

Host-Gut Microbiota Metabolic Interactions

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Abstract:

The composition and activity of the gut microbiota codevelop with the host from birth and is subject to a complex interplay that depends on the host genome, nutrition, and life-style. The gut microbiota is involved in the regulation of multiple host metabolic pathways, giving rise to interactive host-microbiota metabolic, signaling, and immune-inflammatory axes that physiologically connect the gut, liver, muscle, and brain. A deeper understanding of these axes is a prerequisite for optimizing therapeutic strategies to manipulate the gut microbiota to combat disease and improve health.

Article:

Coelomate animals possess an internal body cavity surrounding the gut and other organs and have coevolved with a diverse range of symbiotic gut bacteria and other microorganisms collectively known as the gut microbiota [see Perspective by Gordon (1)]. This mutually beneficial relationship between the host and its resident microbiota results in production of metabolites by microbes that contribute to the evolutionary fitness of the host (2). The diversity and composition of the gut microbiota within and between individuals of a host species is influenced by topographical and temporal variation in the microbial communities, with particular bacterial species occupying specific niches in the body habitat or being associated with particular growth or maturation phases of the host (1, 3). In humans, the primary individual microbiota may reflect the maternal hand-over of “seed ecology” species at birth (4, 5). Subsequent shaping of the microbial landscape is then driven by a series of complex and dynamic interactions throughout life, including diet, life-style, disease, and antibiotic use. This developmental trajectory of the microbiome, incorporating the microbes and their collective genomes, modulates the metabolic phenotype of the host and greatly influences host biochemistry and susceptibility to disease (6).

Interactions between the gut microbiota and the host immune system begin at birth. The microbiota shapes the development of the immune system, and the immune system in turn shapes the composition of the microbiota. This cross-talk between the microbes and the host immune system is transmitted through a vast array of signaling pathways that involve many different classes of molecules and extend beyond the immune system. These immune-mediated

signaling processes, together with direct chemical interactions between the microbe and host, act upon multiple organs such as the gut, liver, muscle, and brain. Together these complex interactions comprise a series of host-microbe metabolic axes. We define a host-microbe metabolic axis as a multidirectional interactive chemical communication highway between specific host cellular pathways and a series of microbial species, subecologies, and activities. Within these metabolic axes, multiple bacterial genomes can sequentially modulate metabolic reactions, resulting in combinatorial metabolism of substrates by the microbiome and host genome, exemplified by production of bile acids, choline, and short-chain fatty acids (SCFAs) that are essential for host health (6). In addition, the production of these metabolites by microbes contributes to the host metabolic phenotype and hence to disease risk. The profound influence of the gut microbiota on the host immune system is strongly associated with long-term health prospects [see Review by Hooper *et al.* in this issue (7) and Review by Blumberg and Powrie (8)]. The composition of the core gut microbiota is considered to be essentially stable throughout adulthood. However, there are components that are dynamic and biologically and metabolically flexible, responding to perturbations such as environmental stresses or changes in diet by alteration in species composition that may influence health or disease risk (9).

The increased incidence of gut dysbiosis (an imbalance in the intestinal bacteria leading to disease) in western populations over the past 60 years is associated with a variety of factors, ranging from the now-textbook story of gastric ulcers caused by infection with the bacterium *Helicobacter pylori* to life-style-related diseases such as diabetes and obesity. Hence, there is much interest in developing new therapeutic tools for manipulating the composition of the gut microbiota to benefit host health. A better understanding of how variations in the symbiotic supraorganism contribute to disease risk and health sustainability will point the way to new therapeutic interventions and disease prevention strategies [see Review by Holmes *et al.* (10)].

THE MICROBIAL AGES OF MAN

The microbiota of the infant is seeded at birth and is initially undifferentiated across the various body habitats. A variety of factors—including method of delivery (vaginal versus Cesarean section), breast feeding, and weaning—influence the infant microbiota (Fig. 1). For example, the microbiota of babies delivered vaginally are dominated by *Lactobacillus*, *Prevotella*, and *Atopobium*, whereas babies delivered by Cesarean section have a microbiota that more closely resembles that of the maternal skin community, with staphylococci being a dominant early member (4). Evidence is beginning to emerge that the in utero environment may not be sterile as originally thought, with bacteria such as *Enterococcus fecalis*, *Staphylococcus epidermidis*, and *Escherichia coli* having been isolated from the meconium (earliest feces) of healthy neonates (11). The dominance of aerobic bacteria at birth is altered during peri- and postnatal development. The microbiota diversifies over the first few weeks of life to form a complex anaerobe-dominated microbial community (12). This early colonization period coincides with activation of the hypothalamic-pituitary-adrenal (HPA) axis, which has an impact on the enteric nervous system that innervates the gastrointestinal (GI) tract. Enteroendocrine cells of the gut secrete a variety of metabolically related peptides all known to be connected to food intake, lipid storage, and energy homeostasis and can be activated by microbial metabolites, such as SCFAs, that act through heterotrimeric guanine nucleotide-binding protein (G protein)-coupled receptors, such as the GPR41 receptor expressed by enteroendocrine cells (13). The gut microbiota appears to become more stable throughout adulthood, although some studies have

reported that adolescents have a higher abundance of bifidobacteria and clostridia than adults (14). A final set of age-related shifts in the composition and function of the gut microbiota occurs during old age. Aging is associated with altered physiological functions, including immune system function, that affect gut microbiota composition. Age-related differences reported in gut microbiota composition include an increase in the total number of facultative anaerobes, shifts in the ratio of Bacteroidetes to Firmicutes species, and a marked decrease in bifidobacteria in people >60 years old, around the time that the immune system starts to decline (Fig. 1) (15). Metabolic changes coinciding with the evolution and maturation of the gut microbiota can be found in the excretion profiles of bacterial products of amino acid metabolism and in energy-related metabolites. For example, bacterial products of choline metabolism may be inversely associated with age in children under 12 years old (16). Changes in the urinary excretion of 4-hydroxyphenylacetic acid, indoleacetic acid, and tricarboxylic acid cycle metabolites have been found during early-onset puberty (17). Integration of age-specific urinary or fecal profiles with bacterial genome sequencing data should provide new insights into the functional coevolution of the microbiota and host.

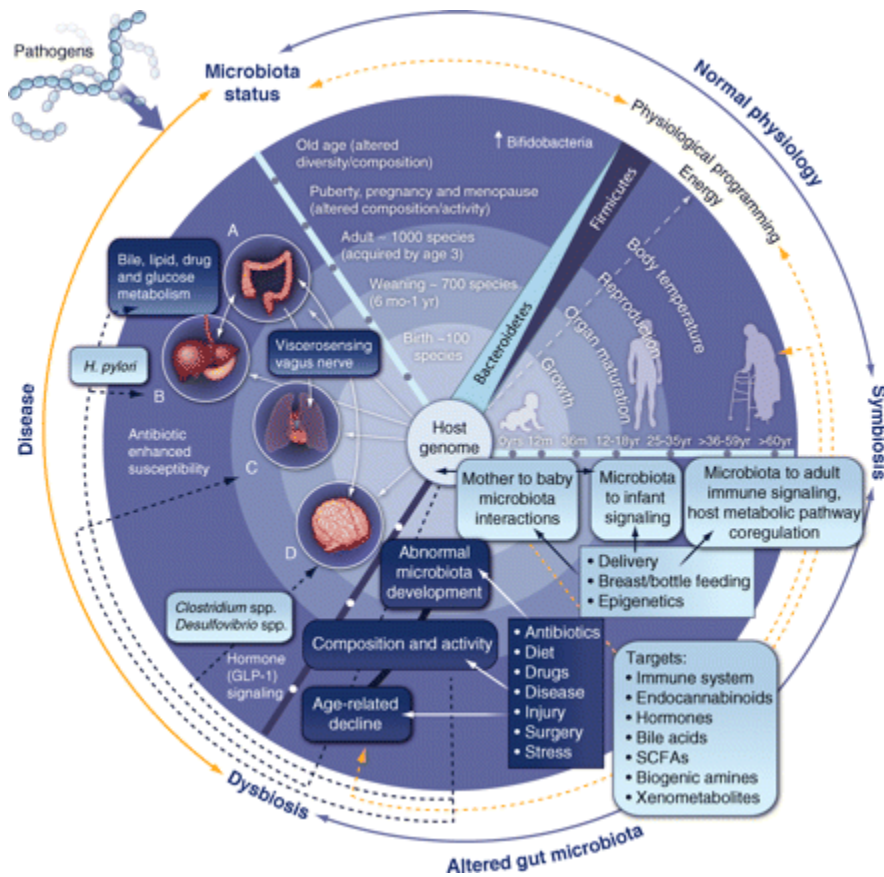


Figure 1: The gut microbiota in development and disease. The influence of the gut microbiota on human health is continuous from birth to old age. The maternal microbiota may influence both the intrauterine environment and the postnatal health of the fetus. At birth, about 100 microbial species populate the colon. Early environmental factors (e.g., method of delivery), nutritional factors (e.g., breast or bottle-feeding), and epigenetic factors have been implicated in the development of a healthy gut and its microbial symbionts. Changes in gut microbial composition in early life can influence risk for developing disease later in life. During suckling, the microbial community develops rapidly; shifts in microbial diversity occur throughout childhood and adult life; and in old age, there is a decrease in

the Bacteroidetes and an increase in Firmicutes species. The gut microbiota is important for maintaining normal physiology and energy production throughout life. Body temperature regulation, reproduction, and tissue growth are energy-dependent processes that may rely in part on gut microbial energy production. Extrinsic environmental factors (such as antibiotic use, diet, stress, disease, and injury) and the mammalian host genome continually influence the diversity and function of the gut microbiota with implications for human health. Disruption of the gut microbiota (dysbiosis) can lead to a variety of different diseases, including (A) inflammatory bowel disease, colon cancer, and irritable bowel syndrome; (B) gastric ulcers, nonalcoholic fatty liver disease, and obesity and metabolic syndromes; (C) asthma, atopy, and hypertension; and (D) mood and behavior through hormone signaling (e.g., GLP-1). The gut microbiota is also important for drug metabolism and preventing the establishment of pathogenic microbes.

HOST AND MICROBIOTA COMETABOLISM AND SIGNALING

The host and its gut microbiota coproduce a large array of small molecules during the metabolism of food and xenobiotics (compounds of nonhost origin that enter the gut with the diet or are produced by the microbiota), many of which play critical roles in shuttling information between host cells and the host's microbial symbionts. Different regions of the human GI tract vary in terms of the composition of indigenous microbiota (1, 4). For each compartment of the GI tract, a chemical dialogue exists among different microbial species (direct substrate provision in the microbial food web, quorum sensing, contact-dependent signaling, and potentially gasotransmitters) (18) as well as between microbial symbionts and host cells (3, 19). This chemical dialogue includes signaling via low molecular weight metabolites, peptides, and proteins or may take place indirectly through immune-mediated pathways. Some of the main chemical classes that regulate host-microbiota interactions are described in Table 1, but it is likely that many more exist.

Humans excrete between 50 to 100 mg of volatile phenols per day, predominantly in the form of 4-cresol and phenol (mainly as glucuronide and sulfate conjugates) with lower amounts of 4-ethylphenol (20). The production of cresols from tyrosine in mammals has been attributed to various species of *Clostridium*, *Bifidobacterium*, and *Bacteroides fragilis*. Other bacteria such as *E. coli* are associated with phenol production. Altered levels of 4-cresol metabolites in human urine have been associated with diverse physiological and pathological conditions from weight loss to inflammatory bowel disease. These conditions are also associated with altered microbiota composition, namely a reduction in the diversity of the microbiota because of loss of *Lactobacillus* and *Bacteroides* species in the case of inflammatory bowel disease (21) and differences in the ratio of the Firmicutes to Bacteroidetes species in the case of weight loss (22).

Dietary fiber (complex carbohydrates) can be digested and subsequently fermented in the colon by gut microbes into SCFAs such as *n*-butyrate, acetate, and propionate and are sensed by the G protein-coupled receptors, GPR41 and GPR43, expressed by gut enteroendocrine cells (13). In addition to being a local nutrient source for colonocytes and a minor nutrient source for microbes such as *Desulfotomaculum* spp. in the GI tract (23), *n*-butyrate has also been shown in cell-culture models and mice to regulate energy homeostasis by stimulating leptin production in adipocytes, as well as inducing glucagon-like peptide-1 (GLP-1) secretion by the intestinal enteroendocrine L cells (13). The main producers of butyrate are clostridia, eubacteria, and roseburia microbes. *n*-Butyrate regulates neutrophil function and migration, inhibits inflammatory cytokine-induced expression of vascular cell adhesion molecule-1, increases expression of tight junction proteins in colon epithelia, and exhibits anti-inflammatory effects

Table 1: Gut bacteria and the metabolites they contribute.

Metabolites	Related bacteria	Potential biological functions	References
Short-chain fatty acids: acetate, propionate, butyrate, isobutyrate, 2-methylpropionate, valerate, isovalerate, hexanoate	Clostridial clusters IV and XIVa of <i>Firmicutes</i> , including species of <i>Eubacterium</i> , <i>Roseburia</i> , <i>Faecalibacterium</i> , and <i>Coprococcus</i>	Decreased colonic pH, inhibit the growth of pathogens; stimulate water and sodium absorption; participate in cholesterol synthesis; provide energy to the colonic epithelial cells, implicated in human obesity, insulin resistance and type 2 diabetes, colorectal cancer.	(13, 24, 57)
Bile acids: cholate, hyocholate, deoxycholate, chenodeoxycholate, α -muricholate, β -muricholate, ω -muricholate, taurocholate, glycocholate, taurochenoxycholate, glycochenodeoxycholate, taurocholate, tauro- α -muricholate, tauro- β -muricholate, lithocholate, ursodeoxycholate, hyodeoxycholate, glycodeoxycholate, taurohyocholate, taurodeoxycholate	<i>Lactobacillus</i> , <i>Bifidobacteria</i> , <i>Enterobacter</i> , <i>Bacteroides</i> , <i>Clostridium</i>	Absorb dietary fats and lipid-soluble vitamins, facilitate lipid absorption, maintain intestinal barrier function, signal systemic endocrine functions to regulate triglycerides, cholesterol, glucose and energy homeostasis.	(30, 31, 33)
Choline metabolites: methylamine, dimethylamine, trimethylamine, trimethylamine- <i>N</i> -oxide, dimethylglycine, betaine	<i>Faecalibacterium prausnitzii</i> , <i>Bifidobacterium</i>	Modulate lipid metabolism and glucose homeostasis. Involved in nonalcoholic fatty liver disease, dietary induced obesity, diabetes, and cardiovascular disease.	(29, 58)
Phenolic, benzoyl, and phenyl derivatives: benzoic acid, hippuric acid, 2-hydroxyhippuric acid, 2-hydroxybenzoic acid, 3-hydroxyhippuric acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 3-hydroxyphenylpropionate, 4-hydroxyphenylpropionate, 3-hydroxycinnamate, 4-methylphenol, tyrosine, phenylalanine, 4-cresol, 4-cresyl sulfate, 4-cresyl glucuronide, 4-hydroxyphenylacetate, 3,4-dihydroxyphenylacetate, phenylacetylglutamine, phenylacetylglutamine, phenylacetylglutamine, phenylacetate, phenylpropionate, phenylpropionylglycine, cinnamoylglycine	<i>Clostridium difficile</i> , <i>F. prausnitzii</i> , <i>Bifidobacterium</i> , <i>Subdoligranulum</i> , <i>Lactobacillus</i>	Detoxification of xenobiotics; indicate gut microbial composition and activity; utilize polyphenols. Urinary hippuric acid may be a biomarker of hypertension and obesity in humans. Urinary 4-hydroxyphenylacetate, 4-cresol, and phenylacetate are elevated in colorectal cancer. Urinary 4-cresyl sulfate is elevated in children with severe autism.	(59, 60)
Indole derivatives: <i>N</i> -acetyltryptophan, indoleacetate, indoleacetylglutamine (IAG), indole, indoxyl sulfate, indole-3-propionate, melatonin, melatonin 6-sulfate, serotonin, 5-hydroxyindole	<i>Clostridium sporogenes</i> , <i>E. coli</i>	Protect against stress-induced lesions in the GI tract; modulate expression of proinflammatory genes, increase expression of anti-inflammatory genes, strengthen epithelial cell barrier properties. Implicated in GI pathologies, brain-gut axis, and a few neurological conditions.	(61–63)
Vitamins: vitamin K, vitamin B12, biotin, folate, thiamine, riboflavin, pyridoxine	<i>Bifidobacterium</i>	Provide complementary endogenous sources of vitamins, strengthen immune function, exert epigenetic effects to regulate cell proliferation.	(64, 65)
Polyamines: putrescine, cadaverine, spermidine, spermine	<i>Campylobacter jejuni</i> , <i>Clostridium saccharolyticum</i> , <i>Bifidobacterium</i>	Exert genotoxic effects on the host, anti-inflammatory and antitumoral effects. Potential tumor markers.	(66, 67)
Lipids: conjugated fatty acids, LPS, peptidoglycan, acylglycerols, sphingomyelin, cholesterol, phosphatidylcholines, phosphoethanolamines, triglycerides	<i>Roseburia</i> , <i>Lactobacillus</i> , <i>Klebsiella</i> , <i>Enterobacter</i> , <i>Citrobacter</i> , <i>Clostridium</i>	Impact intestinal permeability, activate intestine-brain-liver neural axis to regulate glucose homeostasis; LPS induces chronic systemic inflammation; conjugated fatty acids improve hyperinsulinemia, enhance the immune system and alter lipoprotein profiles. Cholesterol is the basis for sterol and bile acid production.	(42, 68)
Others: D-lactate, formate, methanol, ethanol, succinate, lysine, glucose, urea, α -ketoisovalerate, creatine, creatinine, endocannabinoids, 2-arachidonoylglycerol (2-AG), <i>N</i> -arachidonylethanolamide, LPS, etc.	<i>Bacteroides</i> , <i>Pseudobutyrvibrio</i> , <i>Ruminococcus</i> , <i>Faecalibacterium</i> , <i>Subdoligranulum</i> , <i>Bifidobacterium</i> , <i>Atopium</i> , <i>Firmicutes</i> , <i>Lactobacillus</i>	Direct or indirect synthesis or utilization of compounds or modulation of linked pathways including endocannabinoid system.	(43, 60, 69)

by reducing cytokine and chemokine release from human immune cells. Therefore, *n*-butyrate or specific species of butyrate-producing gut bacteria may be a new target for restoring host immune function and barrier integrity and for regulating energy metabolism. *n*-Butyrate can also be directly used by colon epithelial cells to produce ketone bodies and carbon dioxide. Other SCFAs such as propionate and acetate are carried in the bloodstream to a variety of different organs, where they are used as substrates for oxidation, lipid synthesis, and energy metabolism, particularly by the hepatocyte cells of the liver, which use propionate for gluconeogenesis (13, 24). SCFAs have been reported to regulate the function of histone deacetylases (HDACs), stimulate the sympathetic nervous system, and may influence social behavior in rodents (25). SCFAs have also been shown to stimulate gut motility and intestinal transit and at physiological concentrations have been shown to induce an eight- to 10-fold increase in serotonin release in an in vitro colonic mucosal system (26). SCFAs are clearly one of the most important gut microbial products and affect a range of host processes—including energy utilization, host-microbe signaling, and control of colonic pH—with consequent effects on microbiota composition, gut motility, and epithelial cell proliferation (27).

Choline is an essential dietary nutrient and is primarily metabolized in the liver. However, gut microbial enzymes also catalyze the conversion of dietary choline to trimethylamine (28), which is then further metabolized by the flavine monooxygenase system in the liver to produce trimethylamine-*N*-oxide. The microbial conversion of dietary choline is emerging as a metabolic hallmark that is associated with liver and cardiovascular diseases. For example, 129S6 mice are known to be susceptible to developing obesity, impaired glucose tolerance, and nonalcoholic fatty liver disease. When they are fed a high-fat diet, there is an increase in microbial enzyme activity that leads to reduced choline bioavailability, resulting in symptoms that mimic nonalcoholic fatty liver disease; these symptoms are also seen in mice fed a choline-deficient diet (28). A study by Wang *et al.* (29) recently suggested a potential pathological role for trimethylamine-*N*-oxide in the development of atherosclerosis, providing a potential link between the intestinal microbiota, dietary choline, and cardiovascular disease risk.

Bile acids (or bile salts) are steroid acids that are produced in the liver from cholesterol and secreted in bile and whose main function is to facilitate the metabolism of dietary fat and the absorption of fat-soluble vitamins and cholesterol. They complete an enterohepatic cycle between the gut and liver about eight times per day, with 90 to 95% of bile acids being reabsorbed by the intestine and returned to the liver, whereupon they are conjugated predominantly with taurine and glycine to form bile salts. About 5 to 10% of bile acids are biotransformed largely through degradation by intestinal bacteria and some are lost in the feces. The transformation of bile acids in the intestines is mainly performed by anaerobic bacteria of the genera *Bacteroides*, *Eubacterium*, and *Clostridium* and involves deconjugation of taurine- and glycine-conjugated bile acids through the action of bile salt hydrolases to their respective unconjugated free bile acids. These free bile acids then form secondary bile acids such as deoxycholate and lithocholate (30, 31), which in turn undergo reabsorption, mainly by bile acid transporters in the ileal epithelium but also by passive absorption throughout the intestine (32). Bile salt hydrolase enzymes have been identified in several bacterial species, mainly anaerobes of the genera *Bacteroides*, *Clostridium*, *Eubacterium*, *Lactobacillus*, and *Escherichia*. Deconjugation and 7 α -dehydroxylation of bile salts increases their hydrophobicity and hence absorption, which has also been associated with increased pathological effects (30). A minor

portion of bile acid biotransformation may also be conducted by various groups of aerobic bacteria, such as actinobacteria and proteobacteria. As further evidence for the importance of the microbiota in bile salt metabolism, a study has shown that rodents raised under germ-free conditions or treated with antibiotics have altered hepatic gene expression patterns with changes in genes associated with cholesterol, steroid, and bile acid synthesis as well as altered conjugated (especially taurine-conjugated) bile acid signatures in multiple body compartments (33). Bile acid metabolites formed by the interaction of both mammalian and gut microbial metabolisms (cometabolites) are major ligands for nuclear hormone receptors and strongly activate an important member of this family, the farnesoid X receptor. Farnesoid X receptor signaling affects many target genes, including those involved in bile acid synthesis and transport and lipid and carbohydrate metabolism, and is involved in the regulation of intestinal innate immunity (34). The gut microbiota could, through effects on bile acid metabolism in the gut lumen, influence signaling pathways involved in energy and lipid metabolism, leading to alterations in lipid peroxidation, production of hepatic fatty acids, and triglyceride storage. High concentrations of secondary bile acids in various biological fluids have been associated with diseases such as colon cancer (30). Interestingly, it has been demonstrated that bile salt hydrolase activity is a feature of all major bacterial groups, and modulation of bile salt hydrolase activity may be an effective target in the management of obesity and metabolic syndrome (35).

LIVER FUNCTION AND INFLAMMATION IN METABOLIC DISEASE

Over the past 50 years, the prevalence of metabolic diseases such as diabetes and obesity has steadily increased in the developed world (36), the principal culprits being originally attributed to poor diet and lack of exercise. Events early in life, such as delivery mode, maternal pre-pregnancy body mass index, and antibiotic treatment during infancy, influence obesity in later childhood (37). Studies in monozygotic and dizygotic twin pairs concordant for leanness or obesity and their mothers showed that the human gut microbiome is shared among family members with a comparable degree of covariation in gut microbiota species between the twin pairs (38). In seminal work, Gordon and his colleagues demonstrated that the intestinal microbiota can cause metabolic disease in mice independently of genetic background (39, 40). Briefly, the colonization of a germ-free mouse with the intestinal microbiota from an obese mouse donor induced a body weight gain that was more substantial than when the microbiota from a lean mouse was transferred (41). This study provided the first insight into the potential contribution of the microbiota to the obesity epidemic. The same group also observed that the intestinal microbiota of obese individuals differed in microbial diversity compared with that of lean persons, with a lower prevalence of Bacteroidetes and a higher prevalence of Firmicutes (39). Because of differences in the composition of their gut microbiota, obese persons may be more effective at extracting energy from food and stimulating lipogenesis (41). An increase in lipopolysaccharide (LPS), a component of the outer membranes of Gram-negative bacteria, generates low-grade chronic inflammation (metabolic endotoxemia) in mice, resulting in insulin resistance (42) that may act through endocannabinoids produced by the host (43). Prebiotics are nondigestible food substrates that promote the growth of intestinal bacteria that confer health benefits on the host. It has been shown that a high-fat diet increases the proportion of Gram-negative to Gram-positive microbes in the gut by favoring their growth and hence increases the liberation of LPS, which is responsible for inflammation (42). Importantly, this effect can be suppressed with a prebiotic that specifically boosts growth of Gram-positive microbes (44). From this transplantable “second genome,” another causal mechanism for metabolic disease has

been characterized, namely increased capacity of the gut microbiota from obese mice to harvest energy for the host. The transplanted microbiota from obese mice promoted absorption of monosaccharides from the gut lumen, selectively suppressed the production of fasting-induced adipocyte factor (Fiaf), a circulating lipoprotein lipase inhibitor, with resulting induction of de novo hepatic lipogenesis and deposition of triglycerides in adipocytes and the liver (40). Nontransplanted germ-free lean mice were resistant to becoming obese on a fat-enriched diet, with an increase in skeletal muscle and liver of phosphorylated adenosine monophosphate-activated protein kinase (AMPK). AMPK phosphorylates acetyl coenzyme A (CoA) carboxylase, which results in decreased malonylCoA levels. Given that malonylCoA controls the rate-limiting step of long-chain fatty acylCoA entry to the mitochondria by blocking the enzyme carnitine-palmitoyltransferase, fatty acid oxidation is promoted, which is associated with lower storage of fat (40, 45). The contribution of bacterial LPS to metabolic syndrome and other inflammatory conditions is indisputable, and the use of peptides with antimicrobial properties to achieve immunomodulation by sequestering LPS is an attractive therapeutic avenue.

Another metabolic disease with comorbidity associated with obesity and metabolic syndrome is nonalcoholic fatty liver disease. The incidence ranges from 20 to 30% in the general population and up to 75 to 100% in obese individuals. The intestinal microbiota may contribute to the development of nonalcoholic fatty liver disease through the complex and cooperative activities of two microbe-sensing protein families, namely nucleotide oligomerization domain receptors (NLRs) and Toll-like receptors (TLRs) (46, 47), and through inflammasomes (48) that shape metabolic events such as lipid accumulation. The loss of NLRP3 and NLRP6 inflammasomes in mice is associated with intestinal dysbiosis and results in abnormal accumulation of bacterial products such as LPS and bacterial DNA in the hepatic portal circulation. These bacterial products stimulate TLR4 and TLR9, respectively, leading to enhanced liver expression of the pro-inflammatory cytokine tumor-necrosis factor (TNF)- α , which in turn drives progression of nonalcoholic steatohepatitis, a severe form of fatty liver disease. Prebiotics and other dietary interventions have been shown to affect the expression of TLR-encoding genes (49), further underscoring the importance of the tripartite relationship between the host, microbiota, and nutrition.

A key issue is to identify how factors produced by bacteria interact with the host. An oral origin of some bacterial components has been proposed, given that periodontal disease has been linked to atherosclerosis and hence cardiovascular disease. For example, atherosclerotic plaques contain more Proteobacteria species and fewer Firmicutes, and a correlation was found between the combined abundance of *Veillonella* and *Streptococcus* in the oral cavity and atherosclerotic plaque formation (50). Furthermore, changes in diet can result in loosening of the tight junctions between gut epithelial cells, which may result in the accumulation of bacterial components in the hepatic portal vein with downstream effects on inflammation (51). A chronic high-fat diet increases plasma LPS concentrations two- to threefold, a threshold that could be defined as metabolic endotoxemia (43, 51). In mice, continuous subcutaneous infusion of LPS induced characteristics of metabolic disease such as infiltration of F4/80-positive cells (macrophage-like Kupffer cells) and an increase in liver triglyceride content because of CD14, TLR4, NOD1, and Myd88-dependent mechanisms (52). This process of LPS-induced activation of TLR4 and other receptors suggests an important link between the intestinal microbiota, its rapid evolution over recent decades resulting from a change in dietary habits, and low-grade inflammation. Lastly, the

advent of bariatric surgery, the surgical treatment of obesity, further validates the importance of the intestinal microbiota on the control of low-grade inflammation, which is associated with hepatic steatosis and metabolic disease (53). Bariatric surgery (particularly the Roux-en-Y gastric bypass) conducted on both humans and rodent models induces both metabolic alterations in energy metabolism and the urinary excretion of gut microbial metabolites such as 4-cresyl sulfate, phenylacetate, and choline degradation products (53). Bariatric surgery also induces a marked shift in the composition of the gut microbiota from a predominantly Firmicute and Bacteroidetes–dominated microbiota toward one where gammaproteobacteria predominate (53, 54). After surgery, *Faecalibacterium prausnitzii* has been shown to correlate inversely with markers of inflammation such as C-reactive protein and interleukin-6 (55).

CHARACTERIZING METABOLIC INTERACTIONS BETWEEN HOST AND MICROBE

The gut microbiota act in a concerted manner to achieve metabolic communication with the host, and, as Table 1 shows, many different bacterial genera and species are involved in metabolite production. Although there is a global understanding of metabolite flow across the microbe-host food web, for many reasons, including the difficulty in culturing anaerobic bacteria, our knowledge of which bacterial species synthesize which metabolites is currently limited. Taking a specific example, we do not have a comprehensive understanding of which bacteria are involved in synthesis of hippurate, a glycine conjugate of benzoic acid. Yet hippurate is the most widely detected urinary metabolite of host-microbial origin in humans, dogs, ruminants, and rodents, and its urinary concentrations are modulated by diet, stress, disease, and microbial presence or activity (56).

There are various methods for establishing biological or statistical links between the microbes and the metabolites that they may produce. Next-generation sequencing methods can provide a reconstruction of DNA sequences and insight into the capability of organisms to perform metabolic functions but are not able to provide a window on the functionality of particular bacteria under complex and changing environmental conditions. Another strategy for assigning metabolic origin to specific bacteria is to pursue *in vitro* experiments by using different model systems of the gut (ranging from batch to multiple-stage continuous culture), as well as individual pure culture experiments. The principal substrates for these *in vitro* systems are dietary residues (carbohydrates, proteins, and amino acids) and indigenous secretions (mucins). Although much can be learned from these *in vitro* systems, they do not represent the full *in vivo* capacity of the intact gut. Metabolic profiling of biofluids (such as urine, plasma, or fecal water) that uses high-resolution spectroscopy offers an alternative strategy for characterizing metabolites of microbial origin, and the profiles can subsequently be statistically integrated with metagenomics data by using multivariate computational modeling. The challenges for future research lie in optimizing the computational capacity and frameworks for coanalysis of vast quantities of high-density data acquired on disparate analytical platforms with differing processing requirements.

THE UNDISCOVERED COUNTRY: UNDERSTANDING FUTURE MICROBIAL IMPACT ON HUMAN HEALTH

There appear to be sustained long-term shifts in activities and composition of the microbiota that are linked to changing life-styles. It is thus possible that, in just a few generations, the evolutionary relationships between ourselves and our symbionts could change forever. Given

that our gut microbiota influence human development, susceptibility to disease, and even the outcomes of drug treatment, perhaps we should think now about microbiota biobanking for future generations facing unknown biological and infectious disease challenges.

In order to leverage information about the gut microbiota effectively to design new therapeutics, the following knowledge gaps need to be addressed.

1. We need an improved understanding of the dynamics and impact of maternal microbiota transfer and the influence of infant nutrition on development of the gut microbiota in early childhood. We also need to elucidate the influence of host genome variations and the fetal environment on the future gut microbiota.
2. It will be important to map the impact of early antibiotic use on the developmental ecology, function, and resilience of the microbiota during childhood. As the microbiota develops over the first few years of life, there may be greater potential for disruption of the long-term microbial state than would be encountered in adults.
3. A deeper knowledge is required regarding how variation in the gut microbiota influences drug metabolism, drug bioavailability, and drug toxicity with repercussions for patient stratification and personalized health care.
4. Strategies should be developed for the *in vitro* culture of the complete microbiota in order to elucidate bacterial species biology and microbial interactions in engineered ecological constructs and synthetic ecosystems.
5. Comprehensive top-down systems biology analyses should be applied to the changing immunological and metabolic interactions between the host and its gut microbiota to elucidate how these changing interactions affect gut, liver, and brain function.

Perhaps the greatest challenges in this research field, apart from pure metagenomic complexity, relate to understanding the temporal dynamics of metabolic communication between the host and its gut microbiota, not only on the time scale of a human life but also on an evolutionary time scale, in relation to global changes in diet and environmental stressors. Alterations to the gut microbiota affect human biological fitness at multiple levels that will need to be better understood if we are to elucidate the role of the gut microbiota in specific diseases and the best ways to manipulate the microbiota therapeutically to garner human health benefits.

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