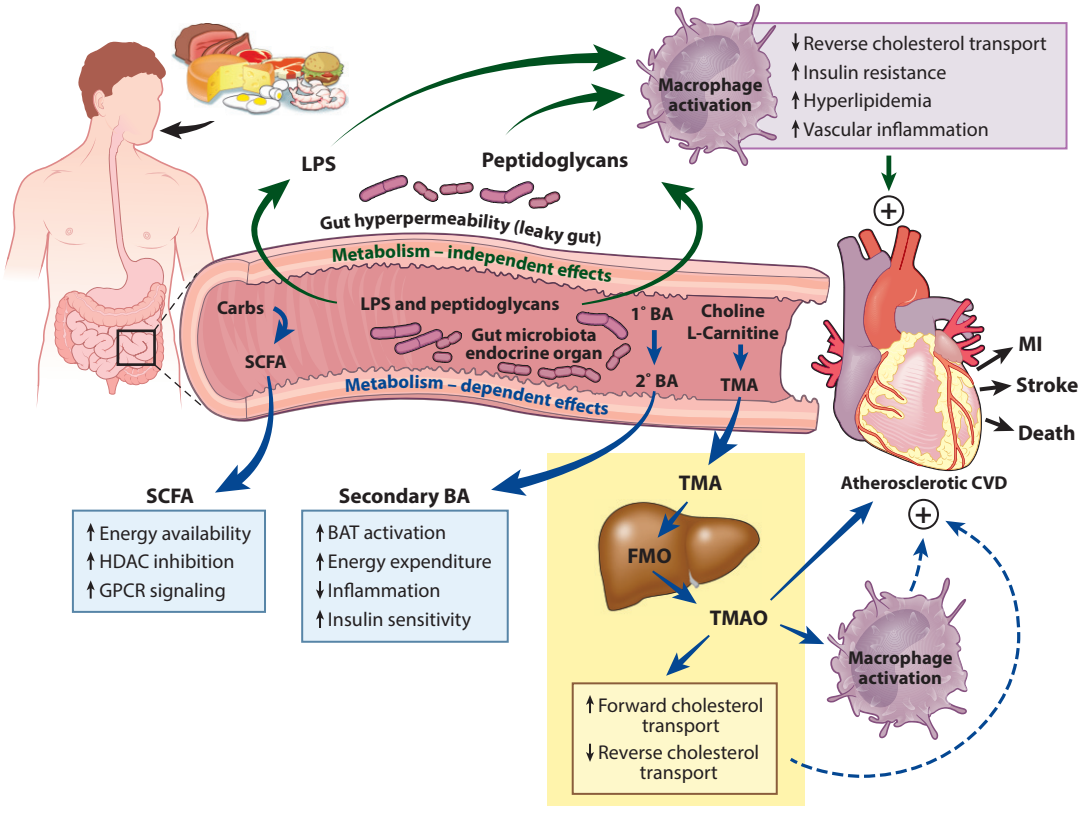

Figure 1

Model of gut microbial participation in the progression of atherosclerotic cardiovascular disease. Following dietary exposures of certain nutrients, gut microbiota can elicit both metabolism-dependent and metabolism-independent effects on the host. Metabolism-dependent effects include: (1) Microbial fermentation of dietary carbohydrates to generate short chain fatty acids (SCFAs), which signal to the host to increase energy expenditure, inhibit histone deacetylase (HDAC) activity, and enhance G protein–coupled receptor (GPCR) signaling. (2) Microbial conversion of primary bile acids to secondary bile acids signals to increase host brown adipose tissue (BAT) activation, energy expenditure and insulin sensitivity, while dampening inflammation. (3) Microbial conversion of choline and L-carnitine to trimethylamine (TMA). TMA is subsequently converted by the host flavin monooxygenase (FMO) enzyme family to trimethylamine-N-oxide (TMAO) in the liver. TMAO accelerates atherosclerosis in animal models, via mechanisms including altering sterol and bile acid metabolism, increases in macrophage activation, and likely other effects. Clinical studies show plasma levels of TMAO are associated with increased risk for cardiovascular disease (CVD), including incident risks for myocardial infarction (MI), stroke, and death. Metabolism-independent effects include gut hyperpermeability (leaky gut), allowing bacterial cell wall products such as lipopolysaccharide (LPS) and peptidoglycans to enter the bloodstream. Low circulating levels of these bacterial components collectively activate macrophages, which can reduce reverse cholesterol transport and increase insulin resistance, hyperlipidemia, and vascular inflammation. Collectively, metabolism-dependent and -independent effects of the gut microbial endocrine organ converge to modulate risk of developing atherosclerotic CVD, MI, stroke, and death. Other abbreviations: BA, bile acids; NOD1, nucleotide oligomerization domain-containing 1; TLR4, toll-like receptor 4.

of microbes present do not cause harm to the host, and in many cases provide benefit through symbiotic relationships. However, recent evidence suggests gut microbial-driven pathways may actually be causally linked to several chronic diseases in humans (8–27a). Collectively, these realizations have prompted large-scale projects to define microbe–host interaction, such as the United States' Human Microbiome Project (HMP) and the European Metagenomics of the Human Intestinal Tract (MetaHIT) Consortium (30, 31). It is now well established through

SCFA: short chain fatty acid

TMAO: trimethylamine-N-oxide

TMA: trimethylamine

such metagenomic sequencing efforts that the human gut possesses a core bacterial microbiome that is predominated by phyla such as Bacteroidetes and Firmicutes. However, lower-abundance phyla such as Proteobacteria, Actinobacteria, and Verrucomicrobia are commonly present in the human gut (30, 31). It is key to note that the human gut microbiome, though resilient, is also highly dynamic and can be dramatically altered by antibiotic use, as well as less rapidly affected by age, host genetics, chronic dietary patterns, and other environmental exposures (32–38). The types of microbes present and the dynamic nature of these symbionts in the human gut are the topic of several excellent recent reviews (10, 38) and are not expanded upon here.

The Merriam-Webster definition of an endocrine organ is as follows: "producing secretions that are distributed in the body by way of the bloodstream." Although the field of endocrinology has historically focused on host organ systems with endocrine properties (hypothalamus, pituitary gland, pineal gland, thyroid gland, pancreas, adipose tissue, etc.), the gut microbiome also fits this classic definition, forming a pseudo-organ with unparalleled endocrine signaling potential. Unlike host endocrine organs, which produce only a few key hormones, the gut microbial endocrine organ has the unique potential to produce hundreds if not thousands of humoral agents generated either through metabolism-dependent or metabolism-independent pathways (**Figure 1**). Much like hormones derived from human endocrine organs, bacterially derived hormones are sensed by highly selective host receptor systems that elicit diverse biological responses (**Figure 2**). The current list of bacterially derived hormones includes trimethylamine/trimethylamine-N-oxide (20–27a), short chain fatty acids (SCFAs) (39–45), secondary bile acids (46–52), polysaccharide A (53), 4-ethyl phenyl sulfate (54), and catecholamines (55). Gut microbial products have also been shown to functionally interact with the host endocrine system to indirectly alter classic hormonal responses to cortisol (56), ghrelin (57), leptin (58), glucagon-like peptide 1 (59), and peptide YY (60). In fact, there is now a growing appreciation for gut microbiota-driven alteration of neurotransmission in the brain, supporting the idea that hormone-like compounds from the gut can act at distant sites in the central nervous system (61). Collectively, these data position the gut microbial endocrine organ as a central player in producing its own hormones (20–27a, 39–55), as well as establishing the signaling tone of host hormones (56–60). Clearly, gut microbes form a largely neglected endocrine organ with important relevance to human disease.

THE GUT MICROBIAL ENDOCRINE ORGAN AS A BASIS OF CARDIOVASCULAR DISEASE: THE TRIMETHYLAMINE-N-OXIDE STORY

With particular relevance to human CVD risk, we have recently described a meta-organismal metabolic pathway that involves multiple interactions between the gut microbial endocrine organ and host metabolic and signaling machinery (20–27a). Initially, we used a metabolomic approach to unbiasedly identify small-molecule metabolites associated with CVD risk in human plasma (20). We found that three metabolites of the dietary lipid phosphatidylcholine (PC) were highly predictive of CVD risk: choline, trimethylamine-N-oxide (TMAO), and betaine (20). In follow-up studies, we have shown that feeding atherosclerosis-prone mice diets enriched in either choline or TMAO enhances atherosclerosis development and alterations in cholesterol and sterol metabolism (20, 21). Importantly, the enhanced atherosclerosis seen with dietary choline supplementation is entirely dependent on gut microbiota, given that antibiotic treatment or germ-free conditions abolished dietary choline-driven TMAO generation and atherosclerosis development (20). These original studies uncovered a novel meta-organismal metabolic pathway linking dietary PC intake to CVD risk. This novel pathway involves gut microbiota-dependent metabolism of dietary PC to generate the gas trimethylamine (TMA), which is subsequently metabolized by enzymes of the

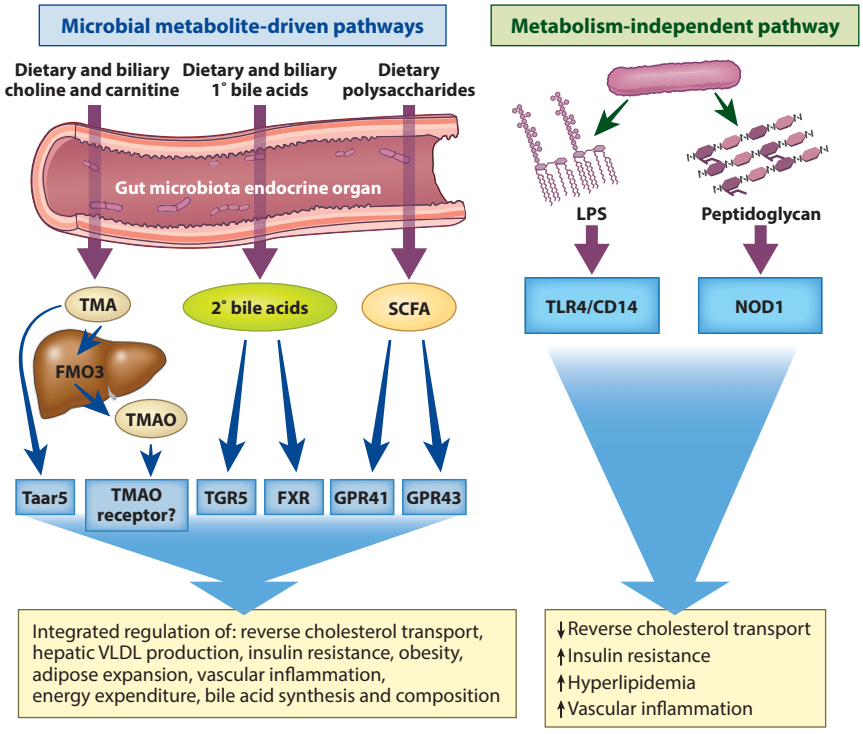

Figure 2

Host receptor systems for sensing bacterial products relevant to cardiovascular disease. Microbial products or metabolites are sensed by the host through dedicated receptor systems to elicit a biological response. Host receptors have been identified for both microbial metabolite-driven pathways and metabolism-independent pathways that signal to reorganize host metabolism and inflammation to alter cardiovascular disease risk. Abbreviations: CD14, cluster of differentiation 14; FMO3, flavin monooxygenase 3; FXR, farnesoid X receptor; GPR41, G protein–coupled receptor 41; GPR43, G protein–coupled receptor 43; TGR5, G protein–coupled bile acid receptor 1; LPS, lipopolysaccharide; TLR4, toll-like receptor 4; NOD1, nucleotide-binding oligomerization domain-containing 1; SCFAs, short chain fatty acids; TMA, trimethylamine; TMAO, trimethylamine-N-oxide; VLDL, very low-density lipoprotein.

flavin monooxygenase (FMO) family in the host's liver to generate the circulating proatherogenic compound TMAO (20, 21, 24).

Subsequent studies have revealed that other nutrients can feed into this meta-organismal pathway producing TMA and TMAO, broadening its relevance to diet-driven alterations in CVD risk. L-carnitine, a particularly abundant nutrient in red meat, contains a trimethylamine structure very similar to choline. Much like dietary choline (20), the quaternary amine structure of L-carnitine is readily converted to TMA by gut microbes (21). In fact, bacterial metabolism of L-carnitine can provide an abundant source of TMA that ultimately produces proatherogenic TMAO in both mice and humans. In agreement, feeding L-carnitine to hyperlipidemic mice alters gut microbiota composition, increases blood TMAO levels, and promotes atherosclerosis in a gut microbiota-dependent fashion. Importantly, plasma L-carnitine levels predicted increased risks for both prevalent CVD and incident major adverse cardiac events (myocardial infarction, stroke, or death) in a large clinical cohort (n = 2,595), but only in subjects whose TMAO levels were also elevated. Moreover, comparisons of omnivores (who have frequent dietary

FMO: flavin monooxygenase

L-carnitine exposure) versus vegans or vegetarians revealed striking differences in the capacity to convert dietary L-carnitine into TMA and TMAO, with vegans/vegetarians showing virtually no TMA/TMAO formation (21). Collectively, these results suggest that chronic exposure to dietary L-carnitine promotes the production of the proatherogenic compound TMAO both in mice and man. Further, they may in part explain why high red meat consumption has been associated with increased CVD and mortality risks (62).

LDL: low-density lipoprotein

Although plasma levels of several metabolites (choline, betaine, trimethylamine, L-carnitine, TMAO) relevant to this meta-organismal pathway are associated with increased CVD risk, subsequent analyses have revealed that the prognostic value is largely confined to TMAO (21–23). Recently, we demonstrated that elevated TMAO levels associate with increased risk of incident major adverse cardiovascular events in a large independent clinical cohort (n = 4,007) (22). People in the highest quartile of circulating TMAO levels had a 2.5-fold increased risk of having a major adverse cardiac event compared to those in the lowest quartile. Within the cohort examined, the hazard ratio for TMAO was much higher than for traditional risk factors such as LDL cholesterol. Further, elevated TMAO levels retained strong prognostic value for predicting incident adverse cardiovascular events even after adjustment for traditional risk factors and renal function (23). In addition to the clear link between TMAO and atherosclerotic CVD risk (20–24), TMAO levels have also more recently been linked to heart failure development, poor prognosis in heart failure patients (25), as well as high-fat-diet-induced obesity and insulin resistance in a rodent model (63). Given the clear links between circulating TMAO levels and human cardiometabolic diseases, we have recently developed a quantitative analytical assay for measuring TMAO levels (27), which will be useful for validation studies in additional cohorts. Collectively, these new data suggest that the meta-organismal pathway responsible for the production of TMAO is an important new determinant for increased CVD risk in humans.

Given that blood levels of TMAO are linked to CVD risk in humans, the next obvious question is whether circulating TMAO is simply a biomarker of disease or whether TMAO is mechanistically involved in CVD pathogenesis. Current evidence suggests a causative link, given that dietary supplementation of TMAO in hyperlipidemic mice promotes atherosclerosis in a gut microbiota-dependent manner (20, 21). However, the mechanism(s) by which circulating TMAO promotes CVD is currently unclear and under active investigation. TMAO has historically been thought of as a key osmolyte, as well as a small-molecule chaperone that stabilizes proteins under denaturing conditions such as high urea or elevated water pressure (64, 65). In the broader context of cardiovascular physiology, we have recently shown that TMAO can impact distinct steps in cholesterol and sterol metabolism and macrophage foam cell formation. Dietary choline or TMAO supplementation results in increased expression of scavenger receptors (CD36 and SR-A1) on macrophages and subsequently promotes foam cell formation (20). In addition, TMAO feeding reduces macrophage reverse cholesterol transport (21), which would be predicted to advance atherosclerosis. Although TMAO feeding clearly impacts multiple steps of both forward and reverse cholesterol transport (20, 21), the underlying molecular mechanisms behind these observations remains unclear. There are many unresolved questions, such as how circulating TMAO levels are sensed to elicit pathological responses, and additional research is needed to elucidate mechanisms by which TMAO promotes CVD.

OTHER EXAMPLES OF MICROBIAL ENDOCRINOLOGY IN CARDIOVASCULAR DISEASE

TMAO is one of many bacterially derived products that have hormone-like properties in the host. Alternative gut microbiota-derived metabolites with hormone-like properties are SCFAs,

including acetic acid, butyric acid, propionic acid, and valeric acid (36–42) (**Figures 1** and **2**). It is well established that a subset of anaerobic bacteria found in the cecum and proximal colon produce SCFAs via fermentation of nondigestable carbohydrates (39). Although the human genome does not encode enzymes capable of breaking down many common forms of complex carbohydrates, anaerobic bacteria serve as a dietary filter to ferment several classes of carbohydrates, including pectins, gums, hemicelluloses, and galactose-oligosaccharides, to produce key metabolites that are then subsequently metabolized by the host or alternatively act as hormones (39). Well-documented roles for bacterially derived SCFAs are to provide fuel for the colonic epithelium, as well as to regulate intestinal immune homeostasis (39–41). In particular, gut microbial production of butyrate interacts with host receptors on leukocytes and endothelial cells in the intestine to balance Th1 and Th2 immune responses (41). In addition, gut microbial-derived SCFAs have potent effects on insulin action in peripheral tissues (42, 43). Most recently, gut microbe-generated SCFAs have been found to act distally in the central nervous system to regulate integrated metabolic responses (44). Serving as a prime example of meta-organismal endocrinology, gut microbiota-derived SCFAs are sensed by dedicated G protein–coupled receptors (GPR41 and GPR43) that reside in diverse host cell populations in peripheral tissues (42–45) (**Figure 2**). Collectively, by regulating energy metabolism, insulin sensitivity, and immune cell programs and responses, SCFAs act as key meta-organismal hormones regulating physiology relevant to numerous processes involved in CVD.

Another elegant example of meta-organismal endocrinology with implications in CVD involves a stepwise interaction of host synthesis, bacterial modification, and subsequent host sensing of bacterially modified bile acids (46–52) (**Figures 1** and **2**). Bile acids have long been known to be important in solubilizing dietary fat and cholesterol for absorption into the body (52), but within the last decade bile acids have been recognized as hormones, regulating many physiological processes such as energy expenditure, insulin sensitivity, and cholesterol balance (52). This meta-organismal pathway is initiated when host enzymes convert cholesterol to primary bile acids, which is a process that is highly regulated by classic feedback regulation (52). Once primary bile acids are synthesized in the host liver, they are secreted into bile, enter into the lumen of small intestine, and traverse through the intestine until they reach the terminal ileum, where they are almost completely recovered (>95%) by selective ileal bile acid transporters. The bile acids that are not reabsorbed then encounter a subset of facultative and anaerobic bacteria resident in the large bowel, where they undergo complex deconjugation and hydroxyl group oxidation modifications generating microbe-dependent secondary bile acids (52). Importantly, a small amount of these microbiota-derived secondary bile acids is released into the bloodstream, typically in the postprandial state, where the bile acids act as hormones to signal to the host (46–52).

There are two major host receptor systems known that sense bacterially derived secondary bile acids (**Figure 2**). The most well-characterized bile acid receptor is a nuclear hormone receptor known as farnesoid X receptor (FXR), which can be activated to elicit transcriptional responses involved in feedback regulation and intestinal bile acid transport (49–51). More recently, a G protein–coupled receptor called TGR5 has been shown to sense plasma bile acid levels to regulate multiple steps in energy balance and insulin sensitivity (46–48). Collectively, microbe–host crosstalk in bile acid metabolism and signaling represents an important endocrine axis regulating cardiometabolic pathways relevant to host physiology and CVD (46–52).

NONENDOCRINE PROPERTIES OF GUT MICROBES IMPACTING CARDIOVASCULAR DISEASE

In addition to the ability of bacterially derived metabolites to act as hormones modulating CVD risk (20–27a, 39–55), components of the bacterial cell wall (lipopolysaccharide and peptidoglycan)

can also be recognized by the host's innate immune system to potentiate CVD pathogenesis (**Figure 1**). Microbe-associated molecular patterns (MAMPs) such as lipopolysaccharide and peptidoglycan are selectively recognized by host toll-like receptors (TLRs) and nucleotide oligomerization domain-containing receptors (NODs), respectively (66, 67) (**Figure 2**). Although it was long thought that this microbial interaction with the innate immune system was most active in the distal gut (70, 71), more recently it has begun to be appreciated that low levels of bacteria can actually make it into the bloodstream to cause chronic low-grade inflammation systemically (72). The concept that low levels of gut-derived bacteria can appear in the circulation is commonly referred to as "metabolic endotoxemia" because it has been found to be prevalent in many chronic metabolic diseases such as obesity, type II diabetes, and atherosclerosis (72). Although this type of microbe–host signaling cannot be classified as endocrine in nature, it has clear potential to alter CVD risk. In fact, human mutations and mouse knockout studies demonstrate a key role for TLR4 or NOD2 activation in atherosclerosis development (66–74). Furthermore, microbial activation of these innate immune receptors promotes inflammation that dampens reverse cholesterol transport, while augmenting insulin resistance, hyperlipidemia, and vascular inflammation (75, 76) (**Figure 1**). Collectively, metabolic endotoxemia and engagement of peripheral pattern recognition receptors play a potentially contributory role in the pathogenesis of CVD by reorganizing lipid metabolism and promoting inflammatory responses (75, 76).

MOVING FORWARD: A META-ORGANISMAL VIEW OF CARDIOVASCULAR MEDICINE

As we move forward considering a meta-organismal view of cardiovascular medicine, several key considerations will need to be taken into account. First, it is clear that gut microbes impact host physiology by generating metabolic intermediates from dietary substrates (20–27a, 39–55). Therefore, it is imperative that we continue to focus on identifying bacterially derived metabolites that have relevance to human disease. Given major advances in the field of metabolomics over the last decade (77), we are now well positioned to identify the entire microbial-generated metabolome by coupling unbiased metabolomic platforms to germ-free or antibiotic-treated model systems. In parallel, we must move forward from correlative metagenomics to mechanistic studies linking bacterially derived metabolites to disease phenotypes in human and animal model systems where gut microbe levels can be experimentally manipulated and determined (20–27a). Once key bacterially derived metabolites are found to be causally linked to human physiology or disease, we must adopt a true endocrine philosophy to subsequently identify the host receptor systems that sense the metabolic hormones produced by gut microbes.

In addition to nutrient-derived metabolites, gut microbes also metabolize a number of xenobiotics to produce modified compounds with broad implications in human health. Although there are many instances of bacterial modification of drugs in humans (78), several elegant examples highlight the importance of our microbial counterparts in shaping the way we respond to drugs. One seminal study employed a metabolomic profiling approach to identify a microbial metabolite (p-cresol) of the commonly used drug acetaminophen. This work showed that microbial production of p-cresol reduces the ability of the host liver to properly metabolize acetaminophen, likely due to competition with sulfotransferases (79). With relevance to CVD, the drug digoxin can be metabolized and functionally inactivated by a common actinobacteria (80). Also, the chemotherapeutic drug irinotecan is metabolized in a gut microbe-dependent manner, generating a metabolite linked to adverse side effects. Inhibition of the bacterial β-glucuronidase enzyme responsible for this reaction was shown to improve the effectiveness of irinotecan while decreasing deleterious side effects (81). Most recently, several studies have shown that gut microbial metabolism of

chemotherapeutic drugs can actually increase their efficacy, providing novel evidence of "symbiosis" (82, 83). These studies highlight the central role that gut microbial metabolism can play in drug metabolism, and also provocatively suggest that dual therapies containing xenobiotics and bacterial modifiers may provide more benefit than either drug alone (81).

Since the invention of the microscope, the field of microbiology has matured over almost four centuries of research. However, even today, translating microbiological knowledge into new therapies that impact human physiology and disease presents many challenges. For instance, despite our wealth of knowledge surrounding the taxa of microbes that inhabit the human gut (28–38), only a minority of these species are amenable to laboratory culture (84). This limitation generates selection bias in our knowledge base. Our appreciation of the secretory repertoire of distinct microbial taxa has been constrained by our inability to culture and study them in a reduced clonal population system, and improvements in methods to culture diverse types of human gut-derived microbes are needed. Progress on this front is slowly beginning (85), and will be central to generating novel probiotic approaches to human health, as is discussed in detail below. Another key consideration for translating microbiology into human health is the concept of horizontal gene transfer (HGT) (86). HGT refers to the transfer of genes between organisms in a manner that does not rely on reproduction. Interbacterial gene transfer was first describe by Ochiai and colleagues (87), who demonstrated that antibiotic resistance could be laterally transferred from one bacterial strain to another. HGT is very common among all bacteria, but interestingly, microbes within the human gut have a 25-fold higher rate of HGT than microbes in other ecosystems (88). The effect of HGT in human physiology is exemplified by the recent demonstration that bacterial transfer of a marine bacterial gene to a human resident symbiont confers the ability to digest seaweed polysaccharides (89). These microbiological concepts, as well as others, will be critical to consider as we move forward to design CVD drugs targeting the microbiome.

THE FUTURE OF CARDIOVASCULAR DRUG DISCOVERY: OPPORTUNITIES TO TARGET THE GUT MICROBIAL ENDOCRINE ORGAN

Historically, drug discovery has been dominated by targeting host pathways driving disease pathology. Accordingly, current and future CVD drug discovery efforts will include approaches targeting the gut microbial endocrine organ (**Figure 3**). As a frame of reference, here we discuss therapeutic opportunities within each of these categories relevant to the gut microbial-driven TMAO pathway (20–27a). However, as additional meta-organismal pathways are discovered, these same approaches will no doubt also be utilized in a broad array of cardiometabolic drug discovery targets.

The simplest point of intervention is to limit consumption of dietary constituents that serve as substrates for metabolism-dependent generation of proatherogenic hormones such as TMA/TMAO (**Figure 3**). For instance, we know that both free choline and PC (20), as well as L-carnitine (21), γ-butyrobetaine, and, to a lesser extent, betaine (22), can serve as dietary substrates for the sequential microbial production of TMA and subsequent host formation of proatherogenic TMAO (20–27a). Therefore, limiting the consumption of foods rich in total choline and L-carnitine can be an effective strategy to limit circulating TMAO (20–24). In fact, people suffering from the condition trimethylaminuria, which is caused by mutations in the host enzyme flavin monooxygenase 3 (FMO3), can significantly reduce circulating TMA levels simply by eating a low-fat and low-choline diet (90). Importantly, there are likely other trimethylamines in the food supply that can also enter into microbiota-dependent TMA production that have yet to be identified. Hence, it is important that we continue efforts to identify all major dietary amines that

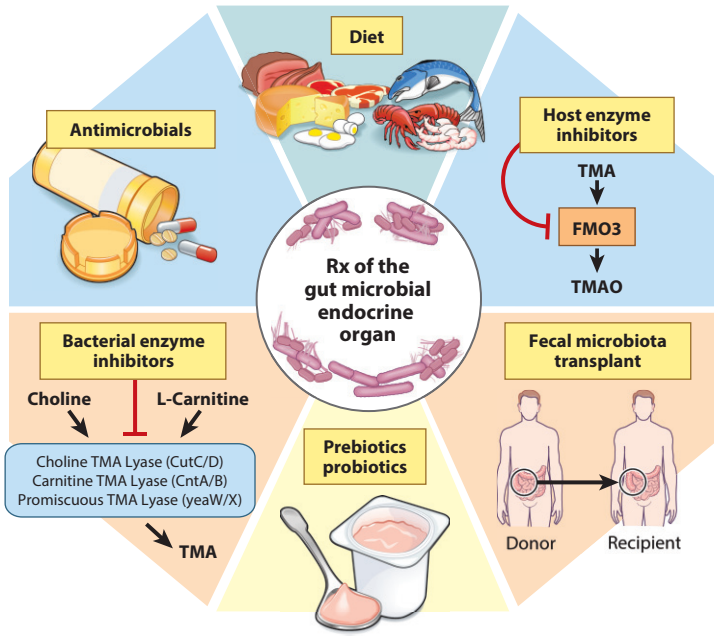

Figure 3

Strategies to target the gut microbial endocrine organ for improving cardiovascular disease treatment. Current strategies for manipulating gut microbiota and potentially impacting CVD include dietary manipulation, prebiotics or probiotics, fecal microbiota transplantation, antimicrobials/antibiotics, bacterial enzyme inhibitors, and host enzyme inhibitors. Abbreviations: FMO3, flavin monooxygenase 3; TMA, trimethylamine; TMAO, trimethylamine-N-oxide.

can enter into the TMAO pathway and apply this knowledge to other relevant meta-organismal metabolic pathways.

Another potential point of therapeutic intervention relies on the use of broad or class-specific antibiotics to eliminate the production of proatherogenic gut microbe-generated hormones. However, this approach is not a likely long-term option because many gut microbial products are beneficial to the host (28–38), and frequent antibiotic treatment can facilitate the emergence of antibiotic-resistant bacterial strains (33, 87, 88). Despite these potential problems, several clinical trials involving prolonged antibiotic treatment have been conducted to examine effects on CVD endpoints such as myocardial infarction and death (91). Collectively, these trials thus far have shown that although long-term antibiotic treatments were well tolerated, no benefit on cardiovascular endpoints was observed (91). These studies are limited by the fact that despite initial diminution of gut microbes by antibiotic treatment that might generate a specific metabolite, antibiotic-resistant strains present in low abundance over time will repopulate and expand their intestinal niche. Further, antibiotic use suppresses good and bad types of commensals alike, making it difficult to target specific taxa and favorably impact cardiometabolic phenotypes.

A more tractable approach for modifying the gut microbial endocrine organ is the use of prebiotics or probiotics. Prebiotic therapy consists of ingestion of select nutrients or dietary constituents (nonmicrobial compositions) that provide a growth advantage of beneficial bacteria (92). A key example of a prebiotic therapy is the ingestion of nondigestible fiber that can enhance the growth of beneficial commensals and alter motility, secondarily impacting gut microbial community structure (92). Probiotic therapy involves the dietary ingestion of one or more live bacterial

strains, attempting to take advantage of the mutualism of microbes and potential for horizontal gene transfer to benefit the host (86–88). Although prebiotic and probiotic therapies are still at the early phases of development, several recent examples highlight their potential. First, the prebiotic ingestion of dietary fructans, which are naturally occurring fructose polymers in many common fruits and vegetables, provides a growth advantage to a beneficial gut microbe family known as *Bifidobacteria* (92). Importantly, dietary-fructan-stimulated *Bifidobacteria* colonization has been associated with improvements in obesity-induced insulin resistance (92). Recent work with probiotics has also shown some promising results. For instance, administration of the probiotic VSL#3 to mice was shown to alter microbial metabolism of bile acids, with resultant potential antiatherogenic alterations in host cholesterol and bile acid metabolism (93). Furthermore, probiotic administration of a genetically modified strain of bacteria designed to generate high levels of a beneficial class of lipids called N-acylphosphatidylethanolamines was recently shown to protect mice from obesity-related disorders (94). These examples highlight the potential utility of both prebiotic and probiotic approaches for the treatment or prevention of CVD.

Although prebiotic and probiotic approaches hold great promise, they lack specificity, given that they impact many types of gut microbial communities due to microbial mutualism and horizontal gene transfer. An alternative therapeutic approach would be to specifically inhibit the bacterial enzyme(s) responsible for the production of TMA or other CVD-relevant microbial metabolites. Craciun & Balskus have identified a bacterial gene cluster responsible for the anaerobic production of TMA from choline (95). Homologues of the choline utilization (*Cut*) gene cluster were identified in 89 bacterial genomes, and the gene products (CutC and CutD) encode a glycyl radical enzyme complex capable of TMA production from choline in vitro (95). Interestingly, a distinct Rieske-type oxygenase/reductase system (CntA and CntB) has also recently been described as a bacterial enzyme complex capable of converting L-carnitine to TMA (96). A related but more promiscuous TMA lyase complex (yeaW/X) has also been reported (27a). It is tempting to speculate that these TMA-producing enzymes, as well as additional not yet identified microbial TMA lyases, may be attractive drug targets within the gut microbial endocrine organ by virtue of their potential to reduce TMA and thus TMAO levels. Another important consideration regarding TMA and TMAO metabolism by gut microbes is that several common taxa of bacteria including *Escherichia coli* can reduce TMAO, providing electron acceptors for energy production under anaerobic conditions (97). This additional level of complexity in the meta-organismal TMAO pathway is driven by the bacterial torCAD operon, which encodes a TMAO reductase (TorA), a c-type cytochrome (TorC), and a TorA-specific chaperone (TorD) (97). A recent report has similarly shown that bacteria present in ocean water can also degrade TMAO through a novel TMAO demethylase enzyme (Tdm) (98). It remains possible that these bacterial TMAO catabolic pathways are another potential avenue of therapeutic intervention, given that gut microbes have a high capacity to metabolize TMAO within the human meta-organism. The identification of the bacterial enzymes responsible for TMA production and TMAO degradation represents an important advance. They will facilitate future studies designed to identify means of favorably altering the TMA/TMAO axis for potential therapeutic benefit. Examples of potential approaches include both the development of selective microbial TMA lyase inhibitors and the development of TMAO-degrading enzymes.

Another potential site of therapeutic intervention lies at the level of the host enzyme machinery necessary for the conversion of TMA to TMAO. The FMO enzyme family carries out this critical oxidation step, with at least five paralogues present in human liver (FMO1, FMO2, FMO3, FMO4, and FMO5) (99). In addition to TMA oxidation, the FMO enzyme family is responsible for the oxygenation of a plethora of nitrogen- and sulfur-containing compounds present in xenobiotics, as well as endogenous substrates (99). FMO3 and FMO1 are the major human FMO

enzymes that can efficiently oxidize TMA to form TMAO, with FMO3 having the highest specific activity toward TMA (24). Interestingly, loss-of-function mutations of the FMO3 gene result in the inherited disorder trimethylaminuria, which is also known as fish odor syndrome (99). This autosomal recessive disease arises from the inability of those affected to convert TMA, which smells like rotten fish, to TMAO (99). The FMO3 gene is under complex transcriptional control, as expression is dynamically regulated by inflammation, sex hormones, and the bile acid–activated nuclear receptor FXR (24, 99). Although pharmacological inhibition of FMO3 is expected to provide therapeutic benefit by reducing host TMAO production, it is unlikely that FMO3 inhibitors would be attractive drug targets. The accumulation of the FMO3 substrate (TMA) would be expected to result in odorous side effects. Also, because FMO3 itself is a xenobiotic metabolizing enzyme, there is a strong potential for drug–drug interactions with FMO3 inhibitors, further dampening enthusiasm for FMO3 inhibition as a therapeutic strategy. Regardless of therapeutic utility, it will be important to better understand how hepatic FMO activity is regulated under physiological and pathophysiological conditions, given its central role in determining circulating TMAO levels (99).

A key therapeutic opportunity lies at the level of blocking the ability of circulating TMAO to elicit a biological response. One potential therapeutic avenue might be development of absorbent agents that can bind and help eliminate TMA and TMAO at the level of the gut. A theoretical alternative would be to intercept TMAO at the level of a molecular receptor (i.e., through a TMAO receptor antagonist). Although a dedicated host TMAO receptor system has not yet been identified, it would not be surprising if such a sensing mechanism exists. In fact, virtually all gut microbial products described to date have dedicated host receptor systems that sense the levels of these biologically active gut microbial products (**Figure 2**). The concept of a TMAO receptor is bolstered by the fact that gut microbe-generated TMA is sensed by the host G protein–coupled receptor Taar5 (68, 69). Identification of a host TMAO receptor system, and subsequent development of TMAO receptor antagonists, would represent an extremely attractive therapeutic approach without the potential drawbacks of dietary manipulation or microbe-modifying drugs. It is quite clear that the interaction between gut microbes and the host they inhabit occurs through delicate host sensing mechanisms (**Figure 2**), and further characterization of such signaling processes will provide exciting new therapeutic opportunities.

CONCLUSIONS AND PERSPECTIVES

The recent discovery that our gut microbiota plays a central role in diet-dependent CVD susceptibility has broad implications. Although largely overlooked, our gut microbiome functions as a dynamic yet resilient endocrine organ. It produces a plethora of metabolism-dependent and metabolism-independent signals that play regulatory roles in CVD development in the host (**Figure 1**). On one hand, commensals present in the human gut serve as a "metabolic filter," significantly influencing how dietary inputs are assimilated by the host. Gut microbiota-derived hormones are already identified that alter energy metabolism, cardiometabolic disease-relevant phenotypes, and CVD pathogenesis. On the other hand, chronic low levels of inflammation driven by metabolic endotoxemia can also apparently reorganize cholesterol balance, insulin resistance, and vascular inflammation (**Figure 1**). Given the central role of the gut microbial endocrine organ in influencing how environmental factors impact CVD in humans, substantial drug discovery opportunity lies ahead in the area of meta-organismal crosstalk. Although the field has been dominated by correlative metagenomic approaches, simply cataloguing microbial communities in disease models is insufficient as we move forward. Instead, functional studies integrating microbial product identification (metabolomics, proteomics, etc.), dietary manipulation, and host receptor

discovery in relevant disease model systems are necessary. Although drug discovery has historically targeted host enzymes, a fertile period in biomedical research lies ahead as instead we target the microorganisms that live within us to either improve human health or prevent disease.

DISCLOSURE STATEMENT

Dr. Hazen is listed as coinventor on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics and therapeutics. He has been a paid consultant to the following companies: Cleveland Heart Lab, Esperion, Liposcience, Inc., Pfizer, Inc., Proctor & Gamble, and Takeda, and has received research funds from Cleveland Heart Lab, Liposcience, Inc., Proctor & Gamble, and Takeda. He also has the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics and therapeutics for Cleveland Heart Lab, Esperion, Frantz Biomarkers, LLC, and Siemens.

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LITERATURE CITED

1. Go AS, Mozaffarian D, Roger VL, et al. 2014. Heart disease and stroke statistics: 2014 update, a report from the American Heart Association. *Circulation* 129:e28–e292
2. Nabel EG. 2003. Cardiovascular disease. *N. Engl. J. Med.* 349:60–72
3. Libby P, Ridker PM, Hansson GK. 2011. Progress and challenges in translating the biology of atherosclerosis. *Nature* 473:317–25
4. Teslovich TM, Musunuru K, Smith AV, et al. 2010. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* 466:707–13
5. Kathiresan S, Willer CJ, Peloso GM, et al. 2009. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat. Genet.* 41:56–65
6. Myocardial Infarction Genetics Consortium, Kathiresan S, Voight BF, Purcell S, et al. 2009. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. *Nat. Genet.* 41:334–41
7. CARDIoGRAMplusC4D Consortium. 2013. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat. Genet.* 45(1):25–33
8. Turnbaugh PJ, Ley RE, Mahowald MA, et al. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444:1027–31
9. Möbius KA. 1877. *Die Auster und dieAusternwirthschaft*. Berlin: Wiegandt, Hempel & Parey
10. Kau AL, Ahern PP, Griffin NW, et al. 2011. Human nutrition, the gut microbiome and the immune system. *Nature* 474:327–36
11. Dumas ME, Barton RH, Toye A, et al. 2006. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proc. Natl. Acad. Sci. USA* 103:12511–16
12. Delzenne NM, Cani PD. 2011. Gut microbiota and the pathogenesis of insulin resistance. *Curr. Diab. Rep.* 11:154–59
13. Aron-Wisnewsky J, Gaborit B, Dutour A, Clement K. 2013. Gut microbiota and non-alcoholic fatty liver disease: new insights. *Clin. Microbiol. Infect.* 19:338–48

14. Sjögren K, Engdahl C, Henning P, et al. 2012. The gut microbiota regulates bone mass in mice. *J. Bone Miner. Res.* 27:1357–67
15. Yoshimoto S, Loo TM, Atarashi K, et al. 2013. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 499:97–101
16. Kostic AD, Chun E, Robertson L, et al. 2013. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 14:207–15
17. Ray K. 2012. Gut microbiota: Colorectal cancer—driven by inflammation and gut bacteria? *Nat. Rev. Gastroenterol. Hepatol.* 9:588
18. Trompette A, Gollwitzer ES, Yadava K, et al. 2014. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* 20:159–66
19. Shulzhenko N, Morgun A, Hsiao W, et al. 2011. Crosstalk between B lymphocytes, microbiota and the intestinal epithelium governs immunity versus metabolism in the gut. *Nat. Med.* 17:1585–93
20. Wang Z, Klipfell E, Bennett BJ, et al. 2011. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 472:57–63
21. Koeth RA, Wang Z, Levinson BS, et al. 2013. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat. Med.* 19:576–85
22. Tang WH, Wang Z, Levison BS, et al. 2013. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N. Engl. J. Med.* 368:1575–78
23. Wang Z, Tang WH, Buffa JA, et al. 2014. Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-N-oxide. *Eur. Heart J.* 35:904–10
24. Bennett BJ, de Aguiar Vallim TQ, Wang Z, et al. 2013. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metab.* 17:49–60
25. Tang WH, Wang Z, Fan Y, et al. 2014. 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.* 64:1908–14
26. Hartiala J, Bennett BJ, Tang WH, et al. 2014. 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.* 34:1307–13
27. Wang Z, Levison BS, Hazen JE, et al. 2014. Measurement of trimethylamine-N-oxide by stable isotope dilution liquid chromatography tandem mass spectrometry. *Anal. Biochem.* 455:35–40
27a. Koeth RA, Levison BS, Culley MK, et al. 2014. γ-Butyrobetaine is a pro-atherogenic intermediate in gut microbial metabolism of *L*-carnitine to TMAO. *Cell Metab.* 20(5):799–812
28. Whitman WB, Coleman DC, Wiebe WJ. 1998. Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci. USA* 95:6578–83
29. The Human Microbiome Project Consortium. 2012. Structure, function and diversity of the healthy human microbiome. *Nature* 486:207–14
30. Qin J, Li R, Raes J, et al. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464:59–65
31. Arumugam M, Raes J, Pelletier E, et al. 2011. MetaHIT Consortium. Enterotypes of the human gut microbiome. *Nature* 473:174–80
32. Schloissnig S, Arumugam M, Sunagawa S, et al. 2013. Genomic variation landscape of the human gut microbiome. *Nature* 493:45–50
33. Jernberg C, Lofmark S, Edlund C, Jansson JK. 2010. Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology* 156:3216–23
34. Claesson MJ, Jeffery IB, Conde S, et al. 2012. Gut microbiota composition correlates with diet and health in the elderly. *Nature* 488:178–84
35. Yatsunenko T, Rey FE, Manary MJ, et al. 2012. Human gut microbiome viewed across age and geography. *Nature* 486:222–27
36. Muegge BD, Kuczynski J, Knights D, et al. 2011. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science* 332:970–74
37. Wu GD, Chen J, Hoffmann C, et al. 2011. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 334:105–8

38. Lozupone CA, Stombaugh JI, Gordon JI, et al. 2012. Diversity, stability and resilience of the human gut microbiota. *Nature* 489:220–30
39. Macfarlane GT, Macfarlane S. 2012. Bacteria, colonic fermentation, and gastrointestinal health. *J. AOAC Int.* 95:50–60
40. den Besten G, Lange K, Havigna R, et al. 2013. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am. J. Physiol. Gastrointest. Liver Physiol.* 305:G900–10
41. Saemann MD, Bohmig GA, Osterreicher CH, et al. 2000. Anti-inflammatory effects of sodium butyrate on human monocytes: potent inhibition of IL-12 and up-regulation of IL-10 production. *FASEB J.* 14:2380–82
42. Kimura I, Ozawa K, Inoue T, et al. 2013. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat. Commun.* 4:1829
43. Gao Z, Yin J, Zhang J, et al. 2009. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* 58:1509–17
44. De Vadder F, Kovatcheva-Datchary P, Goncalves D, et al. 2014. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* 156:84–96
45. Brown AJ, Goldsworthy AA, Barnes AA, et al. 2003. The orphan G protein-coupled receptors GPR41 and GRP43 are activated by proprionate and other short chain carboxylic acids. *J. Biol. Chem.* 278:11312–19
46. Watanabe M, Houten SM, Mataki C, et al. 2006. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 439:484–89
47. Thomas C, Gioiello A, Noriega L, et al. 2009. TGR5-mediated bile acid sensing control glucose homeostasis. *Cell Metab.* 10:167–77
48. Pols TW, Nomura M, Harach T, et al. 2011. TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. *Cell Metab.* 14:747–57
49. Ma K, Saha PK, Chan LL, Moore DD. 2006. Farnesoid X receptor is essential for normal glucose homeostasis. *J. Clin. Invest.* 116:1102–9
50. Downes M, Verdecia MA, Roecker AJ, et al. 2003. A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. *Mol. Cell* 11:1079–92
51. Sayin SI, Wahlstrom A, Felin J, et al. 2013. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab.* 17:225–35
52. Hofmann AF, Hagey LR. 2014. Bile acid chemistry, biology, and therapeutics during the last 80 years: historical aspects. *J. Lipid Res.* 55:1553–95
53. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. 2005. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 122:107–18
54. Hsiao EY, McBride SW, Hsien S, et al. 2013. Microbiota modulate behavioral and physiological abnormalities associated with neuro-developmental disorders. *Cell* 155:1451–63
55. Asano Y, Hiramoto T, Nishino R, et al. 2012. Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 3030:G1288–95
56. Dinan TG, Cryan JF. 2012. Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology. *Psychoendocrinology* 37:1369–78
57. Dockray GJ. 2014. Gastrointestinal hormones and the dialogue between gut and brain. *J. Physiol.* 592:2927–41
58. Schele E, Grahnemo L, Anesten F, et al. 2013. The gut microbiota reduces leptin sensitivity and the expression of the obesity-suppressing neuropeptides proglucagon (Gcg) and brain-derived neurotrophic factor (Bdnf) in the central nervous system. *Endocrinology* 154:3643–51
59. Everard A, Cani PD. 2014. Gut microbiota and GLP-1. *Rev. Endocr. Metab. Disord.* 15:189–96
60. Holzer P, Reichmann F, Farzi A. 2012. Neuropeptide Y, peptide YY and pancreatic polypeptide in the gut-brain axis. *Neuropeptides* 46:261–74
61. Cryan JF, Dinan TG. 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* 13:701–12
62. Pan A, Sun Q, Bernstein AM, et al. 2012. Red meat consumption and mortality: results from 2 prospective cohort studies. *Arch. Intern. Med.* 172:555–63

63. Gao X, Liu X, Xu J, et al. 2014. Dietary trimethylamine-N-oxide exacerbates impaired glucose tolerance in mice fed a high fat diet. *J. Biosci. Bioeng.* S1389–1723:00081–84
64. Yancey PH, Clark ME, Hand SC, et al. 1982. Living with water stress: evolution of osmolyte systems. *Science* 217:1214–22
65. Lin TY, Timasheff SN. 1994. Why do some organisms use a urea-methylamine mixture as osmolyte? Thermodynamic compensation of urea and trimethylamin-N-oxide interactions with protein. *Biochemistry* 33:12695–701
66. Curtiss LK, Tobias PS. 2009. Emerging role of Toll-like receptors in atherosclerosis. *J. Lipid Res.* 50(Suppl):S340–45
67. Philpott DJ, Sorbara MT, Robertson SJ, et al. 2014. NOD proteins: regulators of inflammation in health and disease. *Nat. Rev. Immunol.* 14:9–23
68. Li Q, Korzan WJ, Ferrero DM, et al. 2013. Synchronous evolution of an odor biosynthesis pathway and behavioral response. *Curr. Biol.* 23:11–20
69. Wallrabenstein I, Kuklan J, Weber L, et al. 2013. Human trace amine-associated receptor TAAR5 can be activated by trimethylamine. *PLoS ONE* 8:e54950
70. Wells JM, Rossi O, Meijerink M, van Baarlen P. 2011. Epithelial crosstalk at the microbiota-mucosal interface. *Proc. Natl. Acad. Sci. USA* 108(Suppl.):4607–14
71. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, et al. 2004. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 118:229–41
72. Neves AL, Coelho J, Couto L, et al. 2013. Metabolic endotoxemia: a molecular link between obesity and cardiovascular disease. *J. Mol. Endocrinol.* 51:R51–64
73. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. 2011. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. USA* 108(Suppl. 1):4615–22
74. Yuan H, Zelka S, Burkatovskaya M, et al. 2013. Pivotal role of NOD2 in inflammatory processes affecting atherosclerosis and periodontal bone loss. *Proc. Natl. Acad. Sci. USA* 110:E5059–68
75. Khovidhunkit W, Kim MS, Memon RA, et al. 2004. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. *J. Lipid Res.* 45:1169–96
76. Fisher EA, Feig JE, Hewing B, et al. 2012. High-density lipoprotein function, dysfunction, and reverse cholesterol transport. *Arterioscler. Thromb. Vasc. Biol.* 32:2813–20
77. Griffin JL, Atherton H, Shockcor J, Atzori L. 2011. Metabolomics as a tool for cardiac research. *Nat. Rev. Cardiol.* 8:630–43
78. Haiser HJ, Turnbaugh PJ. 2013. Developing a metagenomic view of xenobiotic metabolism. *Pharmacol. Res.* 69:21–31
79. Clayton TA, Baker D, Lindon JC, et al. 2009. Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. *Proc. Natl. Acad. Sci. USA* 106:14728–33
80. Saha JR, Butler VP Jr, Neu HC, et al. 1983. Digoxin-inactivating bacteria: identification in human gut flora. *Science* 220:325–27
81. Wallace BD, Wang H, Lane KT, et al. 2010. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science* 330:831–35
82. Lida N, Dzutsev A, Stewart CA, et al. 2013. Commensal bacteria control cancer response to therapy by modulating the tumor micrenvironment. *Science* 342:967–70
83. Viaud S, Saccheri F, Mignot G, et al. 2013. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* 342:971–76
84. Eckburg PB, Bik EM, Bernstein CN, et al. 2005. Diversity of the human intestinal microbial flora. *Science* 308:1635–38
85. Ma L, Kim J, Hatzenpichler R, et al. 2014. Gene-targeted microfluidic cultivation validated by isolation of gut bacterium listed in Human Microbiome Project's Most Wanted taxa. *Proc. Natl. Acad. Sci. USA* 111:9768–73
86. Thomas CM, Nielsen KM. 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nat. Rev. Microbiol.* 3:711–21

87. Ochiai K, Yamanaka T, Kimura K, Sawada O. 1959. Inheritance of drug resistance (and its transfer) between *Shigella* strains and between *Shigella* and *E. coli* strains. *Hihon Iji Shimpor* 1861:34 [In Japanese]
88. Smillie CS, Smith MB, Friedman J, et al. 2011. Ecology drives a global network of gene exchange connecting the human microbiome. *Nature* 480:241–44
89. Hehemann JH, Correc G, Barbeyron T, et al. 2010. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature* 464:908–12
90. Busby MG, Fischer L, da Costa KA, et al. 2004. Choline- and betaine-defined diets for use in clinical research and for the management of trimethylaminuria. *J. Am. Diet. Assoc.* 104:11836–45
91. Cleland JG, Huan Loh P, Freemantle N, et al. 2004. Clincial trials update from the European Society of Cariology: SENIORS, ACES, PROVE-IT, ACTION, and the HF-ACTION trial. *Eur. J. Heart Fail.* 6:787–91
92. Delzenne NM, Neyrinck AM, Backhed F, Cani, PD. 2011. Targeting gut microbiota in obesity: effects of prebiotics and probiotics. *Nat. Rev. Endocrinol.* 7:639–46
93. Degirolamo C, Rainaldi S, Bovenga F, et al. 2014. Microbiota modification with probiotics induces hepatic bile acid synthesis via downregulation of the Fxr-Fgf15 axis in mice. *Cell Rep.* 7:12–18
94. Chen Z, Guo L, Zhang Y, et al. 2014. Incorporation of therapeutically modified bacteria into gut microbiota inhibits obesity. *J. Clin. Invest.* 124:3391–406
95. Craciun S, Balskus EP. 2012. Microbial conversion of choline to trimethylamine requires a glycyl radical enzyme. *Proc. Natl. Acad. Sci. USA* 109:21307–12
96. Zhu Y, Jameson E, Crosatti M, et al. 2014. Carnitine metabolism to trimethylamine by an unusual Rieske-type oxygenase from human microbiota. *Proc. Natl. Acad. Sci. USA* 111:4268–73
97. Mejean V, Lobbi-Nivol C, Lepelletier M, et al. 1994. TMAO anaerobic respiration in *Escherichia coli*: involvement of the tor operon. *Mol. Microbiol.* 11:1169–79
98. Lidbury I, Murrell JC, Chen Y. 2014. Trimethylamine N-oxide metabolism by abundant marine heterotrophic bacteria. *Proc. Natl. Acad. Sci. USA* 111:2710–15
99. Cashman JR, Zhang J. 2006. Human flavin-containing monooxygenases. *Annu. Rev. Pharmacol. Toxicol.* 46:65–100