

# Progress and Challenges in Developing Metabolic Footprints from Diet in Human Gut Microbial Cometabolism<sup>1,2</sup>

Linda C Duffy,<sup>3\*</sup> Daniel J Raiten,<sup>4</sup> Van S Hubbard,<sup>5,7</sup> and Pamela Starke-Reed<sup>6,8</sup>

<sup>3</sup>National Center for Complementary and Integrative Health, <sup>4</sup>The Eunice Kennedy Shriver National Institute of Child Health and Human Development, <sup>5</sup>Division of Nutrition Research Coordination, NIH, US Department of Health and Human Services, Bethesda, MD; and <sup>6</sup>Agricultural Research Service, USDA, Beltsville, MD

## Abstract

*Homo sapiens* harbor trillions of microbes, whose microbial metagenome (collective genome of a microbial community) using omic validation interrogation tools is estimated to be at least 100-fold that of human cells, which comprise 23,000 genes. This article highlights some of the current progress and open questions in nutrition-related areas of microbiome research. It also underscores the metabolic capabilities of microbial fermentation on nutritional substrates that require further mechanistic understanding and systems biology approaches of studying functional interactions between diet composition, gut microbiota, and host metabolism. Questions surrounding bacterial fermentation and degradation of dietary constituents (particularly by *Firmicutes* and *Bacteroidetes*) and deciphering how microbial encoding of enzymes and derived metabolites affect recovery of dietary energy by the host are more complex than previously thought. Moreover, it is essential to understand to what extent the intestinal microbiota is subject to dietary control and to integrate these data with functional metabolic signatures and biomarkers. Many lines of research have demonstrated the significant role of the gut microbiota in human physiology and disease. Probiotic and prebiotic products are proliferating in the market in response to consumer demand, and the science and technology around these products are progressing rapidly. With high-throughput molecular technologies driving the science, studying the bidirectional interactions of host-microbial cometabolism, epithelial cell maturation, shaping of innate immune development, normal vs. dysfunctional nutrient absorption and processing, and the complex signaling pathways involved is now possible. Substantiating the safety and mechanisms of action of probiotic/prebiotic formulations is critical. Beneficial modulation of the human microbiota by using these nutritional and biotherapeutic strategies holds considerable promise as next-generation drugs, vaccinomics, and metabolic agents and in novel food discovery. *J Nutr* 2015;145:1123S–30S.

**Keywords:** diet, metagenomics, microbial-host co-metabolism, microbiome, probiotics/prebiotics

## Introduction

Metagenomic sequencing represents a powerful advance for analyzing the profound effect that the microbial genome

(estimated collectively as 100 trillion cells and encoding 100-fold more unique genes than our human genome of 23,000 cells) contributes to energy harvest of food and can impact the global burden of disease (1). A compendium report from the Global Burden of Disease (GBD)<sup>9</sup> Study published in *Lancet* in 2012 reported that, despite substantial reductions in risk exposures for children <5 y of age, child and maternal undernutrition risks accounted for almost 7% of disease burden in 2010 (2). The

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\* To whom correspondence should be addressed. E-mail: duffyf@mail.nih.gov.

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<sup>7</sup> Present address: National Institute of Diabetes and Digestive and Kidney Diseases, NIH, 6707 Democracy Boulevard, Bethesda, MD 20892.

<sup>8</sup> Present address: George Washington Carver Center, 5601 Sunnyside Avenue, Beltsville, MD 20705-5138.

<sup>9</sup> Abbreviations used: BOND, Biomarkers of Nutrition for Development; GBD, Global Burden of Disease; GPR43, G-protein-coupled receptor; HMP, Human Microbiome Project; LAB, lactic acid bacteria; MetaHIT, Metagenomics of the Human Intestinal Tract; *ob/ob*, mice homozygous for obese spontaneous mutation; TLR, Toll-like receptor.

GBD volume cited enormous regional variation in risks associated with noncommunicable diseases and metabolic disorders, infectious disease, and malnutrition (both under- and overnutrition). The GBD findings are consistent with the phenomenon that undernutrition early in life contributes to an increased propensity for obesity in adulthood (3).

Diet-induced obesity and related chronic diseases are the single largest cause of morbidity and mortality, afflicting >50% of the adult population in Western countries (4). Our contemporary lifestyle is associated with an epidemic of metabolic abnormalities, most prominently characterized by excessive body fat accumulation, high BMI, and high fasting plasma glucose. Hildebrandt et al. (5), for example, showed in a diet-induced murine model developed to study obesity and metabolic disorders that a high-fat/high-sugar diet dramatically changed gut microbiota composition and played a crucial role in increased permeability, low-grade inflammation, and metabolic disorders (5). The need to control for dietary variation when evaluating microbial shifts in composition should be a priority in future research. Deeper mechanistic understanding of diet's role in host microbial function, however, imminently depends on integrating *in vitro* and *in vivo* models into a systems-level framework for understanding the coevolution of host microbial cometabolism (6, 7).

Diet plays a pivotal role in shaping the human gut microbiota, one of the most densely populated microbial ecosystems in nature. This complex community of 100 trillion archaeal and bacterial cells is distributed over >1000 species. The estimated 3.3 million nonredundant microbial genes dwarf the human genome's 23,000 genes and help support digestion, carbohydrate degradation, vitamin and amino acid synthesis, SCFA production, detoxification and bile acid metabolism, and immune regulation (1, 8). Despite vast differences in microbiota distributions across individuals, there is consistency across functional components of the microbiome. For example, the addition of any nondigestible, fermentable carbohydrate to the diet increases fermentation capacity for acid production and microbially derived metabolites. From birth, infants are exposed to an abundance of oligosaccharides (10 g/L in human breast milk). Because none of the oligosaccharide-derived glycans can be metabolized by infant digestive enzymes, the evolutionary value of their production is assumed to be selective affinity for these nutrient substrates by *Bifidobacteria* spp. (9). Bioselectivity by *Bifidobacterium* spp. cells able to use glycan substrates as an energy source is assumed to have host energy advantage during periods of reduced dietary intake and may coevolve expanded metabolic capabilities that our mammalian genomes lack (10). Understanding the intricate role of microbial ecology and the variability of microbial gene sets in individual phenotypes that comprise the microbiome within a complex metagenome (aggregate DNA of host and microbiota) has implications for preventing disease susceptibilities and diagnosis of human pathologies.

An important question is to determine whether diet-derived products of microbial metabolism are released under similar conditions in the presence of carbohydrate, fat, and protein food substrates. By understanding how bacterial biotransformation via chemical and enzymatic pathways modulates colonic and systemic inflammation, obesity, and host gut metabolism, the role of macronutrients that drive microbial metabolism, metabolite production, and food matrix byproducts will begin to be more clearly elucidated.

After a meeting in Paris in 2005, the International Human Microbiome Consortium was officially launched in 2008 and continues to generate shared metagenomic-scale data resources for understanding our microbial genomes, their encoded metabolic functions, and interaction with the host ecosystem (11). The

Human Microbiome Project (HMP) sponsored by the NIH has been a member of the international consortium since its inception. An overarching goal of the current ongoing HMP efforts is to create metabolic signatures that further refine our understanding of major implications of coevolution and metabolism of the microbiome for promoting human health and disease prevention (12). The HMP based at the NIH complements other large-scale sequence-based projects such as the European Union's Metagenomics of the Human Intestinal Tract (MetaHIT) project (13). Both projects are focused on examination of the gut microbiome for a wide range of health statuses and physiologic characteristics.

Complementing the international consortium efforts, the first genome sequence of a lactic acid bacterium, *Lactococcus lactis* subsp. *lactis*, was completed in 2001 (14). The U.S. Department of Energy Joint Genome Institute and the Lactic Acid Bacteria Genome Consortium continue to examine polysaccharide glycoside hydrolases encoded in microbial genomes for a broad repertoire of commensal microorganisms and their enzymatic ability to convert between different energy sources (14, 15). Despite the relatively small size of their genomes, a number of biologic systems controlling the internal environment and energy metabolism may be responsible for lactic acid bacteria (LAB) (e.g., *Lactobacilli*, *Bifidobacteria* spp.) survival (15). Function-based "omics" approaches are needed to further examine microbial utilization and degradation of diet-derived polysaccharides, including transport functions and regulatory interactions in commensal LAB organisms. Collectively, these resources serve as invaluable comparative model systems for guiding future investigations and are essential framework components for advancing human microbiome research (16).

## Diet, Microbial Diversity, and Host Metabolism

From phenotypic analysis (metabolomics) and compositional (metagenomic) assessments, diet is a fundamental driver of gut microbial diversity and stability that arise between 2 and 4 y of age (17). Changes in diet and energy expenditure related to energy-dense foods are implicated as causing a major shift in bacterial numbers, SCFA production, satiety signaling, and the increased prevalence of obesity in Western populations (18). The increasing rates of obesity in Western populations are attributed to the evidence that ancestral human populations relied on diets containing more indigestible plant material than do modern high-energy, low-fiber, and high-fat foods (19). Frost et al. (20) found a relation between hormonal appetite pathways and complex metabolites of protein-derived fermentation that plays a greater role in appetite suppression or stimulation than with resistant starch and other dietary fibers. Pig model systems that have similar gastrointestinal tracts and diets to humans and germ-free mice humanized with human intestinal microbes provide comparative approaches for examining microbiota interactions with specific diets (8). Alternatively, in a rodent model, Devkota et al. (21) provided further evidence that dietary fats alter conditions for gut microbial assemblage, which can perturb immune homeostasis. The results implicate immune sensing of microbial metabolites that may be regulating host homeostatic set points in communicating with the microbiota.

Table 1 presents some of the essential properties of the gut microbiome. Metabolomics, defined as the measurement of multivariate metabolic responses to physiologic stimuli or genetic modification, is a high-throughput molecular approach to understanding metabolic regulation of an organism and its microbial

**TABLE 1** Complexity of host microbial cometabolism

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- The human microbiota comprises >100 trillion microbial cells; our adult bodies harbor 10 times more microbial cells than human cells
  - The human gut microbiome is estimated to contain ~3.3 million nonredundant genes, compared with the human genome, which consists of 23,000 genes (1)
  - Microbes make up 90% and human cells ~10% of cells in the human body
  - The human gut microbiome is mostly acquired shortly after birth from the maternal and living environment (diet, genotype, environmental interactions)
  - The metabolic role of the gut microbiota is essential for carrying out functional biochemical processes of human physiology, including the following:
    - salvage of energy
    - generation of absorbable compounds
    - production of SCFAs, vitamins, and essential nutrients
    - xenobiotic metabolism
    - metabolite production
- 

communities (22). In nutritional epidemiology modeling, these technologies have been used to evaluate diet-induced microbial imbalances in obesity and other metabolic phenotypes (23). Other function-based molecular approaches examining how patterns of intestinal fermentation and types of SCFAs produced are determined by how much carbohydrate is consumed have been extensively characterized in the recent literature (24–26).

Ley et al. (26) observed a strong link between obesity and the gut microbiome, reporting that an increased ratio of *Firmicutes* to *Bacteroidetes* in mice homozygous for obese spontaneous mutation (*Lep<sup>ob</sup>* commonly referred to as *ob/ob*) might promote adiposity or, alternatively, could represent a host-mediated adaptive response to limit energy uptake/storage (e.g., reducing capacity to ferment polysaccharides). Cani et al. (27) found that a high-fat diet can trigger metabolic inflammation and lead to endotoxemia and weight gain via transport of LPS out of the gut. Reducing plasma LPS concentration has gained attention as a plausible strategy for the control of metabolic diseases. Moreover, gut microbes contribute to host energy balance by using interactive signaling mechanisms involving innate immune responses and entero-endocrine and epithelial cells. In studying a broad array of microbial functional and metabolic interactions, Holmes et al. (28) argued that integrating biologic knowledge in transgenomic, metabolic, and immune processes is a priority for future research and proposed that interventional windows to disease prevention should be targeted in early life and across the life span.

## Microbial Fermentation of Polysaccharides to SCFAs

The gut microbiota manipulates the provision of calories to the host by hydrolysis of indigestible plant polysaccharides. Complex carbohydrates, such as dietary fiber, are metabolized by the colonic microbiota to oligosaccharides and monosaccharides and then fermented to SCFAs, which function both as an energy source and as a signaling molecule (29). Dietary fiber (e.g., cellulose, pectin) abundance and type are directly related to the species composition of the microbiota and their metabolic interactions. As a fuel source for intestinal epithelial cells and gut epithelial function, SCFAs are suppressed with antibiotic perturbation, are not confined to the intestinal tract, and can disseminate systemically and be detected in the blood (30).

Carbohydrate fermentation in the colon resulting in production of SCFAs provides an intriguing pathway for studying chronic diseases such as obesity, diabetes, and other metabolic

disorders. Energy-harvesting studies by Puertollano et al. (31) showed that the normal gut microbiome converts indigestible plant polysaccharides by scavenging hydrogen during fermentation and methane production into SCFAs (acetate, propionate, and butyrate). In addition to being energy sources, SCFAs control colonic gene expression and metabolic regulation by G-protein-coupled receptors (32). Further studies are needed that examine whether different SCFA signaling receptors [e.g., through G-protein-coupled receptor (GPR43)] are similarly involved in host energy balance and whether selected microbial communities interact differently with these molecules.

## Prebiotics/Probiotics and Energy Metabolism

The gut microbiota is considered a modifiable target for preventing gut metabolic diseases. Microbial products directly affect intestinal function, liver, brain, and adipose tissue, which consequently may affect diet-induced obesity and other metabolic conditions.

Nondigestible food ingredients, including plant cell wall polysaccharides (cellulose, xylan, and pectin) that stimulate specific microbes to improve metabolic regulation, are increasingly being introduced into the Western diet, many of which are claimed to have prebiotic properties (33). A prebiotic is a “nonviable food component that confers a health benefit on the host associated with modulation of the microbiota” (34). Prebiotics such as galacto-oligosaccharides, together with inulins and their fructo-oligosaccharide derivatives, were shown to modify the species composition of the colonic microbiota (35). Changes in carbohydrate intake of a prebiotic inulin, for example, were shown to increase concentrations of *Faecalibacterium prausnitzii* and *Bifidobacterium* spp. in humans and correlated with reduced adiposity (36).

Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a benefit on the host” (37). Probiotics have been available as foods and dietary supplements for decades. *Lactobacillus* and *Bifidobacterium* strains used in foods were initially marketed in yogurts and kefir products, and their use has skyrocketed in consumer products in recent years. Potentially, probiotic-grade strains may affect metabolism of dietary components in the small intestine (partial hydrolysis of lactose in fermented foods; lipid and oxalate metabolism) and metabolism of indigestible carbohydrates, p-cresol excretion, and colonic protein and ammonia metabolism in the large intestine. The benefits of probiotic consumption are still inconclusive, although the role of probiotics in maintaining health is broadly attributed to the combined effect on competitive exclusion of pathogens, epithelial barrier integrity, and immune regulation (38).

Firm conclusions regarding probiotic metabolic effects on modifications of dietary protein or xenobiotic metabolism, gut mucosa, and liver metabolic activities remain priorities for future research. Immunosenescence and effects of aging on the microbiota are less studied areas of research, with reduction in bifidobacterial counts presumed to be mediated by diet changes, including institution or community living. Perez et al. (39) found that probiotic manipulation of the gut microbiota may potentially improve adaptive immune responses and reduce inflammatory secretions, thus compensating for age-related effects. Systematic reviews, meta-analyses, and conference proceedings on the regulatory science of prebiotic/probiotic foods and health supplements are available in the literature and beyond the scope of this article (40–43).

In principle, point-source interventions, including prebiotics/probiotics, are not likely to provide sufficient leverage to shift the entire microbiota system. The use of prebiotic/probiotic systems biology models are desirable targeted approaches, however, for understanding bacterial homeostasis. It may be especially worthwhile to establish a food safety index system of probiotic-grade LAB organisms for assessing obesogenic and related diet-induced metabolic risk conditions based on functional interaction models in simulated in vitro and in vivo biologic systems (44, 45). As the molecular basis of human-microbe interactions is unraveled, selection criteria for probiotics for different clinical indications are gaining increasing attention on the basis of their metabolic, anti-inflammatory, immunomodulatory, and antimicrobial effects (42, 46). Strain-specific analyses remain the standard for current physician recommendations for probiotic use. However, conclusive evidence is lacking to definitively guide the clinical use of prebiotic and probiotic interventions.

Current consumption patterns obligate the need for substantiating the safety and functional value of prebiotic and probiotic products sold as food and dietary supplements, given the increasing number of institutions recommending routine use. Some U.S. policy makers argue that a probiotic regulatory framework should be established where consideration is given for a strain to be regulated simultaneously at different risk levels for different applications (45). In the meantime, practitioner guidelines for consumer health are still unclear. Hence, practitioners must weigh the available evidence, and manufacturers share the responsibility of providing guidance about the type and extent of safety assessments that have been conducted on its products (46).

## Maternal Factors and Developing the Gut Microbiome

Microbial ecology niche colonization profoundly affects immune maturation and disease susceptibility during infancy (47). Table 2 lists key mechanisms by which initial microbiota colonization patterns in neonates are beneficially influenced by maternal factors (48, 49).

As stated earlier, dietary glycans from human breast milk are vitally important in shaping the structure and functional performance of the neonatal intestinal microbiota. Humans lack the various glycolytic enzymes that degrade complex oligosaccharide bonds, whereas selected commensal microbes possess the glycosylhydrolases and transport proteins to break down the glycans humans cannot digest (10, 50). The human milk-oriented microbiota plays a critical role in gut barrier protection during infant growth and development, a period when selective substrates of oligosaccharides allow bifidobacteria to colonize

**TABLE 2** Maternal contributions to the development of a healthy infant microbiome

- 
- Maternal diet/nutritional status
  - In utero exposure to maternal microbiota
  - Vaginal delivery
  - Gestational age
  - Breastfeeding
    - surface contact
    - prebiotic and probiotic potential of human milk
- 

the niche at high abundances (51). Interactions between dietary nutrients and low-molecular-weight molecules of indigenous microbiota during infancy may be key determinants that affect gene expression pathways in the host metagenome via epigenetic processes (i.e., individual genotype and environment) and biochemical mechanisms (52, 53). Understanding how the biochemical and immunologic features of human milk and infant microbiota coevolve during the neonatal period when a mother is healthy, obese, or malnourished is an intriguing question.

With metabolomic and other functional “-omics” technologies available, a full spectrum of microbe-relevant metabolites (e.g., secreted antimicrobials), metabolic signatures, and specific functional benefits of selected commensal probiotic-grade organisms to infant health will be uncovered. Bacteriocins are an abundant and diverse class of ribosomal antimicrobial peptides derived from the gut microbiota (54). These compounds are produced by all known lineages of intestinal bacteria and archaea, which suggests they play an important coevolutionary role in host resilience and niche adaptation. Bacteriocins are among a group of antimicrobial compounds that also may enhance the ability of commensal strains, particularly *Bifidobacterium* and *Lactobacillus* spp. to compete against potentially pathogenic microbes. Although their exact ecological function in niche communities is unknown, metagenomic analyses suggest that bacteriocins may contribute to commensal and probiotic strain selectivity as 1) colonizing peptides facilitating competitive exclusion of pathogens, 2) killing peptides directly eliminating pathogens, or 3) signaling peptides for other bacteria or the immune system (55).

The indigenous microbiota is also essential for the early development and homeostasis of the host immune system. Maslowski et al. (56) found that nutrient- and microbe-derived SCFAs may stimulate potent immunomodulatory effects by acting on G-coupled receptors. *Bacteroides fragilis* was recently used to describe how key microbial players develop into a network and induce a beneficial immune response (e.g., via capsular polysaccharide A) (57). Moreover, the intestinal microbiota plays a vital role in developmental gut barrier structure and function, signaling the innate immune system through pattern recognition receptors [e.g., Toll-like receptors (TLRs)] that bind to specific microbial macromolecules [i.e., LPS, peptidoglycans] and that ultimately lead to release of protective peptides, cytokines, and phagocytes (58).

## Metabolic Footprints of Gut-Microbial Cometalabolism in Global Health

Dietary differences in vegetable and animal protein intake modulate the gut microbiota via metabolic activities that include processing of glycans and isoprenoids, production of vitamins (vitamins A and K and biotin), amino acid synthesis, SCFA effects on cholesterol and glucose metabolism, and bile acid biotransformation (59). In extreme under- and overnutrition states, microbial metabolic activities are of long-term health significance. Nutrition systems biology coupled with “-omics”-based technologies holds an essential key to further probing host-gut microbiome interactions that can inform global public health and the practice of 21st century health care (60–62).

The Biomarkers of Nutrition for Development (BOND) program sponsored by the NIH Eunice Kennedy Shriver National Institute of Child Health and Development, in collaboration with global partnerships representing the food and nutrition enterprise, is designed to support development of

methodologies and biomarkers needed to improve nutrient measurement systems (63). Additional global efforts also identified the need for robust biomarkers to assess the vicious cycles of undernutrition, infectious disease (diarrheal diseases), and physical and neurological development as a global priority (64). Inflammation- and diet-related chronic diseases give rise to microbial perturbation and are emerging as a major target of biomarker surveillance efforts (65). Large-scale sampling methods for population health research to integrate gene-environment predisposition with diet-gut microbiome interactions are a way to generate more informed public health planning (66) and will provide the basis for studies to evaluate the safety and effectiveness of alternative diet-based interventions (67).

The gut microbiota contributes to the risk and pathogenesis of global rates of undernutrition through nutrient metabolism, infection, and disturbances in immune function (68). Complicating this set of conditions, environmental factors cannot be separated in the real world because a number of chemical agents contaminate food chains. Consequently, for much of the world, inadequate dietary and microbial methylation patterns can alter one-carbon metabolism, resulting in hypomethylation and elevated plasma homocysteine concentrations, with increased risk of hepatotoxicity effects. Microecological imbalances caused by impairment of important epigenomic pathways can also lead to the onset of chronic metabolic disturbances (33, 69). Kwashiorkor, a severe acute malnutrition, is exacerbated by environmental insults. Smith et al. (70) observed that the combination of the Malawian diet and kwashiorkor microbiome produced marked weight loss in recipient mice and perturbations in amino acid and carbohydrate metabolism.

## Metabolic Consequences from Modern Diets and Obesity

Obesity is essentially the imbalance between energy intake and energy expenditure, and it is now estimated that ~65% of Americans are overweight (71). The obesogenic state has an increased capacity to harvest energy from the diet and alters gut microbial ecology and metabolic disturbances from fat deposits and gut immune system inflammatory responses, which have strong influences on energy sensing and energy balance in humans. Turnbaugh et al. (72) showed that colonization of germ-free mice with an obese microbiota results in a significantly greater increase in total body fat than does colonization with a lean microbiota.

Ley et al. (73) earlier found that host diet and phylogeny both influence bacterial diversity. Fat in large deposits, for example, evolved in birds and mammals, giving a species-selective advantage in managing wide fluctuations in energy supply and expenditure. When energy consumption overrides energy expenditure, the only tissue capable of expanding to store large amounts of excess nutrients is adipose tissue. This adipocentric view of obesity derives from the hypothesis that chronic health disorders of obesity are secondary to adipose tissue capacity to store leptin (74). Another view describes the innate immune system as a prewired set of cellular and humoral components and that obesity in childhood is associated with the activation of the innate immune system. Increases in inflammatory biomarkers such as C-reactive protein and neutrophilia are seen in obese children as young as 3 y of age, which supports the notion that obesity-induced inflammation may be initiated early in development (75).

Inflammatory effects of obesity are generated by an intricate cascading of immune signaling events that increase the proliferation of macrophages in adipose tissue. There remain many questions in unraveling the precise function of adipose tissue macrophages in different fat depots and pathologic contexts (76). The inflammatory response in obesity is also generated in response to nutrient excess and metabolic dysregulation (77). Collectively, these compounds include proteins, carbohydrates, lipoproteins, nucleic acid species, and pathogen- and microbe-associated molecular patterns. Mammals have also coevolved a set of pattern recognition receptors designed to sense and trigger a response to various antigens they encounter and that play a key role in immune homeostasis. A critical set of the membrane-bound pattern recognition receptors are the set of 10 TLRs in humans (78). TLR2 is essential for epithelial barrier function and in regulating inflammation. How these immunologic receptors form critical links between metabolism and inflammation is unclear.

In summary, the cascade effect evidenced in macrophages recruited to fat depots releases additional proinflammatory molecules. Although this biologic programming works to combat pathogens, it cumulatively causes damage to normal tissue. Hence, by understanding how the gut microbiome may influence calorie balance through the regulation of calorie dissipation (heat generation), the side effects of fat-provoked inflammation, including weight gain, may be better controlled. Along another fascinating line of inquiry, Blum et al. (79) recently reported that the *Drosophila* system may represent an alternative mutualism strategy termed “quotidian replenishment,” defined as the need to obtain daily replenishment from the ecosystem environment to obtain a consistent microbial community in a biologic system, as earlier proposed by Storelli et al. (80). Further study may reveal general principles of why frequent dietary ingestion from an external reservoir might be of mutual host-microbial benefit, promoted by symbiotic microbial farming, and underscores the rationale for probiotics that target host-microbial cometabolism and their consumption in a safe and precise manner.

## Conclusions

Much has been learned about the factors influencing the ontogeny, maintenance, and function of the human gut microbiome. Much remains to be done. Table 3 contains a brief, not exhaustive, list of research priorities to move this important public health agenda forward.

Large database efforts such as the MetaHIT and the NIH HMP continue to play major roles in the International Human Microbiome Consortium and have contributed vastly to our understanding of the complex ecosystem of the gut microbiome that starts at birth, assembling within the human host, and of its role in perturbations and disease across the human life span. Despite the gaps and challenges, the human microbiome holds great potential for accelerating the translational pipeline, equipped with “-omics” precision tools that now allow us to sculpt microbiome interventions with diet, prebiotics, probiotics, and targeted antibiotics to prevent and treat disease. Although many questions remain unanswered, what we do know about key functions of the gut microbiota is primarily related to digestion, with energy production playing a primary role in the digestion of complex polysaccharides and fibers that are otherwise indigestible for humans. A key end product of gut digestion and fermentation is production of SCFAs, which cells need for energy. An additional role is microbial synthesis of chemicals and nutrients, including production of anti-inflammatory

**TABLE 3** Nutritional system and microbiome research priorities

- Accelerate translational pipeline with *in vitro* artificial gut systems, germ-free and gnotobiotic animal models, and humanized model systems
  - identify biomarkers, metabolites, and functional host-microbial cometabolism
- Conduct clinical safety efficacy studies to characterize the impact of diet alteration and probiotic/prebiotic intake, and provide a proof-of-principle needed to direct future human nutritional and clinical interventions
- Construct systems framework for existing and new large cohorts (e.g., the National Children's Study) to study host-microbiome interactions
- Develop integrated models for studying the long-term impact of undernutrition (or overnutrition) in altering host-microbiome interactions and immune regulation, particularly in infants, children, and the elderly
- Develop Nutritional Systems Biology framework for scaling-up "-omics"-based technologies and comprehensive assessments, including the following:
  - metagenomes, epigenomes, microbiomes, metabolome
  - fermenting food and diet compositional measurement
  - prebiotic and probiotic functional biomarker assessments and targeted interventions

compounds, particularly bacteriocins, antibiotic products, and vitamins.

The rapidly expanding product line of probiotics in the marketplace has not been conclusively proven as safe or effective for any specific health claims here in the United States. Targeted models of how single (and combination) bacterial organisms affect homeostasis and can influence human tissue (e.g., bile stress response) will serve as building blocks of the evolving nutrigenomic landscape. Germ-free mice and especially pig models colonized by humanized microbiota could help in identifying biomarkers to characterize the impact of diet and probiotic/prebiotic intake and provide proof-of-principle, host-microbial cometabolism models needed to assess restorative ecology and functional impacts on health.

Programs such as BOND and the Biomarker Initiative sponsored by the Foundation for the NIH are moving to provide food technology and medical communities with robust biomarkers as valid and reliable measurements. One of the ultimate goals of the NIH/Eunice Kennedy Shriver National Institute of Child Health and Human Development National Children's Study and BOND initiatives is profiling metabolites in comprehensive time-series studies to define their relation with diet in healthy individuals at various stages of life (e.g., in women before, during, and after pregnancy and in their children during the first 5 y after birth) (81). Moreover, the realization of the long-term vision of the international consortium and existing microbiome framework of the NIH HMP, MetaHIT, and expanding global networks depends on the long-range commitment of stakeholders in academia, government, philanthropy, and industry to bring the resources available via technology-driven platforms into fuller partnership with better planning strategies for "-omics" science. This requires a broader systems framework including both nutrigenomic and pharmacobiotic data-exchange networks that can handle the increasing complexity of data sets with transdisciplinary insights that can effectively inform public policy.

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### References

1. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010;464:59–65.
2. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, Amann M, Anderson HR, Andrews KG, Aryee M. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012;380:2224–60.
3. Shrimpton R, Rokx C. The double burden of malnutrition: a review of global evidence. Vol. 1. Health, Nutrition and Population (HNP) discussion paper. Washington: The World Bank; 2012.
4. Pendyala S, Walker JM, Holt PR. A high-fat diet is associated with endotoxemia that originates from the gut. *Gastroenterology* 2012;142:1100–1.
5. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh S, Hamady M, Chen Y, Knight R, Ahima R, Buman F, Wu G. High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* 2009;137:1716–24.
6. Borenstein E. Computational systems biology and *in silico* modeling of the human microbiome. *Brief Bioinform* 2012;13:769–80.
7. Dimitrov DV. The human gutome: nutrigenomics of the host-microbiome interactions. *OMICS* 2011;15:419–30.
8. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012;489:242–9.
9. Zaneveld J, Turnbaugh P, Lozupone C, Ley R, Hamady M, Gordon J, Knight R. Host-bacterial coevolution and the search for new drug targets. *Curr Opin Chem Biol* 2008;12:109–14.
10. German JB, Freeman SL, Lebrilla CB, Mills DA. Human milk oligosaccharides: evolution, structures and bioselectivity as substrates for intestinal bacteria. *Nestle Nutr Workshop Ser Pediatr Program* 2008;62:205–18.
11. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 2001;409:860–921.
12. The Human Microbiome Project Consortium. A framework for human microbiome research. *Nature* 2012;486:215–21.
13. The MetaHIT Consortium. MetaHIT: the European Union Project on Metagenomics of the Human Intestinal Tract. In: Nelson K, editor. *Metagenomics of the human body*. Paris: Springer Science+Business Media; 2011.
14. Bolotin A, Wincker P, Mauger S, Jaillon O, Malarme K, Weissenbach J. The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp *lactis* IL1403. *Genome Res* 2001;11:731–53.
15. Klaenhammer TR, Azcarate-Peril MS, Altermann E, Barrangou R. Influence of the dairy environment on gene expression and substrate utilization in lactic acid bacteria. *J Nutr* 2007;137 Suppl:748S–50S.
16. Kolker E. A vision for 21st century U.S. policy to support sustainable advancement of scientific discovery and technological innovation. *OMICS* 2010;14:333–5.
17. Lozupone CA, Stombaugh J, Gordon J, Jansson J, Knight R. Diversity, stability and resilience of the human microbiota. *Nature* 2012;489:220–30.
18. Kant AK. Consumption of energy-dense, nutrient poor foods by adult Americans: nutritional and health implications. The Third National Health and Nutrition Examination Survey, 1988–1994. *Am J Clin Nutr* 2000;72:929–36.
19. Eaton SB. The ancestral diet: what was it and should it be a paradigm for contemporary nutrition? *Proc Nutr Soc* 2006;65:1–6.
20. Frost G, Walton G, Swann J, Psichas A, Costabile A, Johnson L, Sponheimer M, Gibson G, Barraclough T. Impacts of plant-based foods in ancestral hominin diets on the metabolism and function of gut microbiota *in vitro*. *mBio*.ASM.org 2014;5:e00853–14. [cited 2014 Apr 5]. Available from: <http://mbio.asm.org/content/5/3/e00853-14>.
21. Devkota S, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, Antonopoulos DA, Jabri B, Chang EB. Dietary fat-induced taurocholic acid promotes pathobiont expansion and colitis in IL10<sup>-/-</sup> mice. *Nature* 2012;487:104–8.
22. Backhed F. Programming of host metabolism by the gut microbiota. *Ann Nutr Metab* 2011;58:44–52.

23. Kinross J, Muirhead LJ, Nicholson J. Nutritional modulation of the metabolome: applications of metabolic phenotyping in translational nutritional research. *Curr Opin Gastroenterol* 2014;30:196–207.
24. Grundmann O. The gut microbiome and pre-systemic metabolism: current state and evolving research. *J Drug Metab Toxicol* 2010;1:1–7.
25. Nicholson JK, Holmes E, Elliott P. The metabolome-wide association study: a new look at human disease risk factors. *J Proteome Res* 2008;7:3637–8.
26. Ley RE, Backed F, Turnbaugh P, Lozupone C, Knight R, Gordon G. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* 2005;102:11070–5.
27. Cani PD, Amar J, Iglesias M, Poggi M, Knauf C, Bastelica D, Neyrinck A, Fava F, Tuohy K, Chabo C. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007;56:1761–72.
28. Holmes E, Jia V, Marchesi J, Nicholson J. Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. *Cell Metab* 2012;16:559–64.
29. Bazzocco S, Mattila I, Guyot S, Renard CM, Aura AM. Factors affecting the conversion of apple polyphenols to phenolic acids and fruit matrix to short-chain fatty acids by human faecal microbiota in vitro. *Eur J Nutr* 2008;47:442–52.
30. Shank EA, Kolter R. New developments in microbial interspecies signaling. *Curr Opin Microbiol* 2009;12:205–14.
31. Puertollano E, Kolida S, Yagoob P. Biological significance of short-chain fatty acid metabolism by the intestinal microbiome. *Curr Opin Clin Nutr Metab Care* 2014;17:139–44.
32. Samuel BS, Shaito A, Motoike T, Rey FE, Backed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain-fatty-acid binding G protein-coupled receptor, GPR41. *Proc Natl Acad Sci USA* 2008;105:16767–72.
33. Murphy EF, Cotter PD, Hogan A, O’Sullivan O, Joyce A, Fouhy F, Clarke S, Marques T, O’Toole P, Stanton C. Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity. *Gut* 2013;62:220–6.
34. Food Quality and Standards Service (AGNS). Technical report on prebiotics. FAO; 2007 15–16 September; Rome, Italy. [cited 2014 Apr 5]. Available from: [http://www.fao.org/ag/agn/index\\_en.stm](http://www.fao.org/ag/agn/index_en.stm).
35. Macfarlane GT, Macfarlane S. Fermentation in the human large intestine: its physiological consequences and the potential contribution of prebiotics. *J Clin Gastroenterol* 2011;45:S120–7.
36. Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Grietje H, Petra L. Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *Br J Nutr* 2009;101:541–50.
37. World Health Organization. Health and nutritional properties of probiotics in food including powdered milk with live lactic acid bacteria: a joint FAO/WHO Expert Consultation. Geneva: WHO; 2001.
38. McFarland LV. Deciphering meta-analytic results: a mini-review of probiotics for the prevention of pediatric antibiotic-associated diarrhea and *Clostridium difficile* infection. *Benef Microbes* 2014; June 2:1–6 (Epub ahead of print).
39. Perez Martinez G, Bauerl C, Collado MC. Understanding gut microbiota in elderly’s health will enable intervention through probiotics. *Benef Microbes* 2014;5:235–46.
40. Rabot S, Rafter J, Rijkers GT, Watzl B, Antoine JM. Guidance for substantiating the evidence for beneficial effects of probiotics: impact of probiotics on digestive system metabolism. *J Nutr* 2010;140 Suppl:677S–89S.
41. Duffy LC, Duong T, Klein M, Sanders ME, Young H. Meeting report: probiotic foods and supplements: the science and regulations of labelling. *NYAS EBriefings* 2010;1:39.
42. Hempel S, Newberry SJ, Maher AR, Wang Z, Miles JN, Shanman R. Probiotics for prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *JAMA* 2012;307:1959–69.
43. Goldenberg JZ, Ma SSY, Saxton JD, Martzen MR, Vandvik PO, Thorlund K, Guyatt GH, Johnston BC. Probiotics for the prevention of *Clostridium-difficile* associated diarrhea in adults. *Cochrane Database Syst Rev* 2013;5:CD00609
44. Duffy LC, Sporn S, Hibberd P, Pontzer C, Solano-Aguilar G, Lynch SV, McDade-NGutter C. In: Coates PL, Betz JM, Blackman MR, Cragg GM, Levine M, Moss J, White J, editors. *Probiotic biology: Lactobacillus and Bifidobacterium spp.* Encyclopedia of dietary supplements. 2nd ed. London: Informa Business, CRC Press; 2010. p. 469–78.
45. Hoffmann D, Fraswer CM, Palumbo F, Ravel J, Rowthorn V, Schwartz J. Probiotics: achieving a better regulatory fit. *Food Drug Law J* 2014; 69:236–72.
46. Venugopalan V, Shriner K, Wong-Beringer A. Regulatory oversight and safety of probiotic use. *Emerg Infect Dis* 2010;16:1661–5.
47. Brandtzaeg P. Importance of early microbial colonization for intestinal immune development. In: Guarino A, Quigley EMM, Walker WA, editors. *World review of nutrition and dietetics*. Vol. 107. Basel (Switzerland): Karger Publishers; 2013. p. 43–55.
48. Thum C, Cookson AL, Otter DE, McNabb WC, Hodgkinson AJ, Dyer J, Roy NC. Can nutritional modulation of maternal intestinal microbiota influence the development of the infant gastrointestinal tract? *J Nutr* 2012;142:1921–8.
49. Sanz Y. Gut microbiota and probiotics in maternal and infant health. *Am J Clin Nutr* 2011;94 Suppl:2000S–5S.
50. Ruhaak LR, Lebrilla CB. Advances in analysis of human milk oligosaccharides. *Adv Nutr* 2012;3 Suppl:406S–14S.
51. Newburg DS. Neonatal protection by an innate immune system of human milk consisting of oligosaccharides and glycans. *J Anim Sci* 2009;87:26–34.
52. Khodayar-Pardo P, Mira-Pascual L, Collado MC, Martinez-Costa C. Impact of lactation stage, gestational age and mode of delivery on breast milk microbiota. *J Perinatol* 2014;38:599–605.
53. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol* 2007;5:e177.
54. Gálvez A, Abriouel H, Lopez RL, Ben Omar N. Bacteriocin-based strategies for food biopreservation. *Int J Food Microbiol* 2007;120:51–70.
55. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science* 2010;330:1768–73.
56. Maslowski KM, Vieira AT, Ng A. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 2009;461:1282–6.
57. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 2008;453:620–5.
58. Hill DA, Artis D. Intestinal bacteria and the regulation of immune cell homeostasis. *Annu Rev Immunol* 2010;28:623–67.
59. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. *Gut Microbes* 2012;3:289–306.
60. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS. Metagenomic analysis of the human distal gut microbiome. *Science* 2006;312:1355–9.
61. Sauer U, Heinemann M, Zamboni N. Genetics: getting closer closer to the whole picture. *Science* 2007;316:550–1.
62. James WP, Garza C. Summary of the 24<sup>th</sup> Marabou Symposium: Nutrition and the human microbiome. *Nutr Rev* 2012;70(Suppl 1):S87–S94.
63. Raiten DJ, Namaste S, Brabin B, Combs G Jr., L’Abbe MR, Wasantwisut E, Darnton-Hill I. Executive summary—Biomarkers of Nutrition for Development: building a consensus. *Am J Clin Nutr* 2011;94 Suppl:S633–50.
64. Ohlhorst SD, Russell R, Bier D, Klurfeld DM, Li Z, Mein JR, Milner J, Ross AC, Stover P, Konopka E. Nutrition research to affect food and a healthy lifespan. *Adv Nutr* 2013;4:579–84.
65. Raiten DJ, Sakr Ashour FA, Ross AC, Meydani SN, Dawson HD, Stephensen CB, Brabin BJ, Suchdev PS, van Ommen B, and the INSPIRE Consultative Group. Inflammation and nutritional science for programs/policies and interpretation of research evidence (INSPIRE). *J Nutr* 2015;145:1039S–108S.
66. Pang T. Germs, genomics and global public health: how can advances in genomic sciences be integrated into public health in the developing world to deal with infectious diseases? *Hugo J* 2009;3:5–9.
67. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. *Nature* 2011; 474:327–36.
68. Guerrant RL, Oria RB, Moore SR, Oria MO, Lima AA. Malnutrition as an enteric infectious disease with long-term effects on child development. *Nutr Rev* 2008;66:487–505.

69. Lee DH, Jacobs DR, Porta M. Hypothesis: a unifying mechanism for nutrition and chemicals as lifelong modulators of DNA hypomethylation. *Environ Health Perspect* 2009;117:1799–802.
70. Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, Kau AL, Rich SS, Concannon P, Mychaleckyj JC, et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. *Science* 2013;339:548–54.
71. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006;444:1022–3.
72. Turnbaugh PJ, Ley R, Mahowald M, Magrini V, Mardis E, Gordon J. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006;444:1027–31.
73. Ley RE, Hamady M, Lozupone C, Turnbaugh P, Ramey RR, Bircher JS, Schlegel M, Tucker T, Schrenzel M, Knight R, et al. Evolution of mammals and their gut microbes. *Science* 2008;320:1647–51.
74. Spreadbury I. Comparison with ancestral diets suggests dense acellular carbohydrates promote an inflammatory microbiota, and may be the primary dietary cause of leptin resistance and obesity. *Diabetes Metab Syndr Obes* 2012;5:175–89.
75. Lumeng CN. Innate immune activation in obesity. *Mol Aspects Med* 2013;34:12–29.
76. Amano SU, Cohen JL, Vangala P, Tencerova M, Nicoloso SM, Yawe JC, Shen Y, Czech MP, Aouadi M. Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. *Cell Metab* 2014;19:162–71.
77. Faloiu E, Grazia M, De Robertis M, Luconi M, Furlani G, Boscaro M. Inflammation as a link between obesity and metabolic syndrome. *J Nutr Metab* 2012;476380:1–7.
78. Janssens S, Beyaert R. Role of Toll-like receptors in pattern recognition. *Clin Microbiol Rev* 2003;16:637–46.
79. Blum JE, Fischer CN, Miles J, Handelsman J. Frequent replenishment sustains the beneficial microbiome of *Drosophila melanogaster*. *mBio*. ASM.org 2014;4:e00860–13. [cited 2013 Nov 5]. Available from: <http://mbio.asm.org/content/4/6/e00860-13.full.html>.
80. Storelli G, Defaye A, Erkosar B, Hols P, Royet J, Leulier F. *Lactobacillus plantarum* promotes *Drosophila* systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. *Cell Metab* 2011;14:403–14.
81. Raiten DJ, Raghavan R, Porter A, Obbagy JE, Spahn JM. Executive summary: evaluating the evidence base to support the inclusion of infants and children from birth to 24 mo of age in the Dietary Guidelines for Americans—the B-24 project. *Am J Clin Nutr* 2014;99 Suppl:663S–91S.