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# American Diabetes Association and JDRF Research Symposium: Diabetes and the Microbiome

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**From 27–29 October 2014, more than 100 people gathered in Chicago, IL, to participate in a research symposium titled "Diabetes and the Microbiome," jointly sponsored by the American Diabetes Association and JDRF. The conference brought together international scholars and trainees from multiple disciplines, including microbiology, bioinformatics, endocrinology, metabolism, and immunology, to share the current understanding of host-microbe interactions and their influences on diabetes and metabolism. Notably, this gathering was the first to assemble specialists with distinct expertise in type 1 diabetes, type 2 diabetes, immunology, and microbiology with the goal of discussing and defining potential pathophysiologies linking the microbiome and diabetes. In addition to reviewing existing evidence in the field, speakers presented their own original research to provide a comprehensive view of the current understanding of the topics under discussion.**

**Presentations and discussions throughout the conference reflected a number of important concepts. The microbiota in any host represent a complex ecosystem with a high degree of interindividual variability. Different microbial communities, comprising bacteria, archaea, viruses, and fungi, occupy separate niches in and on the human body. Individually and collectively, these microbes provide benefits to the host—including nutrient harvest from food and protection against pathogens. They are dynamically regulated by both host genes and the environment, and they critically influence both physiology and lifelong health. The objective of the symposium was to discuss the relationship between the host and the microbiome—the combination of microbiota and their biomolecular environment and ecology—specifically with regard to metabolic and immunological systems and to define the critical research needed to understand and potentially target the microbiome in the prevention and treatment of diabetes. In this report, we present meeting highlights in the following areas: *1*) relationships between diabetes and the microbiome, *2*) bioinformatic tools, resources, and study design considerations, *3*) microbial programming of the immune system, *4*) the microbiome and energy balance, *5*) interventions, and *6*) limitations, unanswered questions, and resource and policy needs.**

## RELATIONSHIPS BETWEEN DIABETES AND THE MICROBIOME

Globally, the prevalence of both type 1 and type 2 diabetes is increasing. The acceleration of these diseases far outpaces the rate of genetic variation, eliminating genes as singular factors in the trend. Changes in environmental conditions, such as diet, hygiene, antibiotic use, and other medical practices, can be correlated with the growth of these diseases. Such factors may be influencing the composition and function of the microbiome in ways that significantly impact the immune and metabolic systems, contributing to the increased risk for these diseases.

Mark Atkinson (University of Florida, Gainesville, FL) presented an overview of type 1 diabetes and how it might be influenced by the microbiome. The incidence of type 1 diabetes is increasing throughout the world (1), strongly suggesting that factors beyond genetic predisposition—specifically, environmental influences—may contribute to

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the initiation and/or the propagation of the aberrant immune response leading to β-cell loss. In support of this idea, global increases in type 1 diabetes do not manifest the same pattern in all regions. For example, in Finland, the most striking increase in incidence occurs between the ages of 1 and 4 years, whereas in the U.S., substantial increases are noted in adolescents (2). The disease is increasingly reported in individuals who do not carry high-risk genetic alleles (3), which also suggests that environment is a major contributor to type 1 diabetes. Additionally, studies have shown that the concordance of type 1 diabetes in identical twins is only 13–33%, indicating that factors in addition to genetics are involved in disease progression (4,5).

Several lines of evidence suggest that the microbiome may influence the development of type 1 diabetes. Probiotic treatment of NOD mice modifies the course of type 1 diabetes (6). Correspondingly, antibiotic use has complex effects on the development of type 1 diabetes in diabetes-prone BB rat and NOD mouse models. While the increased use of antibiotics in humans correlates with the increased incidence of type 1 diabetes, several studies have shown that specific types and courses of antibiotic exposure are associated with the reduced incidence of type 1 diabetes in these animal models (7,8). In a recent study, NOD mice exposed to antibiotics in early life showed an increased incidence of diabetes (9). While the literature shows varying effects of probiotics and antibiotics on type 1 diabetes, an increasing body of evidence supports the notion that specific microbiome compositions may affect the risk of developing type 1 diabetes (in either direction), although definitive evidence from humans regarding the specific composition and demonstration of causation is still forthcoming. Further interactions of the microbiome with diabetes development in NOD mice include a greatly increased type 1 diabetes disease risk in females than in males. Although this sex bias is not seen in humans, susceptibility in female NOD mice can be reduced through the transfer of intestinal microbiota from males (10). Insulitis, the hallmark of autoimmune reactions leading to type 1 diabetes, has been reported in NOD mice and is accelerated under germ-free (GF) conditions, suggesting an interaction between the immune system and the microbiome (11). Diabetes incidence and age of onset in inbred BB rat strains are associated with differences in microbial diversity, with higher disease rates in animals with lower microbiota diversity (7). The transfer of a single species such as *Lactobacillus johnsonii* from diabetes-resistant animals has been reported to protect diabetes-prone animals from type 1 diabetes (12). Furthermore, several studies have reported lower microbial diversity among people with type 1 diabetes compared with healthy subjects (13–15).

The immune system and the gut microbiome develop coordinately (16,17), and the close functional relationship raises the possibility that microbes or microbial metabolites could be used in the diagnosis, prevention, or treatment of type 1 diabetes. For example, β-cell autoimmunity has been linked to the abundance of specific commensal bacteria, including diminished *Clostridium leptum* in NOD mice (18) and higher abundance of *Bacteroides* species in people who later develop type 1 diabetes (19). Further studies of these relationships may lead to the discovery of a microbial biomarker for type 1 diabetes. As several studies have reported residual β-cell function in type 1 diabetes (20), a better understanding of the function of specific bacteria and their impact on immune function may highlight ways that modification of the gut microbiome could reduce the autoimmune attack of β-cells, allowing the rescue or maintenance of β-cell function.

Clay F. Semenkovich (Washington University, St. Louis, MO) presented an overview of type 2 diabetes and its relationship to the microbiome. While global death rates for most diseases fell over the past two decades, there was a 93% increase in the absolute number of deaths attributed to diabetes between 1990 and 2010, an increase second only to HIV (21). This burden is likely to increase further, as one in three adults is projected to have diabetes by 2050 (22). The rise in the prevalence of type 2 diabetes (about 95% of diabetes cases in the U.S.) is coincident with increases in obesity (23).

The pathogenesis of type 2 diabetes is less well understood than type 1 diabetes. Its onset is insidious. Elevated blood glucose in the disease is a consequence of a progressive insulin secretory defect in the context of insulin resistance, and frequently patients present with vascular complications of the disease at diagnosis, suggesting disease progression without obvious symptoms. The risk for and progression of type 2 diabetes are affected by genetic variants, most of which have small effect sizes. However, similar to type 1 diabetes, genetics alone cannot account for the increasing prevalence of type 2 diabetes, and environmental factors are clearly at play. Increasing evidence suggests that microbiome-host interactions may be one environmental factor that influences type 2 diabetes risk and progression.

Several studies have linked the intestinal bacterial environment to metabolic health. In observational studies, bariatric surgery, which has complex effects on the microbiome, has been reported to improve, or even resolve, type 2 diabetes and decrease cardiovascular risk, even before significant weight loss is realized (24). In gnotobiotic mice, the transfer of microbiota from lean humans can prevent adverse effects of microbiota transferred from obese humans (25). Together, such evidence suggests that the manipulation of the microbiome could improve type 2 diabetes treatment. As β-cell function is preserved in individuals with metabolic syndrome as compared with those with type 2 diabetes, metabolic syndrome patients might be predicted to be more responsive to microbiota-induced metabolic manipulations impacting energy balance.

Andrew Goodman (Yale University, New Haven, CT) presented evidence suggesting that life in the womb begins

free of microbes, followed by the rapid acquisition of commensal microbiota during and after birth. This process is characterized by large-scale acquisition—both an enormous number of bacterial cells and extensive interindividual variation (Fig. 1)—as well as variation over time and inheritance by selection and competition. Importantly, the preponderance of microbe-host interactions is not disease causing (26,27).

An important property of any ecosystem is resilience: the ability of a community to recover from perturbation. Insults can take many forms: toxins, elements in foods, drugs, and microbes. An important question is how the microbiome remains stable during insults involving inflammation. Interactions that determine how the microbiome responds to perturbation are often also established early in life.

As a specific example of the interactions between hosts and their microbiome, Dr. Goodman considered antimicrobial peptides. These host-derived molecules insert into the membranes of bacterial cells and provide innate defenses against infections. Many human gut commensals are resistant to antimicrobial peptides, providing a mechanism for commensal resilience (28).

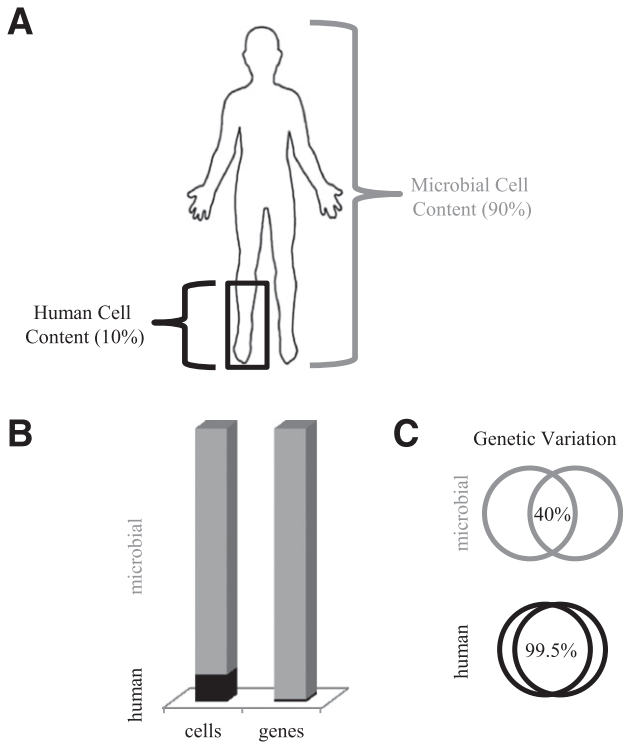


**Figure 1**—Relative composition of microbial and human cell and gene content. Microbial cells represent 70–90% of the cells occupying the human body (*A* and *B*), and microbial genes are about 1,000 times more abundant than human genes (*B*). The microbiome is modifiable through genetic and environmental circumstances, including method of birth, breast-feeding, antibiotics, diet, exposure to toxins, and hygiene. The microbiome is characterized by composition (which species are present), diversity (number of taxa represented), and abundance (total microbial content). It is maintained by competition and resilience. While interindividual variation among human genes is only 0.5%, variation among microbial genes is significantly more substantial, at about 40% (73) (*C*), supporting a critical role for microbe-host interactions in health and pathophysiology.

## BIOINFORMATIC TOOLS, RESOURCES, AND STUDY DESIGN CONSIDERATIONS

The critical factors enabling the study of the microbiome have been access to gnotobiotics—animals that are GF or colonized with only defined microbes—and, in recent years, the rapid advancement of sequencing technologies and the development of elegant and powerful computational and bioinformatic tools. Physiology can be compared between GF and conventional animals, and GF animals can be "conventionalized" through the transfer of a complex microbiome. By varying experimental conditions, it is possible to identify factors that determine resilience of human gut commensal communities and to define their impact.

While sequencing, computational, and bioinformatic tools are crucial, there are important study design and analytical considerations that must be taken into account when investigating the microbiome. Curtis Huttenhower (Harvard University, Boston, MA) provided an introduction to the various approaches in computational analyses of microbial communities. Current techniques are primarily based on nucleotide sequencing due to the technology's efficiency and cost-effectiveness, although functional molecular tools, such as whole-community metabolomics and proteomics, are becoming more accessible. Amplicon sequencing (e.g., 16*S*, 18*S*, or internal transcribed spacers) for taxonomic profiling is currently the most widespread, although 16*S* rRNA sequencing has relatively limited resolution and a narrower range than metagenomic approaches (29). Functional profiling (e.g., metabolic reconstruction) is becoming increasingly important for establishing molecular mechanisms and causality.

C. Ronald Kahn (Joslin Diabetes Center, Boston, MA) presented a study suggesting that the microbiome may be an important aspect of host physiology that plays a role in modulating host responses to environmental influences. His group examined metabolic phenotypes of an inbred mouse strain (129) sourced from two different vendors (The Jackson Laboratory and Taconic Biosciences) and found distinct metabolic responses to high-fat–diet (HFD) feeding, including differences in weight gain, glucose tolerance, insulin resistance, and hepatic steatosis (O. Bezy, S. Ussar, C.R. Kahn, unpublished data). Housing the strains under the same environmental conditions for three generations impacted the microbial diversity, ratio of Firmicutes to Bacteroidetes, and species representation. Interestingly, following cohousing, the responses to HFD—including weight gain, glucose tolerance, and insulin resistance—became nearly identical (O. Bezy, S. Ussar, C.R. Kahn, unpublished data). Antibiotic treatment in strains prone to HFD-induced metabolic derangement improved insulin resistance, lowered blood glucose, and led to improvements in insulin signaling (S. Fujisaka, C.R. Kahn,

unpublished data). Conversely, abrogation of insulin signaling specifically in the gut epithelium induced changes in the composition of the microbiome (T. Haring, S. Fujisaka, C.R. Kahn, unpublished data). While these associations are very interesting, the challenge remains to identify the specific components of the microbiome that drive metabolic changes and to demonstrate causality. Such progress should provide important new insights on how to prevent and treat metabolic disease.

Anthony Fodor (University of North Carolina, Charlotte, NC) extended the theme of the careful experimental design to associate variation in the microbiome with disease phenotypes in type 1 and type 2 diabetes. To illustrate this point, Dr. Fodor highlighted examples from a series of recent publications using interleukin (IL)-10–deficient (*Il10*$^{-/-}$) mice to relate the polyketide synthase locus in *Escherichia coli* to intestinal inflammation and the development of colorectal cancer (30,31). He found that stochastic divergences between cages had a profound impact on murine gut microbial composition, nearing the size of effects observed for large biological differences such as the inflammation induced by dextran sulfate sodium and IL-10 deficiency. Dr. Fodor's conclusions included both quantitative and biological insights. Computational approaches make it easy to perform millions of hypothesis tests, which can rapidly overfit models and lead to spurious conclusions when the numbers of available animals or cages are overwhelmed. Most importantly, causal roles for the microbiome established in animal models may not be causal in humans, even when bioinformatic and statistical best practices are followed assiduously.

Justine Debelius (University of Colorado, Boulder, CO) provided an important reminder that the gut microbiome has the complexity inherent to ecologies composed of hundreds or thousands of interacting species (32–34). Ms. Debelius linked quantitative measures of microbial ecology, such as alpha (within-sample) and beta (between-sample) diversity, to those used in ecological studies of the environment or metazoan biology. She reminded the audience that principles such as colonization (e.g., microbial acquisition at birth) and succession (e.g., recovery from antibiotics) occur at a microscopic scale as well. She concluded with a reminder that one of the recurring themes of the conference, fecal microbiota transplant, is itself an extreme type of ecological intervention, which the field may learn over time to supplement with targeted, preventive modulations of the microbiome through diet or pharmaceuticals.

James Paul Brooks (Virginia Commonwealth University, Richmond, VA) specifically addressed the challenges of distinguishing microbial causality from correlation by using targeted experimental designs and statistically informed analysis approaches. Dr. Brooks outlined four challenges in the interpretation of microbial community data: *1*) identifying the source (taxonomic, phylogenetic, or molecular) of a particular nucleotide sequence or other measurement, *2*) identifying and controlling nonbiological biases, such as interbatch or interlaboratory measurement variability, *3*) balancing discovery-based approaches with directed hypothesis tests and interventions in controlled experiments, and *4*) handling microbial community dynamics and changes in the microbiome over time. While these broad areas should be considered during the design of any microbiome-related experiment, Dr. Brooks provided several specific suggestions for maximizing the effectiveness of investigations into the microbiome, including careful collection of extensive sample metadata, detailed biogeography, longitudinal sampling, replication, and the use of positive and negative controls using mock communities whenever possible.

## MICROBIAL PROGRAMMING OF THE IMMUNE SYSTEM

The interplay between the microbiome and the development of the immune system is particularly relevant to autoimmune diseases such as type 1 diabetes. Understanding the interactions between the microbiome and the developing immune system may spur the development of approaches to modify those interactions and prevent autoimmunity (Fig. 2). Andrew J.S. Macpherson (University of Bern, Bern, Switzerland) addressed the critical role of bacterial compartmentalization by intestinal mucus and its impact on the colonization and function of commensal bacteria (35). He showed that bacteria displayed distinct gene expression patterns reflecting location-specific metabolic demands and found that IgA limits motility and enhances clearance of bacteria in the small intestine but is not required for the production of colonic mucus layers (36). These results underscore the mutualistic roles of microbes and host immunity in intestinal homeostasis.

Alexander Rudensky (Memorial Sloan Kettering Cancer Center, New York, NY) discussed the effects of microbial metabolites on regulatory T-cell generation and downstream immune-mediated inflammation. He defined mechanisms of regulatory T-cell development and function in the thymus and peripheral tissues (induced regulatory T cells). The selective blockade of induced regulatory T cells provokes mucosa-specific allergic inflammation (37) and alters the gut microbiome composition. Microbial metabolites such as butyrate, a short-chain fatty acid, and bile salt hydrolase activity increase regulatory T-cell frequency in the small intestine and colon, respectively. Thus, the host senses commensal metabolites that regulate the balance between regulatory T cells and T-effector cells and the inflammatory status of the gut mucosa (38,39). Understanding the feedback between microbial metabolites and inflammation of the gut may allow for the identification of anti-inflammatory metabolites that could be leveraged therapeutically.

Another T-cell subset that can be proinflammatory in the gut is characterized by expression of the transcription factor RORγt and the cytokine IL-17 (Th17). Dan R. Littman

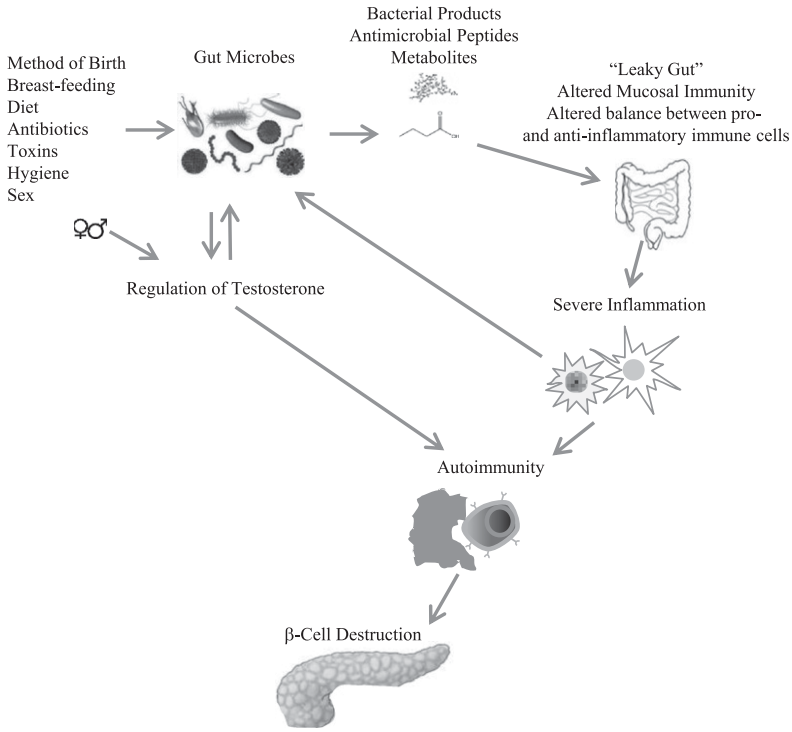


**Figure 2**—Possible interactions between gut microbiome and immune system that may influence the development of type 1 diabetes. Microbial composition may be impacted by host sex or sex steroids, which can also impact autoimmunity (although type 1 diabetes incidence in humans does not display strong sex bias). Microbes, bacterial products, antimicrobial peptides, and microbial metabolites may directly promote inflammation. Microbes, metabolites, or the immune response to them may be involved in promoting permeability of the gut, leading to higher accumulations of microbes in the bloodstream and contributing to increased inflammation. In addition, mucosal immunity and a disrupted balance between pro- and anti-inflammatory immune responses can lead to increased inflammation. A severe inflammatory response may promote autoimmunity, including autoimmune destruction of pancreatic β-cells, causing type 1 diabetes. Inflammation itself may favor the maintenance of specific gut microbes that may produce inflammation-promoting metabolites, maintaining the severe inflammatory state.

(New York University, New York, NY) showed that the colonization of GF mice with segmented filamentous bacteria (SFB) is sufficient to drive Th17 differentiation in the small intestine (40). Strikingly, the antigen receptor (TCR) repertoire of intestinal Th17 cells is biased toward recognition of SFB antigens (41). In transgenic mouse studies, TCR specificity for SFB antigens is not sufficient to drive Th17 differentiation. Dr. Littman speculated that bacteria control T-cell programs through their effects on antigen-presenting cells (dendritic cells and mononuclear phagocytes) in the intestinal niche. Under conditions of dysbiosis, a mononuclear phagocyte subset that expresses the chemokine receptor CX3CR1 can capture luminal bacteria and transport them to mesenteric lymph nodes (42). At steady state, the microbiota limits bacterial transport from the lumen to mesenteric lymph nodes, serving to limit an inflammatory immune response.

Jayne Danska (The Hospital for Sick Children, Toronto, Canada) examined the role of genetics and the gut microbiome in the NOD mouse model of type 1 diabetes, which recapitulates the complex genetics and immune pathogenesis of the human disease, including islet inflammation and islet autoantibodies. As for many autoimmune diseases, the incidence of type 1 diabetes in NOD mice is greater in females than males, but the underlying mechanisms of sexual dimorphism are not understood, and a similar female sex bias for type 1 diabetes is not recapitulated in humans (43–45). Still, the NOD mouse is possibly the best model for the study of type 1 diabetes. In contrast to colonized animals, GF conditions produce equal type 1 diabetes incidence in males and females and depress male testosterone levels. The transfer of adult male gut microbes into immature females elevates testosterone production, blocks islet inflammation and autoantibody production, and protects animals from type 1 diabetes (10). Current longitudinal analyses are measuring changes in human intestinal microbiome composition and function during the progression to islet autoantibodies and type 1 diabetes. Dr. Danska and colleagues tested serum samples from healthy children and children with multiple sclerosis or recent-onset type 1 diabetes and found that each group displayed distinct patterns of antibody reactivity to commensal species (J.D., C. Yau, S. Mortin-Toth, P. Poussier, L. Marandi, A. Bar-Or, unpublished data). Ongoing work will determine whether the host immune system responds to organisms that vary in abundance during progression to type 1 diabetes.

Increasing evidence suggests a critical relationship between the gut microbiome and the development and maintenance of

the immune system. Given this association, there is potential for identifying protective microbial profiles for potential therapeutic use and, alternatively, potentially pathogenic microbial profiles that could be used to determine targets for modification. On the basis of the experimentation in mice, there is hope that microbial biomarkers may be able to better predict the risk for type 1 diabetes and that particular microbes or microbial metabolites could serve as future interventions to combat autoimmune destruction of β-cells, although a critical first step is defining causal relationships in humans.

## THE MICROBIOME AND ENERGY BALANCE

As obesity is a major contributor to type 2 diabetes risk, alteration of energy balance to promote weight loss is a potentially useful strategy to combat type 2 diabetes. Studies in *ob/ob* (leptin-deficient) mice indicate that obesity itself may change the microbiome (46). Several physiological changes make sustaining weight loss difficult, and many of these, such as energy harvest, hormonal control of food intake, inflammation, storage of calories, and energy expenditure, offer potential mechanisms through which microbes in the gut may act to regulate body weight (47–50).

To highlight the relationship between the gut microbiome and obesity, Michael Rosenbaum (Columbia University, New York, NY) showed that the colonization of the gut of gnotobiotic mice through fecal transplant leads to weight gain and that the degree of weight gain is dependent on the weight of the donor, with greater weight gain following transplants from obese mouse (51) or human donors (52) than from lean donors. Furthermore, the gut microbiome of obese or overfed humans is characterized by lower diversity, fewer Bacteroidetes, and enrichment of Firmicutes compared with that of lean or underfed individuals (46,53,54). In mice, the microbiomes of obese and lean subjects respond differently to weight loss (49). To be able to leverage the microbiome for potential therapeutic approaches for obesity, adiposity effects will need to be understood in humans.

Joël Doré (French National Institute for Agricultural Research, Paris, France) presented evidence that individuals with low bacterial gene counts (representing lower bacterial diversity) have less healthy metabolic and inflammatory traits (55,56). Interestingly, the effect size of the microbiome, which is modifiable, on obesity appears greater than can be attributed to any of the 32 validated obesity risk loci identified through genome-wide association studies (57). Dr. Doré emphasized that altered cross talk between microbes and host may impact the molecular signaling machinery responsible for regulating energy metabolism, intestinal nutrient sensing, and inflammation.

Patrice D. Cani (Université catholique de Louvain, Brussels, Belgium) described the strong immune responses elicited by the lipopolysaccharides from gram-negative bacteria in the intestine. In obese rodents, the gut seems to be more permeable than in lean rodents, and Dr. Cani proposed that this "leaky gut syndrome" allows higher accumulation of lipopolysaccharide in the bloodstream, which may contribute to chronic low-grade inflammation and resultant development of insulin resistance and obesity. The hypothesis is that the manipulation of the microbiome through prebiotics can reduce gut permeability (58,59). Specific changes in gut microbiota have been associated with modifications in the production and secretion of gut peptides (e.g., glucagon-like peptide 1 and 2, peptide YY, and ghrelin) and endocannabinoids, ultimately impacting glucose homeostasis and adipogenesis (60,61).

Andrew Gewirtz (Georgia State University, Atlanta, GA) hypothesized that the relationship between the host and the microbiota requires ongoing maintenance and that perturbations to the microbiota activate the mucosal immune system and cause inflammatory responses. Rarely, inflammation is sufficiently severe enough to contribute to inflammatory bowel syndrome or, perhaps, type 1 diabetes. More commonly, low-grade inflammation establishes a new equilibrium and may result in metabolic derangements. To demonstrate this model, Dr. Gewirtz presented data on a Toll-like receptor 5 (TLR5) knockout mouse model, which sustains low-grade inflammation and moderate obesity (62). The metabolic effects of deleting TLR5 are resolved in GF mice and are transferrable to wild-type mice via fecal transplant, indicating the necessary role of the microbiota in the pathogenesis of obesity in this model.

Communication between the host and the microbiota may be regulated through interactions with the intestinal innate immune system, which is able to sense nutritional status and alter host metabolism accordingly (63). If transient states promoting inflammation are maintained over time, they may lead to a sustained alteration of the gut microbiota and sustained inflammation—conditions that support each other. This vicious cycle could make weight loss difficult (Fig. 3).

Current medical treatments target only the host. The microbiota offers another target that may allow medicine to address some of the unique environmental conditions attendant to modern society. Easily accessible, inexpensive interventions such as prebiotics and probiotics (64), as well as certain antibiotics, could be effective in stimulating the development and maintenance of a beneficial microbial community that favors weight loss and weight maintenance.

## INTERVENTIONS

Establishing causal relationships, if they exist, between the microbiome and physiology is critical to the ultimate goal of modifying the microbiome for the prevention, treatment, or cure of diabetes. To examine the current understanding relevant to the development of future interventions, presenters focused on gut signaling pathways and the metabolic effects of clinical approaches that target the gut.

The gut communicates information to the brain through connections to the vagal afferent pathway that regulate

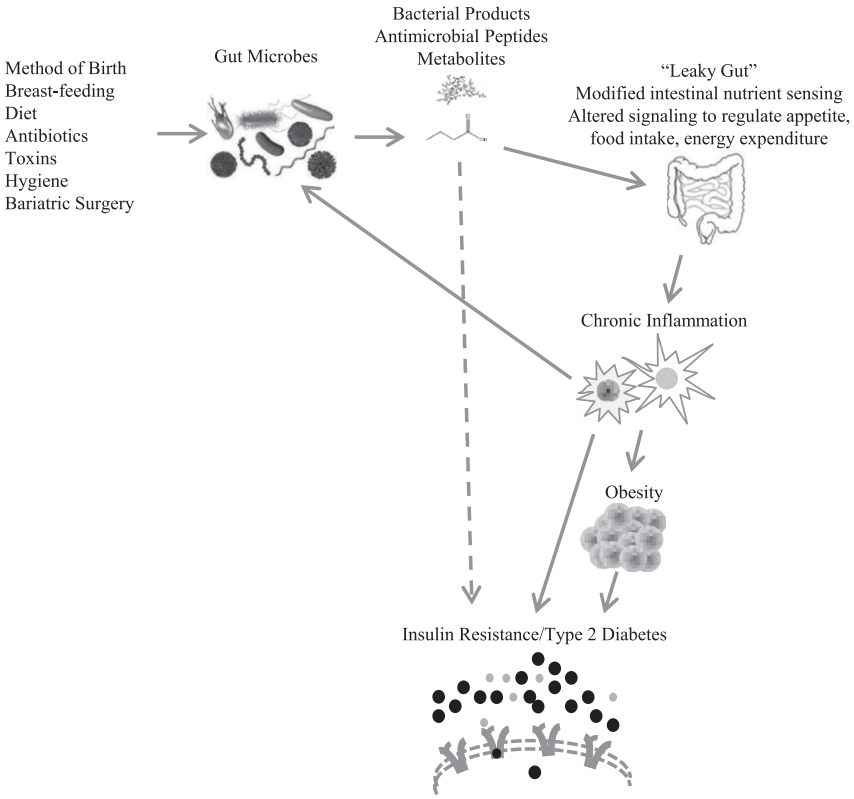


**Figure 3**—Possible interactions between gut microbiome and inflammation that may influence the development of type 2 diabetes. Microbes, bacterial products, antimicrobial peptides, and microbial metabolites may directly promote inflammation. Microbes or metabolites may be involved in promoting permeability of the gut, leading to higher accumulations of microbes and metabolites in the bloodstream and contributing to chronic low-grade inflammation. Inflammation in adipose tissue promotes obesity, which is associated with the development of insulin resistance and type 2 diabetes. Inflammation itself may favor the maintenance of specific gut microbes that may produce inflammation-promoting metabolites, leading to a vicious cycle that maintains itself and makes weight loss difficult.

metabolism and food intake. Helen E. Raybould (University of California, Davis, Davis, CA) discussed an approach to understanding this interface that may present opportunities to identify new therapeutic targets. Vagal afferent neurons contain receptors for nearly every gut peptide, but cholecystokinin (CCK) appears to be the master regulator of their function. Depending on feeding status, CCK drives a signaling switch that changes vagal afferent signaling from orexigenic (in fasting) to anorexigenic (in the fed state). In diet-induced obese animals, the signaling switch in response to CCK is abolished, and the animals are unable to signal satiety to the brain in response to food. Because the switch also requires leptin signaling, this effect is likely due to increased leptin resistance (65).

Dr. Raybould conducted studies using bovine milk oligosaccharides (BMOs), complex indigestible food components. BMOs can be utilized by *Bifidobacteria* and thus are predicted to increase the representation of *Bifidobacteria* in the gut. BMOs shifted the microbiota back to a "healthier" profile, reversing gut permeability induced by an HFD, reducing inflammation, and preventing the onset of diet-induced obesity. Future work is aimed at determining whether these changes in gut physiology also modulate vagal afferent leptin sensitivity and CCK switching and, if so, which molecular entities mediate these effects.

Frank J. Gonzalez (National Cancer Institute, Bethesda, MD) described an intriguing observation from the cancer biology field that serendipitously uncovered potential microbiome-associated therapeutic targets for metabolism. In mice, the antioxidant tempol not only reduced the formation of spontaneous tumors but also significantly decreased the animals' weight and improved metabolic parameters (66). Subsequent metabolomic analyses suggested that tempol treatment changed the metabolite profile in the gut hypothetically through changes in the composition of the gut microbiota that modify bile acid metabolism to favor the production of a farnesoid X receptor (FXR) antagonist (67). In metabolic studies, a relatively low dose of an FXR antagonist led to weight loss, improved insulin sensitivity, increased metabolic rate, and reduction in triglycerides and hepatic steatosis in HFD-fed mice. Each of these effects was abrogated in FXR knockout animals (68), suggesting that modulation of gut FXR signaling could be a successful strategy to improve metabolic function in humans.

G-protein–coupled receptors (GPRs) represent the most common targets for therapeutic intervention. Of the

approximately 400 GPRs that have been characterized, only a small fraction are highly expressed and enriched in enteroendocrine cells (69). Thue W. Schwartz (University of Copenhagen, Copenhagen, Denmark) is focusing his efforts on these receptors to identify potential therapeutic targets. In particular, he showed that short-chain fatty acids produced by gut microbiota as a by-product of the digestion of complex carbohydrates are sensed by the GPR43 receptor and possibly also the GPR41 receptor in adipocytes (A.S. Husted, Z. Gerhart-Hines, R.M. Jones, T.W. Schwartz, unpublished data). As GPR41 is expressed in enteric neurons, and GPR43 in enteric leukocytes, these receptors may also transfer important information regarding metabolite status in the gut to the nervous and immune systems (70). Aromatic amino acids, by-products of protein metabolism, activate GPR142 (O. Rudenko, J. Shang, M. Wu, J. Mokrosinski, M.S. Engelstoft, B. Svendsen, G. Dai, Y. Qian, Y. Feng, K.L. Egerod, J.J. Holst, A.D. Howard, T.W. Schwartz, unpublished data), which is highly expressed on gastric inhibitory polypeptide–secreting cells. A potent selective synthetic agonist of GPR142 can stimulate insulin secretion from β-cells and improve glucose tolerance in both diet-induced obese and lean rodents (O. Rudenko, J. Shang, M. Wu, J. Mokrosinski, M.S. Engelstoft, B. Svendsen, G. Dai, Y. Qian, Y. Feng, K.L. Egerod, J.J. Holst, A.D. Howard, T.W. Schwartz, unpublished data).

The interactions of the receptors with ligands, with each other, and with their cellular surroundings must be considered when developing therapeutics. Agonists that are able to stimulate multiple signaling pathways may be required to optimally regulate effects. Context is also important: receptors expressed in one cell type may have distinct functional characteristics when expressed in a different cell type.

Lee Kaplan (Massachusetts General Hospital, Boston, MA) reviewed the bariatric surgery data that showed sustained reductions in weight and improvements in metabolic health that are greater than other available therapeutic approaches to weight loss. The effects of surgery on metabolism are related to the restrictive nature of the procedure and the ensuing reductions in nutrient intake and weight. However, mechanisms independent of changes in food intake and weight loss are also at play. Dr. Kaplan noted that the physiological changes associated with bariatric surgery are fundamentally different from those resulting from dieting at almost every level, including effects on energy expenditure, appetite, and gut peptide profiles. After dieting, the body attempts to regain lost weight, whereas after bypass surgery, the body appears to undergo a change in physiology favoring a lower body weight set point. Some of the potential mediators of these changes may be linked to the microbiome. For example, Roux-en-Y gastric bypass (RYGB) rapidly and selectively changes the gut microbiota in ways that are distinct from changes seen with diet-induced weight loss and are independent of initial weight and diet following RYGB. The transfer of RYGB microbiota to GF animals results in a significant decrease in weight and adiposity, while the transfer of microbiota from sham-treated animals does not result in weight loss, despite the fact that the RYGB transfer animals eat more than sham transfer animals. These data suggest that bariatric surgery changes the interactions among nutrients, metabolites, mucosa, and microbiota in the gut, which, in turn, can significantly alter signaling from the gut to the rest of the body, resulting in metabolic improvements. Identification of molecular mediators of bariatric surgery that could be leveraged therapeutically to impact metabolism in the absence of surgery has become an important area of metabolic research.

Max Nieuwdorp (University of Amsterdam, Amsterdam, the Netherlands) presented clinical and translational work aimed at identifying specific microbes associated with insulin resistance, metabolic syndrome, and type 2 diabetes (71). In randomized controlled clinical trials of fecal transplantation into male subjects with insulin resistance and metabolic syndrome, some subjects showed enhanced insulin sensitivity and increased levels of fecal short-chain fatty acids. Oral administration of the bacterial metabolite butyrate was associated with increased hepatic insulin sensitivity in healthy subjects. Furthermore, Dr. Nieuwdorp found that daily oral gavage of leptin receptor mutant (*db/db*) mice with butyrate-producing bacteria resulted in increased insulin sensitivity. An ultimate goal is to understand the relative benefits of administering single metabolites like butyrate compared with colonization with organisms that produce them to achieve sustained improvements in insulin sensitivity and to protect against type 2 diabetes.

The focus on interventions highlighted promising areas of research as well as future directions required to realize the potential power of targeting the microbiome therapeutically to improve outcomes for people with, or at risk for, diabetes.

## LIMITATIONS, UNANSWERED QUESTIONS, AND RESOURCE AND POLICY NEEDS

While the microbiome has been linked to many human diseases, there is a need for caution in the design and interpretation of such studies (72). Current limitations in the field were addressed at the conclusion of this conference to formulate recommendations for the standardization of study design and data analyses and for the direction of future research in the field. Limitations include the commonly used techniques for distinguishing meaningful differences in microbial composition, the need to differentiate causation from correlation, and the need to identify mechanisms of action for microbes or microbial metabolites on human health.

Data on emerging relationships of the microbiota with environmental factors, metabolic functions, and immune system activities that might better predict the risk for type 1 diabetes, type 2 diabetes, and obesity are currently limited.

The conferees highlighted areas of need for research that will be required to more clearly define the impact of

the microbiome on diabetes. Consensus from the discussion developed around the need to link physiological function to individual microbes or defined combinations of microbes or microbial metabolic pathways through hypothesis-driven interventional studies using standardized protocols. Exciting research goals include defining functional relationships between hosts and their microbes, which may lead to the development of new therapies; characterization of the effects of drugs and diet on the microbiome and vice versa; and identification of microbes or metabolites that can serve as biomarkers to quantify disease risk or the progression of type 1 diabetes or type 2 diabetes.

## RECOMMENDATIONS FOR PIVOTAL RESEARCH QUESTIONS ABOUT DIABETES AND THE MICROBIOME

### Genetics and Physiology

- How does the host immune system affect the microbiome?
- How does the microbiome directly impact the function of the immune system?
- How does host genetic variation contribute to microbial diversity?
- How do changes in the microbiome in early development influence long-term health?
- Can early interventions to alter microbiome composition potentially prevent diabetes or reduce the complications of diabetes?
- Are there microbiome characteristics predictive for onset or rate of progression of autoimmunity (type 1 diabetes) or metabolic dysfunction (type 2 diabetes)?

### Environment

- How does the microbiome modify pharmacotherapy and vice versa?
- How do components of diet, including changes in the conventional food supply (pesticides, emulsifiers, antimicrobials), impact gut microbes?
- How does microbial acquisition at birth (vaginal vs. cesarean section delivery, effects of prenatal antibiotics) and early-life events, such as breast-feeding, affect human health?

### Defining Causal Relationships

- Can we identify functional roles for the particular microbial characteristics based on studies that show correlation to disease?
- Can we determine whether specific microbes or metabolites reproduce physiological or pathophysiological conditions that revert when the microbe is hindered? This approach might be considered as helping to fulfill Koch's postulates.
- In addition to bacteria, how do other components of the microbial community (i.e., viruses and fungi) impact host health? How do bacteriophages impact microbiome function?
- Are microbiota characteristics functionally related to the distinction between metabolically healthy obese individuals and obese individuals who develop type 2 diabetes?

### Resource and Policy Needs to Address Research Questions

- We need to strengthen collaborations among academia, industry, and government for funding functional studies, developing standardized techniques, and sharing data.
- We need to create National Centers of Excellence in microbiome research to drive consensus around standardization of study design, sample collection, and data analysis. This strategy must be balanced with the broad resources needed for investigator-initiated, hypothesis-driven investigation.
- We need to standardize the reporting of the source and sex of animals and the housing, handling, and dietary conditions in all publications reporting microbiome research so that data can be more effectively compared between studies. Biorepositories of microbiota can help achieve standardization.
- We need to develop animal models with defined microbiota.
- We need to establish best practices in bioinformatics to permit data consolidation and meta-analyses of studies between laboratories, increase collaboration between preclinical and clinical researchers, and build animal models to reflect observations in human studies.
- We need to support longitudinal cohort studies to follow changes in microbiome and health status throughout development and aging. These studies require long-term, stable funding.
- We need to collect stool samples as part of standard clinical protocols in diabetes clinical studies and archive samples for future research.

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