Gut Microbes in Health and Disease

The human intestinal tract is home to at least 1000 distinct species of bacteria, which collectively number over 100 trillion organisms. This diverse ecosystem is shaped by early life events but can evolve over time through interactions between its constituents, host genes, and exogenous factors, such as diet and environmental exposures. Until recently, characterization of the gut microbiome relied mostly on conventional culture-based microbiological techniques, which was a major hindrance because the vast majority of bacteria in the gut are not readily amenable to cell culture. However, advances in next-generation genomic technologies now allow us to identify and classify gut bacterial composition in an unprecedented manner. In this approach, in-depth sequencing of the variable regions of bacterial 16s RNA genes is used to determine the diversity and proportion of bacterial taxa within the microbial community. Although a major step forward, the evolutionary resolution provided by 16s sequencing is still limited with current platforms because bacterial composition with this approach is typically identified only down to the genus level. Overcoming these challenges will not only improve our ability to identify and quantify distinct species of bacteria, but may have implications for understanding the pathological mechanisms through which specific bacteria affect both human health and disease processes.

Metagenomic analyses have revealed that the human gut is mostly composed of a common core of bacteria from 2 major phyla, Firmicutes and Bacteriodetes, with the remainder of the gut microbiota being remarkably diverse. This diversity often includes less abundant representation from the phyla Proteobacteria, Verruromicrobia, Actinobacteria, Fusobacteria, and Cyanobacteria, as well as the domain Archaea. For the most part, metagenomic analyses on the human gut microbiota have used fecal samples, but it is clear from animal studies that certain anaerobic organisms, such as Akkermansia muciniphila, reside primarily in the mucosal layers of the gut and are not readily detected in analyses of only feces. Indeed, the microbial composition throughout the gut varies considerably, both with respect to the anatomic location along the intestinal tract and within a given site, according to the microenvironment. For example, at a given site of intestinal mucosa/lumen, distinct microbes can uniquely reside deep within the crypts, versus on the surface of the mucosal villi, versus within the fecal material. Thus, without invasive procedures to get samples from distinct anatomic regions through the intestines, obtaining a more complete picture of the full spectrum of gut microbiota, at least in humans, poses significant challenges.

It has become widely appreciated that our gut symbionts play integral roles in human health because perturbations of this bacterial community or the products they can produce have been associated with increased susceptibility to a variety of diseases (see Figure). The first indications of these associations were for colitis and inflammatory bowel disease, but altered gut microbial composition or function as a potential contributor to disease risk has now been established in the development of obesity and related metabolic abnormalities, and even neurobehavioral conditions, such as autism.

Association of Gut Microbiota With Lipid Metabolism

Early studies comparing germ-free versus conventionally raised mice first supported a role for gut microbes in both affecting host energy metabolism and modulating lipid levels; however, the design of these early studies did not permit identification of candidate microbes involved in promoting the observed phenotypic changes in conventionalized (microbe colonized) mice. In this issue of Circulation Research, Fu et al now provide some of the first evidence in humans that variation in gut bacteria are associated with blood lipid levels, as well as clues toward discovering the microbes involved. Using a subset of the LifeLines population-based cohort, the fecal microbial composition of 893 subjects was determined by assessing genetic variation of bacterial 16s rRNA genes.

This effort represents one of the largest gut microbial composition studies in humans to date, and the results revealed several interesting observations. First, microbial richness and diversity, as indicated by operational taxonomic units, was inversely correlated with body mass index (BMI) and triglyceride (TG) levels and positively associated with high-density lipoprotein (HDL) cholesterol levels. Second, although some associations were shared across BMI and lipids, there were also microbial taxa whose proportions were primarily

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associated with lipids alone, and some of these were newly identified associations with TG and HDL levels. Third, the correlations between microbial taxa and lipids did not seem to be modified by BMI or host genetic factors, at least based on a risk score calculated from host genetic variants identified from genome-wide association studies for lipids and BMI. Finally, incorporation of the results from gut microbial composition analysis in a risk model that included age, sex, and previous validated genetic risk factors (for lipids and BMI) significantly increased the percent of variance explained for BMI, TG, or HDL each by \approx 5\%.

Overall, the associations reported in the LifeLines cohort are consistent with prior studies. For example, the number of operational taxonomic units, or microbial richness, was inversely related to BMI and TG levels and positively associated with HDL. These observations reinforce the concept that increased diversity of the gut microbiota is associated with more favorable cardiovascular and metabolic profiles. Previously described associations between obesity and certain taxa of bacteria, such as *Akkermansia*, *Christensenellaceae*, and *Tenericutes*, were also confirmed. Importantly, with this new and largest yet study of its kind, the authors were able to identify additional

Figure. Schematic illustration of some of the many phenotypes and diseases where gut microbe involvement is reported. Multiple lines of evidence support a role for altered gut microbial composition or function as a contributor to the development of obesity and related metabolic abnormalities (ie, type 2 diabetes), peripheral and coronary artery disease, and even neurobehavioral conditions, such as autism. Recent observations of significant associations between proportions of specific intestinal bacteria taxa with high-density lipoprotein (HDL) cholesterol and triglyceride (TG) levels in subjects independent of body mass index suggest a role for gut microbes in modifying host lipid metabolism. Gut microbe effects may be mediated through multiple mechanisms, including elaboration of lipopolysaccharide (LPS) or other bioactive metabolites that act fundamentally as hormones, since they can circulate within the host and act at distant sites. Gut microbial production of short chain fatty acids (SCFAs) and secondary bile acids are two such examples. Evidence shows that gut bacteria also generate intermediate precursors (eg, trimethylamine [TMA]) from certain dietary nutrients that can then be further metabolized by the host to generate biologically active products (eg, trimethylamine N-oxide), which then can exert direct effects on lipid metabolism and contribute to disease development or progression. Biological mechanisms impacted by gut microbial metabolites can involve reverse cholesterol transport, hepatic cholesterol and sterol metabolism, intestinal lipid transport, bile acid composition and pool size, glucose and insulin metabolism, energy harvest/expenditure, as well as others. LDL indicates low-density lipoprotein; and VLDL, very low-density lipoprotein.
significant and novel associations between the proportions of *Eggerthella, Pasteurellaceae*, and *Butyricimonas* and host TG and HDL levels. Surprisingly, there were only weak relationships noted between microbial variation and levels of total cholesterol or low-density lipoprotein cholesterol, suggesting that gut bacteria affect specific aspects of lipid metabolism and distinct classes of lipoproteins. Taken together, the observations by Fu et al. provide exciting insights and suggest new avenues worth pursuing both for validation studies and as follow up investigations to understand the underlying mechanism(s) and determine whether these associations are causal. It should be noted, however, that some of the associations observed between bacterial groups and lipid levels were at the level of phyla, whereas others were, at best, to the genus level. It will thus be difficult to narrow down the responsible microbial species, at least based on currently used methods.

Another concept addressed by Fu et al. was the role of host genetic factors in determining variation in gut microbiota. Although the collective effect of ≈250 previously validated variants for lipid levels and BMI were confirmed for these clinical phenotypes, the authors did not find any evidence for genetic effects, either alone or as a risk score, on microbiome composition. This may be explained, in part, by the fact that the only variants tested were, understandably, selected based on their main effects on lipids and BMI. One might speculate that host genetic variants that are involved in regulating bacterial abundance within the host may be more likely to be associated with observed changes in proportions of gut microbiota taxa; however, linking these taxa proportions to changes in host lipid levels would pose its own unique challenges. It is also probable that, even though the present study represents the largest gut microbiome study reported to date, its sample size is insufficient to have permitted identification of genetic associations. Third, the negative findings of a link between host genetic variants that impact lipids and BMI also being associated with gut microbiota proportions does not necessarily indicate there is a lack of host genetic variants influencing microbial composition. Given the enormous genetic diversity of the gut microbiome, the current study may simply have been underpowered to detect such associations. By comparison, a recent genetic analysis with ≈100 inbred mouse strains, all of which were maintained on equivalent diet and housing conditions, identified 7 host loci that were associated with common bacterial genera. In particular, one of these host genetic loci was associated with the proportion of *Akkermansia muciniphila*, a taxa whose proportion was also associated with gonadal fat mass and plasma TG levels. Similar genetic associations likely exist in humans as well, but their identification will require larger sample sizes and broader interrogation of genes, or new discovery approaches.

**Biological Mechanisms Through Which Gut Microbes May Affect Lipid Metabolism**

As with any gut microbiota study that is associative in nature, a major challenge is elucidating the underlying biological mechanisms involved in the associations and proving whether the associations are because of a causal relationship. Given the known clinical correlation between obesity and dyslipidemia, it is possible that the associations with TG and HDL levels reported by Fu et al. are mediated through effects on BMI. The data, however, suggest this not to be the case because statistical analyses showed that the proportions of bacterial taxa identified were independently associated with TG and HDL after adjustments for BMI. Moreover, a statistical model that included addition of microbial operational taxonomic units to age, sex, BMI, and genetic factors significantly increased the percent of the variation in lipid levels that could be explained compared with a model without inclusion of the fecal microbial composition data.

Evidence from other prior studies also supports the notion that the gut microbiome may mechanistically impact host lipid levels. For example, certain facultative and anaerobic bacteria in the large bowel produce secondary bile acids from the pool of bile salts secreted into the intestine. A small fraction of these bacterially derived bile acids are absorbed into the bloodstream and can modulate hepatic or systemic lipid and glucose metabolism through nuclear or G protein–coupled receptors, such as FXR or TGR5, respectively. Notably, several of the bacteria identified by Fu et al. are known to be involved in bile acid metabolism, suggesting that gut microbe–associated alterations in bile acid composition and pool size may at least account for part of the biological basis for the associations between the identified fecal taxa proportions and plasma lipid levels.

Another potential mechanism through which gut microbes could affect lipid metabolism may involve fermentation of nondigestable carbohydrates to short chain fatty acids by a subset of anaerobic bacteria found in the cecum and proximal colon. These short chain fatty acids, such as acetate, propionate, and butyrate, are known to regulate intestinal immune homeostasis and serve as an energy source for colonic epithelial cells. However, short chain fatty acids are also absorbed from the gut and can have potent effects on energy expenditure and insulin sensitivity in peripheral metabolic tissues through different G protein–coupled receptors, such as GPR41 and GPR43.

It is also possible that gut bacteria generate intermediate precursors that are further metabolized by the host to products that exert direct effects on lipid levels. In this regard, studies by our group recently identified trimethylamine N-oxide as a metabolite that increases atherosclerosis in mice and cardiovascular risk in humans, in part through perturbations of reverse cholesterol transport, cholesterol and sterol metabolism, and the quantity and composition of bile acids. In this regard, trimethylamine N-oxide is derived secondarily through hepatic oxidation of TMA, which is first produced through gut microbe–mediated metabolism of dietary choline and L-carnitine.

**The Challenge of Proving Causality**

Fecal microbial composition studies are associative and, thus, hypothesis generating. Ultimately, proof of causality for identified microbial associations with host phenotypes requires additional experimentation, such as manipulating gut bacterial composition and observing changes in physiological parameters that were identified in the initial associations. In both animal models and humans, intestinal microbial transplantation studies have provided evidence for a causal role of gut bacteria in modulating metabolic and cardiovascular phenotypes, as well as treating various intestinal diseases, most notably *Clostridium difficile* infection.
For example, studies in mice have elegantly demonstrated that transfer of gut microbes can transmit obesity phenotypes, as well as efficiency of energy extraction from food. A similar strategy provided evidence that atherosclerosis susceptibility in mice could also be transmitted to a host by gut microbial transplantation. Such approaches have yet to be implemented in humans for treating cardiometabolic traits, but it is of interest that fecal transplantation studies in humans have shown that transfer of gut microbes from lean donors through a duodenal infusion into recipients with metabolic syndrome can increase insulin sensitivity, underlining the therapeutic potential of interventions that alter gut microbial composition or function.

**Targeting the Gut Microbiota for Therapeutic Applications**

An important clinical implication from studies on gut microbiota is how to leverage findings for therapeutic purposes. Selective manipulation of the gut microbial ecosystem might provide new avenues to treat and prevent cardiometabolic diseases, but this will first require a better understanding of which specific bacteria, or alternatively, which bacterial metabolites, are the appropriate targets for intervention and manipulation. The simplest point of intervention may be to limit consumption of dietary constituents that either foster the growth of undesirable bacteria or serve as substrates for microbe-dependent generation of products that disrupt lipid homeostasis or other metabolic processes.

Another viable therapeutic strategy may be the use of probiotics or prebiotics to produce a desired change in microbial composition or function that favorably impacts host global metabolism. Prebiotic therapy consists of ingestion of select nutrients or dietary constituents (nonmicrobial compositions) that provide a growth advantage of beneficial bacteria, whereas probiotic therapy involves the ingestion of one or more live bacterial strains, attempting to take advantage of the mutualism of microbes. Alternatively, therapeutic intervention could rely on the use of broad or class-specific antibiotics to eliminate bacterial species or their products associated with dyslipidemia and other metabolic disturbances. However, this approach is not a sustainable long-term option. For example, many gut microbial products are beneficial to the host, and even infrequent antibiotic treatment, particularly in young children whose gut microbiota has yet to be fully established, can adversely impact host global metabolism via changes in the gut microbial community and facilitate the emergence of antibiotic-resistant bacterial strains.

**Conclusions**

For many years, the community of bacteria living in our gut was largely ignored. However, emerging evidence clearly demonstrates that our microbial symbionts play multiple fundamentally important roles in maintaining normal metabolic homeostasis. These discoveries have broad implications for elucidating bacterially mediated pathophysiological mechanisms that alter lipid metabolism and other related metabolic traits. From a clinical perspective, this newly recognized endocrine organ system can be targeted for therapeutic benefit or prevention of metabolic diseases. The ability to manipulate the gut microbiome for improved health and prevention of diseases is still in the early phases of development, but recent rapid advances in gut microbiome studies highlight both the potential and promise of targeting intestinal microbes for therapeutic gain.

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