Contribution of the Intestinal Microbiota to Human Health and Disease

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Prof. Ferdinand Haschke, MD, PhD
Chairman
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The idea that a large part of our lives can be controlled by our intestinal bacteria is quite frightening! To realize that such things as our mother’s weight, the way we are born, and what we are fed can determine the degree of bacterial diversity that we will have early in life, which in turn will affect our immune system, metabolism, etc., adds more reasons to pay particular attention to what happens in the first 1,000 days of existence. This subject has developed its own terminology, which at times makes it difficult to follow. However, the topic is so fascinating and the ramifications of such relevance that I encourage you to make an effort and read and re-read it until the concepts are understood. In multi-authored publications, there is always the risk of repetition. And I acknowledge that there is some repetition among the four excellent contributions that make this issue of the supplement of Annals of Nutrition and Metabolism.

Dr. W. Allan Walker, Conrad Taff Professor of Nutrition at Harvard Medical School, Boston, Mass., USA, discusses the establishment of the intestinal microbiota. Dr. Walker explains how the newborn, full-term, vaginally-delivered infant initially colonizes its GIT and how, with full colonization, a symbiotic relationship develops between colonizing bacteria and the underlying epithelial and lymphoid tissues. This relationship results in both nonspecific and immunologic (innate and adaptive immune responses) defenses which collectively comprise the intestinal mucosal barrier to pathogens and noxious antigens. An important component of mature intestinal immune homeostasis is the development of oral tolerance.
to benign commensal bacteria and noxious antigens. Disruption of these events results in inadequate colonization which leads to dysbiosis, an undesirable alteration of the microbiota resulting in an imbalance between protective and harmful bacteria, and increased expression of immune-mediated and allergic disease states.

Dysbiosis has been implicated in many human disease conditions including local gastrointestinal and systemic diseases. The second paper by Dr. Deanna Gibson and colleagues from British Columbia, Canada, discusses the fact that dietary patterns alter the intestinal microbiota ecologically and functionally, which results in physiological consequences to the host. Changes to the community structure of the intestinal microbiota are not without consequence, considering the wide effects that the microbes have on both local and systemic immunity. A complex tripartite relationship between diet, microbes and the gut epithelium is the basis for health or certain diseases. This is followed by a summary of clinical evidence of diet-induced dysbiosis as a contributing factor in the development of gastrointestinal diseases like inflammatory bowel disease, irritable bowel syndrome and colorectal cancer, as well as systemic diseases like obesity, diabetes, atherosclerosis and nonalcoholic fatty liver disease. Finally, the current dietary and microbial interventions to promote a healthy microbial profile are reviewed. This article presents a table where papers describing clinical effects of different probiotics are listed.

The paper by Dr. Erika Isolauri’s group in Turku, Finland, addresses the issue of how reshaping the gut microbiota at an early age may have a functional impact on obesity risk. Recent scientific advances point to an aberrant compositional development of the gut microbiota and low-grade inflammation as contributing factors, in conjunction with excessive energy intake. A high-fat/energy diet alters the gut microbiota composition, which reciprocally engenders excessive energy harvesting and storage. Further, microbial imbalance increases gut permeability, leading to metabolic endotoxemia, inflammation and insulin resistance. Local intestinal immunologic homeostasis is achieved by tolerogenic immune responses to microbial antigens. In the context of amelioration of insulin sensitivity and decreased adiposity, the potential of gut microbiota modulation with specific probiotics and prebiotics lies in the normalization of aberrant microbiota, improved gut barrier function and the creation of an anti-inflammatory milieu.

In 2001, the Food and Agriculture Organization and the World Health Organization adopted a definition of the term ‘probiotic’ as ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’. In the last contribution to this issue, Dr. James Versalovic of Houston, Tex., USA, addresses the fact that the relative abundance of probiotic genera and species in the healthy human microbiome is a relevant consideration and discusses whether microbial deficiencies in individual species could be readily corrected by administration of probiotics to children. Alternatively, do probiotics simply enhance the ability of other bacterial genera to proliferate and reduce the numbers of potentially harmful bacteria? Research related to the mechanisms of probiosis during the 1990s and the rapid coalescence of the human microbiome research community globally since 2005 have provided the basis for the current era in metagenomics (the genomic analysis of microorganisms by direct extraction and cloning of DNA from an assemblage of microorganisms). We intentionally chose a more basic science approach to this topic dealing with what we know today about the mechanisms by which probiotics act, rather than offering a more practical review of which probiotic has proven to be good for what.

At a recent meeting at the National Institute of Health, the microbiome research over the last 7 years was reviewed in a 3-day symposium. Individual presentations can be seen at their website (http://www.genome.gov/27554404).

We hope that this issue will trigger your curiosity, modify the way that you think about bacteria, provide you with scientific evidence to try to convince pregnant women to have natural births whenever feasible, breastfeed at least as long as recommended, avoid arbitrary use of non-milk supplements under the age of at least 4 months, and avoid unnecessary antibiotics, in order to allow for a healthy development and preservation of your patients’ intestinal microbiota. Groom your intestinal microbiota from the start!

Carlos Lifschitz
A diverse balanced microbiota is necessary for the development of an appropriate innate and adaptive immune response

Initial Intestinal Colonization in the Human Infant and Immune Homeostasis
by W. Allan Walker

**Key insights**
Initial colonization of the infant gut is an important event in the development of mucosal immune homeostasis. To this end, the infant’s diet plays a major role in shaping the identity of the intestinal flora that will ultimately orchestrate innate and adaptive immune function.

**Current knowledge**
Bacterial colonization of the infant gut occurs in several distinct phases, starting with birth, followed by the introduction of oral feeding and weaning. Intestinal microorganisms establish a symbiotic relationship with the epithelial and lymphoid tissues of the host, evoking the development of the innate and adaptive immune response. Pattern recognition receptors, such as those of the toll-like receptor family, interact with bacterial molecules to trigger specific pro- or anti-inflammatory signaling cascades. The maturation of mucosal immunity is completed with the process of oral tolerance.

**Practical implications**
Normal colonization of the infant gut occurs when full-term infants are born by vaginal delivery and are exclusively breastfed for the first 6 months of life. Nutrition is a key environmental factor that influences the bacterial signature that will prevail throughout later life. The oligosaccharide content of human breast milk facilitates the growth of beneficial bacteria, providing the optimal environment to boost mucosal immunity. Factors such as caesarean delivery and excessive antibiotic usage can have a negative impact on early microbial colonization. The use of pre- and probiotics supports normal microbial flora, thereby restoring immune function in cases of aberrant microbial homeostasis as in necrotizing enterocolitis, infections and immune dysfunction.

**Recommended reading**
Initial Intestinal Colonization in the Human Infant and Immune Homeostasis

W. Allan Walker

Harvard Medical School, Mucosal Immunology and Biology Research Center, Massachusetts General Hospital for Children, Boston, Mass., USA

Key Messages
- Initial bacterial colonization is in part determined by the infant’s diet.
- A symbiotic bacteria-host relationship determines mucosal immune homeostasis.
- Abnormal colonization (dysbiosis) and its accompanying increase in disease expression can be prevented by pre- and probiotics.

Key Words
Intestinal colonization · Immune homeostasis · Breastfeeding · Probiotics

Abstract
The paradigm of disease burden in the developed world has changed drastically in the last few decades from predominantly infections to immune-mediated diseases (autoimmunity and allergy) because of alterations in the Western lifestyle (improved sanitation, immunizations, antibiotic usage and altered dietary intake). A diverse balanced microbiota is necessary for the development of an appropriate innate and adaptive immune response. There is strong evidence that disruption of the normal colonization process can lead to alterations in the important symbiotic relationship that is necessary for immune homeostasis. For example, infants born by cesarean section or receiving excessive perinatal antibiotics have inadequate initial colonization and aberrant mucosal immune function. As a result, later in childhood, they express an increased incidence in asthma and autoimmune diseases (e.g. celiac disease). An important component of initial colonization is the infant’s diet. Breast milk contains a variety of nondigestible oligosaccharides which function as prebiotics preferentially stimulating proliferation of Bifidobacteria and Lactobacilli, important health-promoting bacteria, and cause fermentation of the oligosaccharides into short-chain fatty acids. In the absence of breastfeeding for the first 6 months of life, formula containing pre- and probiotics may overcome an initial inadequate colonization process and help establish a normal mucosal immune system.

Introduction
In this review, I will consider how the newborn, full-term, vaginally-delivered infant initially colonizes its gastrointestinal tract [1]. With full colonization, a symbiotic relationship develops between colonizing bacteria and the underlying epithelial and lymphoid tissues [2]. This relationship results in both nonspecific and immunologic (innate and adaptive immune responses) defenses which collectively comprise the intestinal mucosal barrier to pathogens and noxious antigens [3]. An important component of mature intestinal immune homeostasis is the develop-
ment of oral tolerance to benign commensal bacteria and anoxious antigens [4]. This phenomenon can be achieved with complete colonization of the gut during the newborn period [5]. With complete colonization and development of the mucosal barrier, immune homeostasis occurs and there is no expression of disease. In contrast, circumstances exist in which inadequate colonization occurs (premature delivery, delivery by cesarean section and excessive use of perinatal antibiotics) [6]. Under these conditions, an inadequate colonization occurs leading to dysbiosis and increased expression of immune-mediated and allergic disease states [7]. This dysbiosis of the gut has become the basis for the ‘new’ hygiene hypothesis. Fortunately, clinical evidence suggests that pre- and probiotics can act as ‘surrogate’ colonizers and help prevent the expression of these diseases [8]. Each of these concepts will be discussed in detail in this review.

Normal Initial Bacterial Colonization

A cross-section of the small intestine in the human fetus in utero appears as an immature epithelial surface with prolonged cell turnover and a paucity of lymphoid elements [1]. In contrast, an identical section of the small intestine in the newborn infant in the extrauterine environment appears as an active structure with a rapid turnover, expressing the subtypes of epithelial cells and displaying a plethora of lymphoid elements (fig. 1) [2]. The principal difference in these two situations is that the intrauterine environment is germ free, whereas the extrauterine environment consists of abundant microbiota which colonize the gastrointestinal tract. This observation emphasizes the importance of initial intestinal colonization in the development of gastrointestinal functions. Thus, normal initial colonization of the gut is an important event in the adjustment of the newborn to the extrauterine environment [9]. Several factors influence initial intestinal colonization. These include the infant’s genetic signature, the nature of the delivery process, the use of excessive antibiotics during the perinatal period and whether the mother is under stress or expresses an inflammatory condition [10]. Normal colonization is most likely to occur when the infant is born full term by a vaginal delivery and is exclusively breastfed during the first 6 months of life (table 1). Colonization occurs in phases over 1 year to 18 months in the postpartum period. The full-term infant leaves the germ-free intrauterine environment and passes through the birth canal where it ingests a healthy bolus of maternal vaginal and colonic microbiota. This represents the first and most important phase of colonization [11]. With the introduction of oral feedings, the ingested bolus is further stimulated (phase 2). The nature of initial oral feeding, e.g. breast versus formula feeding, has profound short-term effects on the composition of colonizing bacteria [12]. The role of early nutrition in bacterial colonization will be discussed in greater detail later in this review. At the time of weaning to complementary foods, e.g. after 6 months, colonization is further effected (phase 3). Finally, by 1 year to 18 months of age, the infant’s intestine is completely colonized with a unique signature of microorganisms consist-
ing of more than 1,000 separate species and more cells by 10-fold than cells in the human body [13]. If antibiotic treatment is used during this period, the timing and nature of colonization is disrupted and prolonged [14].

A fully colonized intestine can function as an ancillary organ in the body. It consists of 1–2 kg of body weight in the human adult and has a 10-fold greater number of cells than the cells of the human body as well as a 100-fold greater number of genes than the human genome. Furthermore, the metabolic activity of colonizing bacteria is greater than the most active body organ, namely the liver [15]. Accordingly, investigators over the last decade have expanded exponentially our understanding of the functions that colonizing microbiota have in human body function, particularly intestinal and immune function [16].

**Symbiosis and Immune Function**

Once a normal colonization has been achieved with diverse individual bacterial species, these microorganisms establish a symbiotic relationship with the intestinal epithelial and lymphoid tissues. Conserved molecular patterns, either expressed on the surface of symbiotic bacteria or secreted into the gut, can interact with pattern recognition receptors (PRRs) expressed on or inside epithelial and lymphoid cells to initiate signal transduction and transcription of a host of molecules which mediate host defense or metabolic activities within the intestine [17]. The best-known family of PRRs is the toll-like receptor (TLR) family consisting of 9 identified receptors which interact with components of Gram-positive and Gram-negative bacteria to mediate both innate and adaptive immunities as well as other mucosal barrier cellular functions [18].

**Innate Immune Function**

Colonizing commensal and pathologic organisms can interact with TLRs on the intestinal epithelial cell to evoke an innate immune response. For example, lipopolysaccharides on the surface of Gram-negative organisms, particularly pathogens, stimulate TLR4 by binding to a lipopolysaccharide-binding protein and a surface molecule CD14 on the enterocyte surface [19]. An ancillary protein (MD2) helps to anchor the complex to TLR4 which then activates signaling molecules that allow for the transcription factor NFκB to disassociate from its binding protein IκB in the cytoplasm and traverse the nucleus to activate inflammation, through the transcription of cytokines and chemokines that in turn mediate inflammation. Inflammation prevents bacterial penetration across the epithelium and into the blood stream leading to sepsis [19]. This inflammatory innate reaction to pathogenic bacteria is spontaneous and self-limited in order to prevent chronic inflammation (fig. 2). With sustained interaction between the molecular pattern and its PRR, negative regulators of inflammation are activated to inhibit inflammatory signaling at various steps along the innate immune pathway [20].

**Adaptive Immunity**

In like manner, colonizing bacteria can activate adaptive immunity to create immune homeostasis within the intestine. For purposes of illustrating this phenomenon, three examples of adaptive immunity will be considered (table 2). Polymeric IgA (pIgA) produced by B cells within mesenteric lymph nodes is secreted onto the intestinal surface and acts as ‘aseptic paste’ to protect against invasion by pathologic organisms or noxious antigens (table 3). At birth, full-term, vaginally-born infants are pIgA deficient [21]. It takes a finite period postpartum (1 month) for protective levels of pIgA to appear. The matu-
ration of pIgA corresponds to the first and second phases of colonization (table 1). A classic publication [22] has shown the mechanism of this process. Colonizing bacteria within the lumen are taken up by dendritic cells that penetrate through appendages between enterocytes into the lumen or underlying microfold cells over Peyer’s patches. Engulfing dendritic cells then migrate to the mesenteric lymph node where they present the engulfed bacteria to B cells to activate them into pIgA-producing plasma cells. Secreted pIgA directed against engulfed microbiota are in turn transported to the intestinal surface where they coat the microvillus membrane to protect against invasion [21].

An important component of immune homeostasis is to have a balanced T helper (Th) cell response. Colonizing microbiota help to ensure that this happens. This occurs through ‘crosstalk’ between luminal bacteria and the TLRs on dendritic appendages penetrating into the intestinal lumen. Activation of dendritic cells results in their producing a cytokine environment which allows naive Th cells (Th0) to mature into Th1, Th2, Th17 and T-regulatory (Treg) cells (fig. 3) [23]. Th1 cells mediate cellular immunity, and Th2 cells mediate humoral immunity (e.g. antibody production) including the production of IgE antibodies. A new subclass of Th cells, Th17, mediates tissue inflammation and clearance of extracellular pathogens. Probably the most studied Th subclass over the last few years are Treg cells (TR1 and Th3) which mediate oral tolerance and anti-inflammation [24].

**Oral Tolerance**

Maturation of mucosal immune function leading to immune homeostasis is not complete until the process of oral tolerance occurs. Oral tolerance is a systemic reduction in cellular and humoral immunity to commensal bacteria and noxious antigens through exposure to the intestine via the perioral route. Figure 4 depicts our current understanding of oral tolerance. Antigens or non-pathogenic bacteria interacting with submucosal dendritic cells via TLRs in the presence of colonizing bacteria are stimulated to preferentially produce Treg cells and a specialized microenvironment that facilitates the development of Treg cells. These cells release TGF-β, an oral tolerogenic cytokine, which reduces the Th1, Th2 and Th17 response to antigens/bacteria [25]. It has previously been shown that oral tolerance cannot be achieved in germ-free animals [26] and these animals must be conventionalized to full colonization during the neonatal period for tolerance to be effective [27]. In our laboratory, we have shown that oral tolerance requires an intact TLR4 to be effective, and tolerance can be broken with extensive use of broad-spectrum antibiotics [26]. These observations suggest that normal initial intestinal colonization is needed to establish oral tolerance, and tolerance once achieved can be broken by excessive use of antibiotics.
Infant Nutrition and Initial Colonization

As stated in the introduction to this review, nutrition is an important environmental factor that influences the composition of colonizing bacteria. At no other time in life is nutrition as influential in determining colonization as it is during the newborn period when the infant is initially establishing its lifelong signature of microbiota. Striking short-term differences in colonizing bacteria occur if the newborn infant is exclusively breastfed compared to formula feeding [28]. Breastfed infants during the first month of life have an increase in health-promoting bacteria (Bifidobacterium infantis, Lactobacillus acidophilus, and Bacteroides fragilis) [29]. This was first shown using conventional culture techniques almost 30 years ago [28]. More recently, using metagenomic analysis of infant intestinal contents, it has been shown that breastfed versus formula-fed infants have differences in large families of bacteria (phyla) and more diversity in individual species [29]. Moreover, bacteria stimulated by breastfeeding activate more biologically and immuno-protective genes in the host than by formula feeding [30]. Although not as striking in its effect, diet after weaning and in early childhood over long periods of time can continue to affect bacterial phyla and individual species [31]. It is now suggested that the dietary influence (Western diet) on bacterial colonization may be an important factor in the paradigm shift in disease burden in developed countries from predominately infection to immune-mediated (autoimmune and allergy) diseases [32].

A principal component of influence on colonizing bacteria relates to the oligosaccharide content of breast milk [33]. Oligosaccharides make up 8% of the total nutrient content of human milk. They are not digested in the small intestine but enter the colon where they are fermented by colonic bacteria leading to an acid milieu and an increase in short-chain fatty acids (prebiotic effect). This results in a boost in health-promoting bacteria (e.g., probiotic bacteria) and an early stimulus to mucosal immune defense. In fact, a previous clinical study has shown a direct association with the pIgA levels in the intestine during the first months of life and the number of B. infantis organisms present [34] and an inverse relationship between levels of B. fragilis and the inflammatory cytokine IL-6, suggesting an anti-inflammatory effect [34]. In fact, recent studies measuring the stimulation of B. infantis genes (B. infantis has had its genome sequenced) when grown on human milk oligosaccharides (HMO) versus artificial prebiotics (inulin, fructooligosaccharide or galactooligosaccharide) have shown a striking difference in gene response [35]. Subsequent studies have suggested that HMO-grown B. infantis genes can actively stimulate increased expression of tight-junction proteins and also provide anti-inflammatory effects [36]. These studies collectively suggest that breast milk nutrition in infancy is critical to early colonization of the newborn gut and to the development of mucosal immune-protective function.

Abnormal Initial Bacterial Colonization

Table 3 depicts the conditions in which abnormal initial bacterial colonization occurs. Disruption of phase 1 can occur in premature delivery, delivery by cesarean section and with excessive use of perinatal antibiotics. In each of these circumstances, there is an inadequate initial
colonization. Despite the stimulus of oral feeding and weaning, final colonization can be delayed until 4–6 years of age during which time the infant is more susceptible to both infections and immune-mediated disease states. Inadequate colonization leads to a dysbiosis of intestinal microbiota and the intestine which in turn leads to immune dysfunction and an increased tendency for inflammatory disease [2]. In fact, many chronic intestinal conditions, e.g. necrotizing enterocolitis (NEC), allergy and inflammatory bowel disease, have been shown to be associated with an intestinal microbiota different from age-matched non-disease controls [37]. Furthermore, when germ-free mice are colonized with the microbiota from patients with allergic, obese or malnourished conditions, they develop a phenotypic expression of the actual disease [38], suggesting that a dysbiotic microbiota may contribute to symptoms of disease.

**Diseases Associated with Intestinal Dysbiosis**

Several clinical conditions have been associated with the increased expression of disrupted colonizing bacteria (table 4). For example, NEC represents a condition in premature infants in which the colonizing bacterial phyla are not equally represented compared to age-matched controls and diversity of individual species is lacking [39]. We have also shown that the fetal intestine, like the mature intestine, responds to both pathogens and commensal bacteria with an excessive inflammatory response and that this is due to a developmental expression of innate inflammatory immune response genes which favor an inflammatory response to all colonizing bacteria [40]. We have moreover reported that a fetal cell line, fetal organ cultures and intestinal fetal xenografts respond excessively to exogenous and endogenous inflammatory stimuli [41] due to an overexpression of TLRs, signaling molecules and transcription factors and an underexpression of negative regulators [42]. In like manner, increased episodes of antibiotic treatment, particularly a broad-spectrum antibiotic treatment in the first year of life, have been associated with an increased expression of asthma [43] during adolescence and inflammatory bowel disease [44] during childhood. Furthermore, women with a history of atopic disease deliver infants that are 8-fold more likely to express allergy if they are born by cesarean section rather than by vaginal delivery [45]. These clinical studies strongly suggest that an abnormal intestinal bacterial colonization leading to dysbiosis can increase the incidence of immune-mediated disease.

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**Prebiotics and probiotics or a combination, e.g. symbiotics, may convert a dysbiosis to a symbiosis by balancing potential pathogens with health-promoting bacteria.**

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**Pre- and Probiotics Are ‘Surrogate’ Colonizers**

Fortunately, there are possibilities of dealing with dysbiosis leading to clinical disease. Several clinical studies have been published which suggest that prebiotics and probiotics or a combination, e.g. symbiotics, may convert a dysbiosis to a symbiosis by balancing potential pathogens with health-promoting bacteria. Two circumstances illustrate this approach to rectifying a dysbiosis of intestinal microbiota. A seminal study from Finland [46] has shown that when *Lactobacillus rhamnosus* (LGG) is given to pregnant women with a family history of allergy during the latter stages of pregnancy, this results in infants with a 50% lower incidence of atopic dermatitis than control infants. Furthermore, this protective effect is still apparent at 7 years after birth [47]. However, when these studies were expanded to include multiple test sites using a single protocol, the results were not as clear-cut [48]. Yet, the probiotic used during pregnancy and lactation was helpful if the allergy-prone babies were born by cesarean section. Another example of probiotics stabilizing a dysbiosis occurs with their use in premature infants to prevent NEC [49]. Several studies have been done and when analyzed by a meta-analysis seemed to both prevent and lessen the severity of NEC [50]. A study performed in Taiwan initially used a combination of *L. acidophilus* and *B. infantis* in one nursery to significantly reduce the incidence and severity of the disease. This was followed by an expanded study in five nurseries with similar results [51]. Since the Food and Drug Administration (FDA) in the United States will not allow live organisms to be given to immune-compromised premature infants, we have tested in human fetal intestinal models the effect of secreted...
products of these two bacteria and then secretions from each grown separately. We have reported that secreted products of *B. infantis* have greater anti-inflammatory properties than those of *L. acidophilus*, and the anti-inflammatory function seems to be mediated through the stimulation of immature genes in the innate inflammatory immune response [52]. Further studies are planned to test the secreted factor with expressed breast milk from mothers delivering premature infants to determine if this combination of pre- (breast milk) and probiotic secretions may be protective.

Other examples of a prebiotic protective effect on dysbiosis suggest that when given after birth, prebiotics and probiotics appear to be protective against mild infections occurring during the first 6 months and allergy symptoms in allergy-prone infants during the first 2 years [53], as well as causing an enhancement of specific antibody levels with vaccines for polio [54] and *Salmonella* [55].

**Summary and Conclusions**

In this review, it was emphasized that initial colonization was in part dependent on the infant diet, particularly breastfeeding. Furthermore, it was shown that a symbiotic bacterial-host relationship determines immune homeostasis. An important component of immune homeostasis is the development of oral tolerance which can only occur with complete colonization of the intestine. Under conditions of abnormal colonization (dysbiosis), an increase in immune-mediated disease occurs. Fortunately, pre- and probiotics given to the infant can convert a dysbiosis to a symbiosis and potentially reduce the incidence of disease.

**Disclosure Statement**

The author declares that no financial or other conflict of interest exists in relation to the content of the article. The writing of this article was supported by Nestlé Nutrition Institute.


The gut microbiota, an integral part of the gut barrier, functions at the intersection between host genotype and diet to modulate the host physiology

Reshaping the Gut Microbiota at an Early Age: Functional Impact on Obesity Risk?
by R. Luoto et al.

Key insights
An aberrant homeostasis of the gut microbiota alongside low-grade inflammation are factors that contribute to overweight and obesity. High-fat and high-energy diets alter the composition of intestinal microbes, which in turn disrupts energy storage, immune response and gut function.

Current knowledge
The latest findings suggest that microbial contact may begin prior to birth, within the intrauterine environment. Following birth, breast milk is an excellent source of commensal bacteria. However, the composition of breast milk is highly dependent on the metabolic and immune status of the mother, with the milk of obese mothers containing a less diverse bacterial signature. Deviations in the gut microbiome are associated with greater risk of gastrointestinal and immune disorders, including obesity. Excessive energy intake favors obesogenic bacteria; furthermore, specific bacterial strains may promote the onset of the chronic low-grade inflammation that is a hallmark of obesity-associated metabolic disorders.

Practical implications
Clinical data point towards the contribution of specific gut bacteria alongside lifestyle interventions to maintain microbial equilibrium. Dietary changes and exercise weight loss programs have been shown to modify the activity and composition of the gut microbiome. Given the prevalence of obesity, however, this approach may not suffice. Modifying the gut microbiota in pregnancy and early infancy may be an important means of halting the vicious circle of unfavorable metabolic status that is transmitted between mother and child.

Recommended reading
Reshaping the Gut Microbiota at an Early Age: Functional Impact on Obesity Risk?

R. Luoto, M.C. Collado, S. Salminen, E. Isolauri

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Abstract

Overweight and obesity can currently be considered a major threat to human health and well-being. Recent scientific advances point to an aberrant compositional development of the gut microbiota and low-grade inflammation as contributing factors, in conjunction with excessive energy intake. A high-fat/energy diet alters the gut microbiota composition, which reciprocally engenders excessive energy harvesting and storage. Further, microbial imbalance increases gut permeability, leading to metabolic endotoxemia, inflammation and insulin resistance. Local intestinal immunologic homeostasis is achieved by tolerogenic immune responses to microbial antigens. In the context of amelioration of insulin sensitivity and decreased adiposity, the potential of gut microbiota modulation with specific probiotics and prebiotics lies in the normalization of aberrant microbiota, improved gut barrier function and creation of an anti-inflammatory milieu. This would suggest a role for probiotic/prebiotic interventions in the search for preventive and therapeutic applications in weight management.

Key Words

Bifidobacteria · Breast milk · Children · Diet · Gut microbiota · Infancy · Intestinal microbiota · Obesity · Overweight · Probiotics · Prebiotics

Introduction

Obesity presents a profound pediatric health problem; in fact, it is the most prevalent nutritional disorder among children throughout the world. In Europe, an estimated...
20% of children and adolescents are overweight, one third of these being considered obese [1]. Notwithstanding the extensive and multidisciplinary scientific interest centered on this problem, research so far has been unable to conclusively ascertain the determinants underlying the epidemic of obesity [2]. In contrast, an escalation of the disorder is to be expected, since the velocity of propagation is highest in the pediatric population.

**Vaginally delivered infants acquire a collection of bacterial communities similar to those in their own mother’s vagina and skin, whereas caesarean section-delivered infants acquire different and less diverse bacterial communities.**

A recent article on systematic reviews has sought to identify early-life determinants of obesity [3]. Altogether, 22 eligible reviews from a database of 12,021 publications lent themselves to quality assessment: no review fulfilled high-quality criteria, 11 were considered of moderate standard and 11 low. In these reports, overweight and obesity were associated with maternal diabetes and smoking, rapid infant growth, no or short duration of breastfeeding, obesity in infancy, short sleep duration, less than 30 min of physical activity daily and consumption of sugar-sweetened beverages. Importantly, many of these items remained causally perplexing: sleep duration, socioeconomic status, and above all, breastfeeding. It was shown that despite a significant difference in mean body mass index (BMI) for breastfed compared with formula-fed infants, adjustment for possible confounders (socioeconomic status, maternal smoking and maternal BMI) removed the effect, but in more recent reviews it nevertheless remained, even when the exclusive nature of breastfeeding was not considered. The authors concluded that since it is difficult to disentangle the complex web of associations and reciprocal influences, future research should focus on intervention studies.

Thus far, intervention studies have focused, for obvious reasons, on the amount or quality of dietary intake. The root cause of obesity is energy imbalance: more calories are consumed than expended. However, a gap still exists between food intake and weight gain, and indeed, our knowledge of the cascade of events precipitated by energy intake and expenditure, quality of food, energy storage and body composition is by no means satisfactory. In particular, a more profound understanding of the complex interaction between nutrition and the gut microbiome, the total genetic pool of the microbiota, is called for. There are perhaps 10–100 times as many microbes in our gut as we have cells in the body, and the microbiome is estimated to comprise 100 or 1,000 times as many genes as we have genes in the human genome [4]. Moreover, the gut microbiota, an integral part of the gut barrier, functions at the intersection between host genotype and diet to modulate the host physiology. The utilization of food is influenced by the gut microbiome, and the collective composition and the compositional development of the gut microbiota, co-evolving with the immune system, is highly sensitive to diet [5]. Nutritional status, host defenses and disease all impact on each other [6]. Indeed, advances in the study of the microbiome during this past decade suggest that the gut microbiota in fact modulates intestinal barrier function and immune responsiveness [7–9], and vice versa, and can be affected by specific nutrients or lack of them. It would thus appear simplistic to assume that one mode of prevention or treatment would suffice to counter the obesity epidemic. Rigorous scientific effort is essential to elucidate the mechanisms contributing to the development of obesity and devise new interventions and practical applications.

**Colonization of the Infant Gut**

*Initial Postnatal Microbial Contact*

The recent dogma that the human intestinal microbiota begins to set itself up during and after birth and converges toward an adult-like microbiota by the end of the first 2 years of life has been challenged. The traditional thinking, as indicated in the article by Walker in this issue, suggests that the first pioneer bacteria may originate from the vaginal and fecal microbiota of the mother. Further sources of bacteria include the mammary glands through breastfeeding, the mother’s skin and oral microbes, and the environment through initial contacts by the infant. Initial colonizers are generally facultative anaerobes including enterobacteria, coliforms, *Lactobacilli* and *Streptococci*, which are then replaced by anaerobic genera such as *Bifidobacterium*, *Bacteroides*, *Clostridium* and *Eubacterium* by the end of the first week of life [10]. It has been established that the diversity of the early microbiota is initially relatively low and that interindividual variations in diversity are high [11, 12]. Vaginally delivered infants acquire a collection of bacterial communities similar to those in their own mother’s vagina and skin,
whereas caesarean section-delivered infants acquire different and less diverse bacterial communities which may resemble the microbiota of the assisting personnel and the general delivery environment. Other factors influencing the compositional development of the gut microbiota include gestational age at birth, the use of antibiotics by either the mother or the infant during early life and the need for hospitalization [13–15].

**Bacterial Exposure during Pregnancy**

New findings, which challenge the former dogma of a sterile intrauterine existence, suggest that microbial contact of the human being may in fact begin already prior to birth [16]. Accumulating evidence now suggests that traces of microbes, including microbial DNA and cell structures from intestinal bacteria, are detectable in the placenta, amniotic fluid and fetal membranes, their presence being verified in term pregnancies without signs of inflammation, rupture of membranes or onset of labor [16–19]. Further, microbial contact in utero has been shown to induce changes in the fetal intestinal toll-like receptor (TLR)-related innate immune gene expression [19]. In addition to the previous findings, microbial DNA has also been characterized in the meconium of healthy term neonates, suggesting a prenatal origin [20, 21]. Hence, contact with the complex bacterial communities of the extrauterine world may be initiated already in utero and thus be determined by changes in the mother’s intestinal microbiota during pregnancy. On this basis, factors affecting and also possibilities to modulate the composition of the maternal microbiota during pregnancy warrant further characterization.

**Impact of Diet**

Following birth, the mode of feeding and the timing of different complementary foods have a further impact on the gut microbiota composition and activity in the infant [10]. Breast milk has been shown to be an excellent and continuous source of potentially beneficial and commensal bacteria, including *Staphylococci*, *Streptococci*, lactic acid bacteria and *Bifidobacteria*, with bacterial cell numbers reaching $10^3$ to $10^5$/ml of breast milk. The presence of *Bifidobacteria* in breast milk is of utmost importance for the colonization of the infant gut, since the activation of IgA-producing plasma cells in the human neonatal intestine is known to be dependent on the colonization of the gut by *Bifidobacterium* and also *Lactobacillus* spp. stimulated by fermentation of nondigestible oligosaccharides found also in breast milk. It is well established that a gut microbiota dominated by *Bifidobacterium* typifies that of the healthy breastfed infant [22], breastfed infants harboring twice as many *Bifidobacterium* cells compared to formula-fed infants [23]. On the other hand, formula-fed neonates are likely to harbor a more diverse microbiota including Enterobacteriaceae, *Enterococcus* and, as recently demonstrated, also *Bacteroides* [23–25].

The composition of breast milk, however, depends on the immunological and metabolic status of the mother. In addition to changes in the human milk microbiome over lactation, milk from obese mothers tends to contain a different and less diverse bacterial community compared with milk from normal-weight mothers [26]. In the study in question, breast milk from obese women was found to contain higher total bacteria counts, *Staphylococcus* and *Lactobacillus*, and lower *Bifidobacterium* numbers when compared to the breast milk of normal-weight women over the first 6 months of breastfeeding [26]. Excessive weight gain over pregnancy had an influence on breast milk bacterial numbers similar to that of prepregnancy obesity. Interestingly, the mode of delivery also influences the bacterial diversity of breast milk. Milk samples from mothers who had undergone elective but not emergency caesarean delivery had decreased amounts of *Leucostocaceae* and increased amounts of *Carnobacteriaceae*, among others, compared with those who delivered vaginally, suggesting that it is not the operation per se but rather the absence of physiological stress or hormonal signals which could contribute to an aberrant microbial transmission process to breast milk [26]. Indeed, the release of stress hormones triggers a cascade of cytokines involved in inflammatory pathways [27]. Further, complex interactions of cytokines and microbiota in breast milk have been reported, as transforming growth factor β2 and soluble innate microbial receptor CD14 levels in the breast milk of overweight mothers have tended to be lower than in normal-weight mothers [28].

It may thus be suggested that alterations in the intestinal barrier allow for transfer of bacteria from the intestine, among others, to breast milk, while labor and early lactation further endorse bacterial translocation. The
route of transfer of these bacteria detected in breast milk has not yet been ascertained, although different hypotheses have been put forward. Dendritic cells have been shown to penetrate the intestinal epithelium and to take up commensal bacteria from the gut lumen, to reach the systemic circulation and to retain live bacteria for several days [29]. Recently, transfer of intestinal bacteria to the mammary glands within dendritic cells has been envisaged [17, 30]. Breast milk composition is, however, a complex and multifactorial continuum, which is influenced not only by maternal gut microbiota and mode of delivery, but also by the infant itself, and immunomodulatory constituents of breast milk have been shown to respond to infection in the neonate [31].

**Early Microbial Contact and Risk of Disease**

As described in the article by Walker in this issue, alterations in the compositional development of the gut microbiota of a newborn have been shown to be related to several disorders and to predispose to diseases later in life. The best-documented function of the gut microbiota, an integral part of the gut barrier, is to control antigen exposure to host tissues, thereby lessening the potential for pathological outcomes. Deviations in the early microbiota have already been demonstrated to be associated with a higher risk of allergy, gastrointestinal infections and inflammatory conditions, necrotizing enterocolitis and late-onset sepsis in preterm infants, and also with obesity [32–36]. Furthermore, children born by caesarean section have been shown to carry an increased risk of chronic inflammatory conditions such as coeliac disease, type 1 diabetes mellitus, asthma and also obesity, as compared with children born by vaginal delivery [37–39], indicating that both immunological and metabolic disturbances may be driven by aberrant population among gut microbiota. Likewise, the beneficial health effects of breastfeeding are mediated at least partly via modulation of infant immune responsiveness and gut microbiota composition. This is exemplified in a reduced risk of necrotizing enterocolitis and infections of the gastrointestinal and respiratory tracts in breastfed infants when compared to formula-fed infants, but also in improved cognitive development and a decreased occurrence of coeliac disease, asthma, hypercholesterolemia, type 2 diabetes mellitus and obesity in later life (reviewed by Rautava et al. [40]). On this basis, the role of early microbial inoculum, further shaped by early nutrition, can have a significant impact on later health.

**Gut Microbiota and Metabolic Health – Experimental and Clinical Evidence**

Some metabolic disease trajectories are set early in life. Focusing on the plaque of Western countries, obesity, the complex regulatory mechanisms of the gut microbiota have attracted research interest in terms of nutrient processing, extraction and utilization as well as modifying immunity and inflammation [9]. Although genetic factors can determine the propensity of an individual to become obese, environmental and lifestyle patterns, including dietary habits, are the major contributors to the obesity increment. Altered dietary intake not only affects energy balance but also constantly regulates and modifies the microbiota composition, which influences nutrient accessibility for the host body, and thereby potentially boosts weight gain (fig. 1) [41]. These observations document that the gut microbiota can adapt to excessive energy intake, selecting obesogenic microbiota, which transmits additional energy to the host to be stored. Furthermore, specific strains may favor the onset of a low-grade inflammatory state and consequently obesity-associated metabolic disorders [42, 43].
Fig. 2. Suggested mechanisms linking gut microbiota to obesity. The gut microbiota may regulate energy storage firstly by increasing fermentation of indigestible dietary polysaccharides, by increasing monosaccharide absorption, by producing SCFAs and by increasing hepatic lipogenesis. Another suggested mechanism is suppression of the fasting-induced adipocyte factor (FIAF) in the gut, which in turn increases lipoprotein lipase (LPL) activity in adipocytes. Thirdly, inhibition of adenosine monophosphate-activated protein kinase (AMPK)-dependent fatty acid oxidation may contribute to overweight development. High-fat diet feeding alters the gut microbiota composition in a complex way. This phenomenon is associated with higher gut permeability, leading to higher plasma LPS levels, e.g. metabolic endotoxemia, which promotes low-grade inflammation-induced metabolic disorders such as insulin resistance, diabetes, obesity, steatosis, oxidative stress and adipose tissue macrophage infiltration.

Potential Mechanisms Linked with Gut Microbiota Influence on Obesity

Several mechanisms have been proposed to link the microbiota with obesity (fig. 2). Dysbiosis, perturbation of the gut microbiota composition, could promote intestinal monosaccharide absorption and energy extraction from nondigestible food components (mainly carbohydrates) via short-chain fatty acid (SCFA) production and hepatic de novo lipogenesis [41, 44, 45]. Furthermore, this dysbiosis could increase fatty acid storage in adipocytes by suppressing the fasting-induced adipocyte factor in the gut, which in turn increases enzyme lipoprotein lipase activity [41]. Another mechanism envisaged as linking a balanced gut microbiota composition to protection against diet-induced obesity could be, firstly, inhibition of cellular energy-dependent protein kinase activation [46] and, secondly, association between SCFA signaling molecules, G protein-coupled receptor activation and energy storage [47].

The Role of the Gut Microbiota in Overweight-Related Low-Grade Inflammation

Additionally, it is increasingly recognized that obesity is characterized by chronic activation of inflammatory pathways [48]. Overexpression of proinflammatory cytokines in adipocytes activates various signal transduction cascades, many of them being critical inhibitors of insulin action. An important feature of inflammation is infiltration of inflamed tissues by immune cells, especially macrophages, which contribute to the maintenance of inflammatory responses. An aberrant gut microbiota composition may trigger a low-grade inflammatory state, 'metabolic endotoxemia', by rendering the host liable to systemic exposure to the lipopolysaccharide (LPS), a large glycolipid derived from the outer membrane of Gram-negative bacteria [49]. LPS is known to be a powerful trigger for the innate immune system response and is causally linked with adiposity, insulin resistance and de novo synthesis of triglycerides. Upon binding to TLR-4 and its co-receptors, LPS triggers a cascade of responses ultimately resulting in the release of proinflammatory molecules which interfere with the modulation of glucose and
insulin metabolism. These inflammatory signaling pathways are causally linked to insulin resistance, which is a prerequisite for numerous overweight-associated pathologies, including non-insulin-dependent diabetes, hypertension and dyslipidemia, and favor progression of fatty liver disease to steatohepatitis and promote the development and rupture of the atherosclerotic plaque [50]. Interestingly, the dietary fatty acids, whose circulating levels are often increased in obesity, induce insulin resistance through TLR-4 signaling, this linking the innate immune system to insulin resistance also in response to changes in the nutritional environment and reflecting the complex interaction between diet, microbes and host metabolism [51].

**Experimental Evidence**

Pioneer experimental studies have provided evidence of the gut microbiota facilitating the extraction of energy from ingested diet and its storage in the host adipose tissue [41, 46, 52, 53]. The transferable nature of the obese phenotype was demonstrated by colonization of germ-free mice with ‘obese microbiota’, resulting in a significantly greater increase in total body fat than by colonization with ‘lean microbiota’ [54]. Furthermore, a positive correlation between an increment in **Bifidobacterium spp.** and normalization of the inflammatory status in obese mice has been demonstrated [49]. Recent reports also suggest that the presence of **Akkermansia muciniphila** bacteria correlates inversely with body weight, restores mucus layer in high-fat diet-fed mice and furthermore decreases fat mass and LPS levels, thus improving metabolic profile [55].

**Clinical Evidence**

The aforementioned publications were followed by human demonstrations of alterations in the gut microbiota in obese individuals compared to those of normal weight [53, 55]. These studies reported a reduced amount of bacteria belonging to the phylum Bacteroidetes in obese individuals as well as an enrichment of genes involved in carbohydrate and lipid metabolism of obese host microbiomes [56]. Thus far, a relative abundance of various types of gut bacteria in obese and lean humans, adults and children, have been demonstrated in several studies, although the results have not led to the same conclusion (reviewed by Angelakis et al. [57]). However, in light of the most recent findings, smaller changes in the gut microbiota community, rather than those occurring at a wide phylum level, might be involved in overweight development. As a continuum, dietary changes and also an exercise weight loss program have been demonstrated to modify the gut microbiota composition and activity [58–60]. It is of note that in the aforementioned study by Santacruz et al. [59], the response of overweight adolescents to a diet and exercise weight loss program was shown to be dependent on the gut microbiota prevailing prior to treatment. Similarly, the initial composition of the gut microbiota was suggested to be an instrumental contributor in a study by Walker et al. [60], where a marked increase in the relative abundance of **Ruminococcus bromii** and **Eubacterium rectale** phylotypes was demonstrated as a result of a diet rich in resistant starch.

On the basis of this data, it is conceivable that modification of the gut microbiota by specific dietary or pharmacological interventions may favorably affect host metabolism. Probiotics, ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’, have been shown to influence gut mucosal barrier functions and the interaction between host and bacteria. Some of these functions may be related to the development of overweight and may thus serve as targets of both prevention and treatment. The contribution of specific gut bacteria together with lifestyle interventions to maintain microbial equilibrium has been studied in some prospective, randomized clinical trials with metabolic markers or other cardiovascular risk factors as outcomes. The evidence of a direct impact of gut microbiota modulation on weight development is, however, thus far scant. Kadooka et al. [61] administered probiotic **Lactobacillus gasseri** SBT2055 (LG2055) to overweight subjects and found the intervention to have a significant diminishing effect on abdominal adiposity, body weight and also on other measures reflecting adiposity. A few studies have also provided clinical evidence of the beneficial effect of prebiotics in weight management, as reviewed by Delzenne et al. [62]. Similarly, the active role of an aberrant gut microbiota composition in the pathogenesis of obesity and low-grade inflammation might be one further putative explanation for the rapid weight loss, reduced adiposity and especially dramatically improved glucose metabolism after bariatric surgery [63, 64].
Recently, also fecal microbiota transplantation has attracted great scientific interest in the treatment not only of *Clostridium difficile* infection, inflammatory bowel disease and irritable bowel syndrome, but also of obesity [65]. A recent clinical study showed that transfer of intestinal microbiota from lean donors increased insulin sensitivity in individuals with metabolic syndrome [66].

**Transfer of intestinal microbiota from lean donors increased insulin sensitivity in individuals with metabolic syndrome.**

**Current Thinking on the Functional Interaction between the Gut Microbiota and the Development of Obesity in Childhood**

The traditional approach in the prevention of weight gain and obesity has been to modify the lifestyle habits of an individual or group of people who are obese or appear to be at risk of becoming obese. Positive outcomes have been described in the literature [67], but considering the extent to which obesity is becoming more common, this approach does not suffice. There is a need to act prior to the appearance of any signs of obesity. To halt the vicious circle, early interventions are called for [2, 3]. The most exciting insight to date is that early life conditions determine the risk of developing disease in later life, the ‘programming effect’. The programming theory envisages health to be determined by early life events in utero and during early infancy, whereby the nutritional environment permanently alters the body’s structure, physiology and metabolism and leads to disease in adult life [68]. This developmental programming is promoted by nutritional, hormonal and metabolic factors, as well as by the microbiota composition, afforded by the mother during the critical periods when the system is plastic and sensitive to the environment [69]. The mother transfers environmental information to the fetus through the placenta or to her infant through lactation. This shaping information may include the mother’s weight status (under- or overnutrition), unbalanced dietary intake or microbiota composition, and breast milk composition.

**Perinatal Window of Opportunity**

Hence, pregnancy and early infancy are to the current understanding the most interesting critical stages and targets for interventions aiming to reduce the risk of overweight development in future generations. Modification of the gut microbiota by probiotics early in life has thus attracted interest, since there is a critical period during the first months of life which affords an important opportunity for immune education, while the establishment of the intestinal microbiota and maturation of the immune system are not yet completed. Initial microbial colonization of the gastrointestinal tract, linked with lifestyle determinants, may be an instrumental contributor to the infant’s weight development, newborns thus constituting one of the populations most likely to benefit from the use of probiotics. In view of the aforementioned phenomenon that the immune education of an infant may begin already in utero [40], the administration of probiotics during pregnancy is also under consideration in view of the positive effects some strains exert on certain clinical conditions both in pregnant women and in the child. In overweight and obese pregnant women, an intergenerational vicious circle of unfavorable metabolic development may be generated if the aberrant gut microbiota associated with overweight or excessive weight gain during pregnancy is transferred to the infant.

**Clinical Evidence of the Impact of Maternal Nutritional Status on Infant Microbiota Development**

The association between maternal nutritional status and gut microbiota composition during pregnancy has been reported by groups under Collado [70] and Santacruz [59]. Interestingly, both studies are supportive of the view that a gut microbiota profile favoring a higher number of *Bifidobacteria* and a lower number of *Staphylococcus aureus* may provide protection against maternal overweight development. Additionally, the infant fecal microbiota composition has been shown to be related to maternal weight and weight gain over pregnancy [71]. Mother’s higher BMI and excessive weight gain during pregnancy were related in the study population in question to lower levels of the *Bifidobacteria* and higher concentrations of Bacteroidetes, *Clostridium* and *Staphylococcus*. The instrumental role of microbial stimulus during pregnancy for the later metabolic programming of the offspring may also partly explain the findings in a large prospective cohort study, where most of the association between maternal weight gain during pregnancy in overweight and obese women and later offspring BMI proved attributable to intrauterine mechanisms other than shared familial (genetic and early environmental) characteristics [72]. In another large longitudinal prospective study, a combination of early exposures, including delivery mode, maternal prepregnancy BMI and antibiotics in infancy, were shown to influence the risk of overweight in later childhood [73].
Clinical Evidence from Probiotic Studies

We have shown in a clinical placebo-controlled study that a perinatally administered probiotic combination, *Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium lactis*, attains consistently improved plasma glucose concentrations and insulin sensitivity in metabolically healthy women during pregnancy and 12 months postpartum when an advantageous dietary intake was combined with probiotics [74]. Furthermore, the beneficial effects were shown to extend to neonates and infants. Interestingly, nutrition counseling and probiotic intervention were demonstrated to have a distinct effect on gestational diabetes, probiotics reducing the risk, while dietary counseling reduced the risk of fetal overgrowth associated with it [75]. This same intervention study has provided clinical evidence that probiotic consumption lowers the risk of central adiposity in mothers over the 6-month postpartum period [76]. Considering that early bifidobacterial colonization can have far-reaching impacts on infant weight development, it is of note that maternal consumption of LGG in another double-blind placebo-controlled probiotic study before and after delivery induced specific changes in the transfer and initial neonatal colonization of *Bifidobacteria* compared with placebo [77]. In the study in question, infants whose mothers received probiotics showed an increase in bifidobacterial diversity, a higher prevalence of *Bifidobacterium breve* and a lower prevalence of *Bifidobacterium adolescentis* during the first year of life than the placebo group. Further, in this same cohort, differences in early gut microbiota composition were shown to predict overweight in children early in life, those becoming overweight by 7 years of age having had lower levels of *Bifidobacteria* and higher levels of *S. aureus* at 6 and 12 months of age compared to those remaining normal weight [36]. In line with this observation was a finding whereby this perinatal probiotic intervention with LGG moderated excessive weight gain especially among children who subsequently became overweight during the first years of life, the impact being most pronounced at the age of 4 years [78].

Conclusion

It is acknowledged that dysbiosis might be a pivotal factor and the ‘missing link’ in the fight against the obesity epidemic. However, before the term dysbiosis can be characterized, the composition of a healthy ‘normal’ microbiota has to be defined in evaluating the compositional development of the gut microbiota in healthy breastfed infants who also remain normal weight and healthy long term. On the basis of the data presented in this review, specific strategies to modify the gut microbiota to enhance *Bifidobacteria* in infancy and childhood may thus emerge as a measure to reduce the incidence of overweight development and, as a corollary, restrain the Western lifestyle disease epidemic. Further mechanistic studies, especially in humans, are needed in order to better understand how the gut microbiota may interact with the host immune response in the context of obesity and obesity-related disorders. Furthermore, pregnancy and early infancy are to the current understanding the most interesting critical stages and targets for interventions aiming to reduce the risk of overweight development in future generations. In other words, by influencing the nutritional and microbial environment of the mother and her fetus today, the health of the next generation may be modified.

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Gut Microbiota and Obesity in Children

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A healthy microbiota is defined by high diversity and an ability to resist change under physiological stress. In contrast, microbiota associated with disease is defined by lower species diversity, fewer beneficial microbes and/or the presence of pathobionts.

Clinical Consequences of Diet-Induced Dysbiosis
by Yee Kwan Chan et al.

Key insights
Various disease states are associated with alterations in the balance between beneficial and harmful bacteria that reside in the intestine. This dysbiosis has far-reaching effects on local and systemic immunity, and underpins the pathogenesis of disorders such as inflammatory bowel disease, colorectal cancer, diabetes, atherosclerosis and nonalcoholic fatty liver disease. Interventions that target the microbial profile of the gut have tremendous potential for addressing these disorders.

Current knowledge
There is a complex tripartite relationship between diet, microbes and the gut epithelium. Beyond the postnatal period, long-term dietary patterns have a strong influence on the composition of gut microbes. For example, regular red meat consumption favors a Bacteroides-rich microflora, whereas Prevotella species tends to dominate in vegetarians. A high-fat diet may induce dysbiosis through the actions of bile, which could affect the growth of some microbes. An examination of the digestive process may offer greater insight into the mechanisms through which diet can influence dysbiosis and disease.

Practical implications
Not surprisingly, diet and gut microbes are two critical factors in the pathogenesis of gastrointestinal diseases. Intestinal dysbiosis has also been linked to systemic conditions such as metabolic and cardiac disorders. Although diet is a tempting intervention, our understanding of how to manipulate diet to promote a healthy microbiota is still in its early days. Bacteriotherapy provides a novel approach for restoring healthy homeostasis through the gut microbes. This is achieved through the use of various interventions, including the removal of pathogenic bacteria with antibiotics, supplementation with prebiotics and/or probiotics, and most recently, introduction of a new healthy microbial ecosystem by transplanting fecal bacteria from a healthy donor.

Recommended reading
Clinical Consequences of Diet-Induced Dysbiosis

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Key Messages

- An undesirable alteration of the microbiota resulting in an imbalance between protective and harmful bacteria is termed dysbiosis.
- Dietary patterns alter the intestinal microbiota ecologically and functionally and this results in physiological consequences to the host.
- Dysbiosis has been implicated in many human disease conditions including local gastrointestinal and systemic diseases.
- Restoration and maintenance of a healthy gut microbiota may be an effective, inexpensive and safe remedy to diseases associated with dysbiosis.

Key Words

Intestinal microbiota · Dysbiosis · Nutrition · Inflammation · Disease susceptibility · Bacteriotherapy

Abstract

Various disease states are associated with an imbalance of protective and pathogenic bacteria in the gut, termed dysbiosis. Current evidence reveals that dietary factors affect the microbial ecosystem in the gut. Changes to community structure of the intestinal microbiota are not without consequence considering the wide effects that the microbes have on both local and systemic immunity. The goal of this review is to give insight into the importance of gut microbiota in disease development and the possible therapeutic interventions in clinical settings. We introduce the complex tripartite relationship between diet, microbes and the gut epithelium. This is followed by a summary of clinical evidence of diet-induced dysbiosis as a contributing factor in the development of gastrointestinal diseases like inflammatory bowel disease, irritable bowel syndrome and colorectal cancer, as well as systemic diseases like obesity, diabetes, atherosclerosis and nonalcoholic fatty liver disease. Finally, the current dietary and microbial interventions to promote a healthy microbial profile will be reviewed.

Colonization and Diversity of Gut Microbes

Humans have co-evolved with vast amounts of microorganisms that inhabit the body. The average human being harbors 10 times more bacterial cells than their own cell numbers. These microbes colonize the skin, nasal and oral cavity, urogenital and gastrointestinal tract (GIT). Among all sites, the GIT is the most densely populated area with the colon alone harboring over 10^{10}–10^{12} colony-forming units per gram of feces, or 70% of all microbes in the human body [1].
While it has been thought that a fetus is sterile in utero, there is some evidence that microbial DNA and potentially even microbes are exposed to the fetus and fetal gut through the placenta [discussed by Luoto et al. in this issue]. During birth, microbial colonization of the GIT occurs and develops rapidly thereafter with maternal and environmental microbes. Colonization does not appear to be random but preprogrammed; yet, the mode of infant delivery, antibiotic exposure, nutrition and other extrinsic factors influence microbial ecology (fig. 1). Microbial diversity increases during the first few years of life and then stabilizes by 2–4 years of age resembling that of an adult [2]. Most of these bacteria associate with the intestinal mucosal surface and maintain their specific niches over time as indigenous populations. Newly introduced bacteria either pass through the GIT in the stool or compete with indigenous bacteria to create their niche. While there is evidence that the intestinal microbiota is relatively stable throughout life, extrinsic factors such as stress, alcohol consumption, exercise and dietary choices do change the ecology and function of the microbiota in adults. We do not yet understand how dynamic the ecology of the microbiota is, so microbial changes may only be transient and reversible, but more research is required to understand this plasticity.

Humans carry 500–1,000 bacterial species in the GIT of which the majority belongs in only two phyla: the Firmicutes and Bacteroidetes (>90%). Other phyla present to a lesser extent include: Actinobacteria, Proteobacteria, Fusobacteria, Spirochaetae and Verrucomicrobia. While the dominating phyla are relatively constant between individuals, diversity increases along the taxonomic line with each individual harboring over a hundred unique species. Three distinct clusters of gut microbiota have been identified in humans. These ‘enterotypes’ are mainly driven by species composition and are not geographical, age or gender specific [3]. An undesirable alteration of the microbiota resulting in an imbalance between protective and harmful bacteria is termed dysbiosis and may cluster as a specific enterotype.
type. In support of this, enterotypes have been shown to associate with chronic ailments such as colonic inflammation [3], symptomatic atherosclerosis [4] and nonalcoholic steatohepatitis [5]. Factors such as nutrient load, macro- and micronutrients induce changes to the ecology and functionality of the gut microbiota, and long-term dietary patterns can alter the original enterotype [6]. Identifying dietary factors that promote beneficial microbes and prevent pathobiont intrusion may be an important tactic in the prevention of dysbiosis-associated diseases.

**The GIT, Microbes and Diet**

The gut microbial ecosystem has tremendous influence on the overall health status of the human host. The microbiota lies at the interface of the internal and external environment in the gut forming a tripartite relationship with the intestinal epithelial cells and dietary antigens (fig. 2). Due to this conspicuous location, the microbiota is able to liaise with both the intestinal mucosal surface and the luminal environment that contain partially digested food. Dietary antigens interact with both the microbes and the intestinal epithelium. Microbes impart physiological changes to the host by interacting with the...
intestinal epithelial cells via innate immune receptors [discussed by Walker in this issue]. The intestines contain the largest mass of lymphoid tissue in the body: the gut-associated lymphoid tissue (GALT). The GALT relays signals from the mucosal surface to the rest of the body through various immune cells and immune receptors including innate toll-like receptors (TLRs) and NOD-like receptors (NLRs). The intestinal microbiota plays crucial roles in the GIT development, systemic immunity and colonic homeostasis. Gut microbiota can modulate the function and responsiveness of intestinal immune cells, like T regulatory cells, to bacterial products. This is required to regulate mechanisms that keep both mucosal and systemic immunity in balance, allowing for mucosal surfaces to tolerate harmless bacteria, yet adequately respond to invading pathogens. Production of short-chain fatty acid (SCFA) by gut microbes also plays an important role in regulating homeostasis in the gut. For example, butyrate produced by colonic microbes is not only the main energy source for colonocytes, but also inhibits intestinal cell proliferation which can reduce colitis symptoms [7]. Given the vital relationship between microbes and intestinal health, a normal functioning microbiota is crucial to maintaining a balance of local and systemic immunity. As discussed below, in the absence of a healthy microbiota, immune disorders may arise. Identifying dietary factors that control the intestinal microbial ecology and their role in enteric disease susceptibility could provide insight into the functioning of the microbiota in healthy and diseased individuals. Yet, due to the vast diversity of dietary antigens and gut microbes, we are challenged to define the exact interactions between microbes, dietary antigens and epithelium and their consequences to the host.

Dietary antigens can interact with both the microbiota and the intestinal mucosa, initiating biological reactions in the host. Food contains numerous compounds that shape the chemistry of the gut as well as the whole body. For example, dietary antigens are absorbed through the intestine which results in metabolites in the circulating fluids like blood and lymph [8]. The association of specific metabolites in the body with dominant bacterial taxa in infants suggests that the chemical composition of the diet can define the gut microbial ecology [9]. While dietary factors can directly affect the functionality of intestinal epithelial cells and the underlying immune cells [10], dietary antigens also alter the intestinal ecosystem by enabling certain microbial populations to proliferate and dampening the dominance of others (reviewed by Brown et al. [11]). The consequences of dysbiosis are not innocent, but detrimental when pathobionts (any disease-causing microorganism) become prominent in the microbial communities. To support this idea, oral microbes sequenced from ancient teeth found in skeletons from various periods of time have become increasingly cariogenic dominant, or rich in microbes that promote dental disease [12]. These microbial changes have occurred during the two greatest dietary shifts in human evolution: the transition from the hunter-gatherer ‘Paleolithic’ period to the carbohydrate-rich farming ‘Neolithic’ period (∼10,000 years ago) and the initiation of the industrialized period characterized by processed foods (∼160 years ago). These findings support the notion that diet induces dysbiosis which alters the health of the host.

Evidence suggests that dietary factors alter intestinal ecology in both rodent models (reviewed by Brown et al. [11]) and in humans, and the changed ecology is associated with clinical consequences (table 1). Neonatal nutrition is critical in the initial development of microbial ecology [13]. For example, formula-fed infants have higher levels of pathobionts like Enterobacteriaceae and less beneficial microbes like Bifidobacteria spp. compared to breastfed infants [14]. Interestingly, infants fed cow’s milk but not infant formula supplemented with fish oil had increased Bifidobacteria spp. [15] suggesting that postnatal nutrition could be used to target specific changes in microbial diversity. Beyond the postnatal period, long-term dietary choices are strongly associated with the gut microbiota composition [6]. In humans, diets that include regular red meat consumption tend to favor a predominantly Bacteroides-rich gut ecosystem [16], while Prevotella species dominate in vegetarians [17]. European children are deficient in Bacteroidetes and enriched with Enterobacteriaceae compared to rural African children who consume diets rich in fiber [18]. This study may be an important key to understanding the increase in noncommunicable diseases in European children. While it is generally agreed that high-fat diets promote dysbiosis, recent evidence from our laboratory suggests that the specific type of dietary fatty acid as opposed to total calories from fat appears to be important. For example, diets rich in ome-
### Table 1. Summary of studies showing that dietary factors change microbial profiles in humans and the associated clinical consequences

<table>
<thead>
<tr>
<th>Dietary factor implicated</th>
<th>Specific diet</th>
<th>Sample size</th>
<th>Location of microbes analyzed in host</th>
<th>Bacterial population altered</th>
<th>Method of bacterial detection</th>
<th>Associated host effect</th>
<th>Ref. PubMed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>high fat (shortening) and high sugar</td>
<td>1 man, 15 mice</td>
<td>feces of men</td>
<td>↑ Clostridium innocuum, Enterobacteriaceae spp.</td>
<td>multiplex amplicon pyrosequencing</td>
<td>↑ obesity when transplanted into mouse</td>
<td>20368178</td>
</tr>
<tr>
<td></td>
<td>fish-oil-supplemented infant formula versus cow’s milk</td>
<td>65 feces of 10-month-old infants</td>
<td>consumption of cow’s milk and infant formula resulted in different microbial patterns; fish oil supplementation affects the microbial pattern of the cow’s milk group only</td>
<td>DGGE not examined</td>
<td>not examined</td>
<td>17460496</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate-rich foods</td>
<td>increased carbohydrate-rich foods</td>
<td>34 mouth of ancient skeletons</td>
<td>cariogenic-dominant</td>
<td>454 pyrosequencing</td>
<td>↑ dental disease</td>
<td>23416530</td>
<td></td>
</tr>
<tr>
<td></td>
<td>diets high in resistant starch compared to non-starch polysaccharides and low carbohydrate</td>
<td>14 feces of overweight men</td>
<td>↑ Firmicutes, Eubacteriaceae rectale, Roseburia, Ruminococcus bromii (R-ruminococci)</td>
<td>qPCR</td>
<td>↑ digestibility of starch</td>
<td>20686513</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dietary fiber-rich diets found in rural Africa compared to Western European diets</td>
<td>29 fecal microbiota of children aged 1–6 years</td>
<td>↑ Bacteroidetes, ↑ Firmicutes, ↑ Prevotella and Xylanibacter Enterobacteriaceae</td>
<td>454 pyrosequencing</td>
<td>↑ bacterial genes for cellulose and xylan hydrolysis</td>
<td>20679230</td>
<td></td>
</tr>
<tr>
<td></td>
<td>inulin and Brussels sprouts</td>
<td>1 man, 48 rats</td>
<td>feces</td>
<td>↑ Bifidobacterium and Lactobacillus</td>
<td>TTGE</td>
<td>↑ cecal butyrate and acetate when transplanted into rats</td>
<td>15975167</td>
</tr>
<tr>
<td></td>
<td>kiwi fruit</td>
<td>10 feces</td>
<td>↑ Bifidobacterium and Bacteroides-Prevotella-Porphyromonas group</td>
<td>qPCR</td>
<td>↑ microbial glycans and SCFAs</td>
<td>22576129</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sucrose-free chocolates + maltitol + bulking agents (polydextrose and resistant starch)</td>
<td>40 feces</td>
<td>↑ Bifidobacterium and Lactobacillus</td>
<td>FISH</td>
<td>↑ SCFAs propionate and butyrate</td>
<td>20370946</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bread enriched with arabinoxylano-oligosaccharides</td>
<td>40 feces</td>
<td>↑ Bifidobacterium and Lactobacillus</td>
<td>FISH</td>
<td>↑ butyrate</td>
<td>22657950</td>
<td></td>
</tr>
<tr>
<td></td>
<td>protein</td>
<td>29 feces</td>
<td>↑ in overall bacterial DNA, ↑ the amount and changing the diversity of Clostridium cluster IV</td>
<td>DGGE, qPCR not examined</td>
<td>↑ isovalerate and fatty acids associated with protein fermentation</td>
<td>19641302</td>
<td></td>
</tr>
<tr>
<td></td>
<td>high red-meat diet</td>
<td>24 mice</td>
<td>feces</td>
<td>↑ Bacteroides spp.</td>
<td>qPCR</td>
<td>no functional changes observed when transplanted into mouse</td>
<td>23239972</td>
</tr>
<tr>
<td></td>
<td>gluten-free diet</td>
<td>10 feces</td>
<td>↑ Bifidobacterium and Lactobacillus Enterobacteriaceae</td>
<td>not mentioned</td>
<td>↑ TNF-α, IFN-γ, IL-8 and IL-10 in peripheral blood mononuclear cells</td>
<td>21327021</td>
<td></td>
</tr>
<tr>
<td>Breastfeeding versus formula feeding</td>
<td>not reported</td>
<td>feces of 3-month-old infants</td>
<td>↑ Bacteroidetes, ↑ Firmicutes and Verrucomicrobia</td>
<td>454 pyrosequencing</td>
<td>gene networks (inflammation, cell adhesion, barrier function, histamine, etc.) differentially expressed in exfoliated intestinal epithelial cells</td>
<td>22585924</td>
<td></td>
</tr>
<tr>
<td></td>
<td>breastfeeding compared to formula feeding</td>
<td>207 mouth of 3-month-old infants</td>
<td>Lactobacillus spp.</td>
<td>culturing, qPCR</td>
<td>inhibited growth of the cariogenic Streptococcus spp.</td>
<td>22955450</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>ready-to-use therapeutic food composed of peanut paste, sugar, vegetable oil and milk fortified with vitamins and minerals</td>
<td>634 feces of Malavian twin pairs over the first 3 years of life</td>
<td>↑ Actinobacteria in kwashiorkor twin compared to healthy twin</td>
<td>multiplex shotgun sequencing</td>
<td>severe acute malnutrition caused when kwashiorkor microbiota transplanted into mouse</td>
<td>23363771</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 cups of coffee daily for 3 weeks</td>
<td>16 feces</td>
<td>↑ Bifidobacterium spp.</td>
<td>DGGE, FISH</td>
<td>↑ metabolic activity of Bifidobacteria spp.</td>
<td>19217682</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dark chocolate</td>
<td>30 urine</td>
<td>not examined</td>
<td>↑1H NMR, MS analysis</td>
<td>different energy profiles, hormonal metabolism and gut microbial activity</td>
<td>19807004</td>
<td></td>
</tr>
</tbody>
</table>

DGGE = Denaturing gradient gel electrophoresis; FISH = fluorescence in situ hybridization; ↑1H NMR = proton nuclear magnetic resonance; MS = mass spectrometry; qPCR = quantitative polymerase chain reaction; TTGE = temporal temperature gradient electrophoresis.
ga-6 polyunsaturated fatty acids (PUFAs) cause blooms of pathobionts, but isocaloric diets supplemented with omega-3 PUFA can reverse such microbial alterations in mice [19, 20].

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**Diets rich in omega-6 PUFAs cause blooms of pathobionts, but isocaloric diets supplemented with omega-3 PUFA can reverse such microbial alterations in mice.**

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One of the main functions of the microbiota is to break down food to make it available to the host and as a result, the effect of dysbiosis on metabolism has received considerable attention in current research. ‘Humanized’ mice, or germ-free mice transplanted with human fecal microbiota, are now being used to test the effects of human gut microbiota on mammalian physiology. Using this model, humanized mice fed a ‘Western’ diet high in fat and sugar were shown to have increased adiposity as a result of decreased ratios of Bacteroidetes to Firmicutes in the fecal microbiota [21]. Similarly, the gut microbiome was shown to play an important role in the development of kwashiorkor disease, a severe form of malnutrition [22]. In this study, the fecal microbiota of Malawian twins that were discordant for kwashiorkor was transplanted into mice. When fed a Malawian diet, weight loss and metabolic perturbations were more severe in the mice that received microbiota from the twin that had kwashiorkor compared to those that received microbes from the unaffected twin. Another study elegantly links specific nutrition factors to microbial ecology and the complex biological consequences that occur in the intestinal epithelial cells [23]. This study examined infant fecal microbiota with varying human milk oligosaccharide consumption and found that differences in microbiota modulated major gene networks including signal transduction, inflammation, histamine, cell migration and adhesion.GIT motility is another major function that is affected by the intricate interactions amongst diet and microbes. When humanized mice were fed a diet containing fermentable fructooligosaccharides (FOS), gastrointestinal transit time was altered [24].

Dietary factors alter the microbial ecology in the small intestine where food antigens are primarily digested, as well as the cecum and the distal colon where digestion is not a main function of the host but an important function of the microbes. High-fat feeding induces dysbiosis through the direct antimicrobial activity of bile. Insoluble lipid molecules are broken into small droplets by bile and lipases which become soluble free fatty acids and monoglycerides, which then enter the bloodstream. As shown, bile secreted during high-fat feeding could affect the growth or survival of some microbes [25], although we have found that varying types of fatty acids play more of a role in dysbiosis than high-fat feeding alone [19, 20]. The process of lipid digestion may give more clues as to how microbes could be related to various diseases.

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**Clinical Evidence for Intestinal Dysbiosis-Associated Diseases**

A healthy microbiota is defined by high diversity and an ability to resist change under physiological stress. In contrast, microbiota associated with disease is defined by lower species diversity, fewer beneficial microbes and/or the presence of pathobionts. Given the role of the microbiota in mediating host metabolism and immunity, disruption of the microbiota has been associated with various human diseases of the GIT and systemically throughout the body. Here, we review evidence from recent clinical studies connecting dysbiosis to various diseases, with an emphasis on the involvement of dietary factors.

**Intestinal Dysbiosis in Gastrointestinal Diseases**

The functional roles of the human GIT include nutrient absorption, waste removal via peristalsis, defense against ingested pathogens and prevention of translocation of food or antigens into the bloodstream. The gut microbiota regulates several of these functions including peristalsis, barrier function and maintaining balanced inflammatory and homeostatic responses. Disruption of the gut microbiota renders the GIT vulnerable to local disease states (fig. 3).

**Inflammatory Bowel Diseases**

Clinical studies have identified dysbiosis in patients with inflammatory bowel disease (IBD), including both Crohn’s disease (CD) and ulcerative colitis (UC). Studies examining twins have shown enriched Actinobacteria and Proteobacteria and reduced Bacteroidetes in the twins with UC as compared to their healthy siblings [26]. An increase in sulfide-generating Desulfovibrio subspecies and Fusobacterium varium that can invade the epithelium are present in UC patients [27], while anti-in-
Inflammatory-associated *Faecalibacterium prausnitzii* is reduced [28]. A typical trait of human IBD patients is reduced gut microbial biodiversity [29, 30]. For example, patients with CD had reduced levels of *Faecalibacterium* and *Roseburia*, increased *Ruminococcus* [30] and Enterobacteriaceae including adherent-invasive *Escherichia coli* [31]. Excessive milk fat [32] and omega-6 PUFA [19] were shown in rodents to exacerbate IBD through dysbiosis, which is supported by a 30% increased risk for UC by excessive consumption of omega-6 PUFA [33].

Colorectal Cancer

The adaptation of African-Americans to Western diets has been shown to increase the incidence of and mortality due to colorectal cancer (CRC) corresponding to altered fecal microbial profiles [34]. CRC patients are shown to have increased levels of certain bacterial species such as *Bacteroides fragilis*, *Enterococcus*, *Escherichia/Shigella*, *Klebsiella*, *Streptococcus*, *Peptostreptococcus*, *Roseburia* and decreased abundance in butyrate-producing *Lachnospiraceae* [35]. Growing evidence supports an inverse relationship between dietary fiber, fruit and vegetable intake to CRC development risk. Long-term fiber intake can result in a microbiota enterotype that positively associates with Firmicutes and Proteobacteria and inversely with Bacteroidetes, Actinobacteria [6] and *Bifidobacteria* [36]. This may be through improved intestinal barrier function since beneficial microbes improve barrier integrity and this is associated with decreased complications in patients undergoing colectomy [37]. Dietary fiber intake can also reduce the risk of CRC development by promoting a gut microbiota that is enriched by SCFA production [38].

Irritable Bowel Syndrome

Diet and gut microbiota are two crucial components implicated in the pathogenesis of irritable bowel syndrome (IBS). Poorly absorbed dietary carbohydrates induce prolonged hydrogen production in the intestines of patients with IBS (Rome III criteria), which is important since the amount of methane produced corresponds with disease symptoms [39]. IBS patients have an altered carbohydrate and protein energy metabolism in the gut, accompanied by changes in the diversity of particular gut bacterial genera [40], where enriched Firmicutes and reduced Bacteroidetes are found to be associated with a distinct subset of IBS patients [41]. Studies conducted in diarrhea-dominated IBS patients show reduced fecal

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Chan/Estaki/Gibson

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DOI: 10.1159/000354902
aerobic bacteria, *Bifidobacteria* and *Verrucomicobium* and an increase in *Lactobacillus, Veillonella, Prevotella* and *Parasporo* [42, 43]. In addition, the increase in *E. coli* and decrease in *Leptum* and *Bifidobacteria* and bacteria involved in bile acid transformation is accompanied by an increase in fecal bile acids, which acts as an endogenous laxative further exacerbating disease symptoms [44].

**Intestinal Dysbiosis in Systemic Diseases**

In addition to local GIT diseases, intestinal dysbiosis is also associated with systemic diseases such as obesity, diabetes, atherosclerosis and nonalcoholic fatty liver disease (NAFLD) (fig. 3). Indeed, many metabolic diseases are associated with chronic inflammation induced by lipopolysaccharide, a major component of the outer membrane of Gram-negative bacteria. Other causative factors associated with the intestinal microbiota include gut barrier dysfunction, immunomodulation, production of SCFA and other metabolites, as well as changes to metabolic pathways involved in nutrient or energy harvest.

**Obesity**

Current evidence reveals that gut microbes are critical in overall energy harvest influencing obesity [45]. Fat- and carbohydrate-restricted diets lead to increased Bacteroidetes and decreased Firmicutes [46]. Other diets with low carbohydrate/high protein content, resistant starch [47] or high dietary fiber [48] also lead to distinct increases in various bacterial populations. Obese children have a microbiota enriched with Enterobacteriaceae [49], reduced *Bacteroides* and Bacteroidetes to Firmicutes ratio that are negatively correlated with body mass index [50]. Obese children also have increased Desulfovibrio and Akkermansia muciniphila [49] found important for gut barrier dysfunction [51]. Moreover, obese children also have increased SCFAs and more exhaustive substrate utilization implicating that microbes are capable of increased energy harvest [52]. Similarly, obese adolescents had gut microbes that were more engaged in de novo B12 synthesis and butyrate production [53]. Finnish women with metabolic disorders were found to have increased *Eubacterium rectale-Clostridium coccoides* that efficiently harvest energy and nutrients positively correlated with several metabolic markers [54].

**Diabetes**

Type 2 diabetes (T2D) is a metabolic disorder defined by insulin resistance, impaired intestinal permeability, endotoxemia and chronic inflammation, all of which are linked to diet-induced dysbiosis [55]. Patients with T2D have been shown to have a fecal microbiota with reduced populations of Firmicutes including microbes from the Clostridia clusters [56]. Recently, a metagenome-wide association study involving 345 Chinese subjects had their fecal microbiota shotgun sequenced and dysbiosis was confirmed in patients with T2D. The results revealed that the patients' fecal microbiota was enriched with more opportunistic pathogens and less microbes involved in butyrate production. This resulted in increased microbial functions involving sulfate reduction and oxidative stress resistance [57]. Another study found that T2D patients of Chinese origin also displayed a microbiota decreased in *Bifidobacteria* spp. [58], a beneficial microbe often shown to be decreased in rodent models of T2D. Although mounting evidence reveals that intestinal microbes are important in T1D pathogenesis, so far, little evidence has linked dietary factors to disease progression.

**Atherosclerosis**

Recent evidence reveals that gut microbiota participates in atherosclerosis, a chronic inflammatory condition of the arteries with the formation of multiple plaques that restrict blood flow. Various microbial byproducts or so-called microbial-associated molecular patterns (MAMPs) play a pivotal role in atherogenesis [59]. Additionally, the metabolism of dietary phosphatidylcholine and the subsequent generation of cardiovascular disease risk markers are gut microbiota dependent [60]. Specific bacterial phylotypes are present in atherosclerotic plaques that are common to oral or gut samples from patients with atherosclerosis, where the amount of bacterial DNA correlated with the amount of leukocytes found in the atherosclerotic plaque [61]. Shotgun sequencing of fecal samples revealed that the *Bacteroides* - and *Ruminococcus*-domi-
Nated enterotypes were under- and overexpressed, respectively, in atherosclerotic patients. The disease microbiome was enriched in genes encoding peptidoglycan synthesis but depleted in phytoene dehydrogenase required for metabolism of lipid-soluble antioxidants [4]. Although there are limited clinical studies to date, they present a powerful message to potential therapeutic strategies against atherosclerosis targeted to the gut.

Nonalcoholic Fatty Liver Disease

NAFLD is associated with small intestinal bacterial overgrowth (SIBO) and the resulting effects of increased acetaldehyde, trimethylamine, trimethylamine N-oxide and tumor necrosis factor-α [62]. Given that the gut and liver are connected by the portal venous system, it makes the liver more vulnerable to translocation of bacteria, bacterial products, endotoxin or secreted cytokines. An obesogenic microbiota can alternate liver function by stimulating hepatic triglyceride and modulating systemic lipid metabolism that indirectly impact the storage of fatty acids in the liver [63]. In support of this, SIBO correlates with a leaky gut in humans [64] and hepatic steatosis in obese patients [65]. The severity of NAFLD is associated with chronic endotoxin exposure in humans [66]. Choline deficiency and fatty liver development have also been associated with changes in abundance of γ-Proteobacteria and Erysipelotrichi [67]. The diet-induced change of such bacterial abundance further helps predict the risk of fatty liver development.

Bacteriotherapy to Promote a Healthy Microbial Profile

Diet is considered a modifiable intervention; however, our understanding of how to manipulate diet to promote a healthy microbiota is in its infancy, since the effects of many dietary factors are frequently changed, disputed or simply lack evidence. A novel approach to alter our intestinal microbes is by the use of bacteriotherapy (fig. 4). While bacteriotherapy may be an alternative approach to preventing, treating or even curing ailments, there is a lack of clarity as to its efficacy in humans.
Table 2. Summary of clinical studies using probiotics against dysbiosis-induced diseases

<table>
<thead>
<tr>
<th>Probiotics</th>
<th>Dose, CFU/day</th>
<th>Duration</th>
<th>Sample size</th>
<th>Subject's condition</th>
<th>Gut/fecal microbiota change</th>
<th>Method of bacterial detection</th>
<th>Outcome (generalized)</th>
<th>Ref.</th>
<th>PubMed accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B. bifidum</strong></td>
<td>$1 \times 10^9$</td>
<td>4 weeks</td>
<td>122</td>
<td>IBS</td>
<td>improved IBS symptoms and QOL</td>
<td>NA</td>
<td>improved IBS symptoms and QOL</td>
<td>21418261</td>
<td></td>
</tr>
<tr>
<td><strong>VSL#3</strong></td>
<td>$9 \times 10^{11}$</td>
<td>8 weeks</td>
<td>24</td>
<td>IBS, diarrhea</td>
<td>no change</td>
<td>microarray hybridization</td>
<td>no major changes</td>
<td>22247743</td>
<td></td>
</tr>
<tr>
<td><strong>L. acidophilus</strong></td>
<td>$1 \times 10^9$</td>
<td>4 weeks</td>
<td>60</td>
<td>IBS</td>
<td>improved IBS symptoms</td>
<td>qPCR</td>
<td>improved IBS symptoms</td>
<td>22837798</td>
<td></td>
</tr>
<tr>
<td><strong>L. plantarum</strong></td>
<td>$1 \times 10^9$</td>
<td>6 weeks</td>
<td>26</td>
<td>IBD, mild left-sided UC</td>
<td>no change</td>
<td>direct sequencing, DGGE</td>
<td>decreased inflammation</td>
<td>20737710</td>
<td></td>
</tr>
<tr>
<td><strong>B. longum</strong></td>
<td>$1 \times 10^9$</td>
<td>4 weeks</td>
<td>60</td>
<td>IBS</td>
<td>improved IBS symptoms</td>
<td>qPCR</td>
<td>no major changes</td>
<td>17312986</td>
<td></td>
</tr>
<tr>
<td><strong>L. casei</strong></td>
<td>$1.6 \times 10^9$</td>
<td>8 weeks</td>
<td>26</td>
<td>IBD, mild left-sided UC</td>
<td>↑ Lactobacillus spp.</td>
<td>↑ Bifidobacteria spp.</td>
<td>↑ Lactobacillus spp.</td>
<td>20737210</td>
<td></td>
</tr>
<tr>
<td><strong>L. plantarum</strong></td>
<td>$2.6 \times 10^{10}$</td>
<td>6 days pre- and 10 days postoperatively</td>
<td>100</td>
<td>CRC</td>
<td>no change</td>
<td>↑ Enterobacteriaceae</td>
<td>↑ Bacterial variety,↑ Lactobacillus spp.,↑ Enterobacteriaceae</td>
<td>21083585</td>
<td></td>
</tr>
<tr>
<td><strong>L. acidophilus</strong></td>
<td>$2 \times 10^9$</td>
<td>15 days</td>
<td>8</td>
<td>constipation</td>
<td>no change</td>
<td>↑ Lactobacillus spp.</td>
<td>↑ Lactobacillus spp.</td>
<td>22899904</td>
<td></td>
</tr>
<tr>
<td><strong>L. casei</strong></td>
<td>$1.95 \times 10^{10}$</td>
<td>3 months</td>
<td>26</td>
<td>metabolic syndrome</td>
<td>no change</td>
<td>↑ Lactobacillus spp.</td>
<td>↑ Lactobacillus spp.</td>
<td>22872030</td>
<td></td>
</tr>
<tr>
<td><strong>L. salivarius</strong></td>
<td>$1 \times 10^9$</td>
<td>12 weeks</td>
<td>60</td>
<td>IBD, mild left-sided UC</td>
<td>no change</td>
<td>↑ Enterobacteriaceae</td>
<td>↑ Lactobacillus spp.</td>
<td>22837798</td>
<td></td>
</tr>
<tr>
<td><strong>L. rhamnosus</strong></td>
<td>$1 \times 10^10$</td>
<td>6 weeks</td>
<td>16</td>
<td>overweight</td>
<td>no change</td>
<td>↑ Lactobacillus spp.</td>
<td>↑ Lactobacillus spp.</td>
<td>20231842</td>
<td></td>
</tr>
<tr>
<td><strong>B. longum</strong></td>
<td>$2.6 \times 10^{10}$</td>
<td>6 days pre- and 6 weeks post-delivery</td>
<td>159</td>
<td>pregnant</td>
<td>no change</td>
<td>↑ Lactobacillus spp.</td>
<td>↑ Lactobacillus spp.</td>
<td>22095470</td>
<td></td>
</tr>
<tr>
<td><strong>L. acidophilus</strong></td>
<td>$2 \times 10^8$</td>
<td>56 weeks</td>
<td>76</td>
<td>healthy</td>
<td>no change</td>
<td>↑ Lactobacillus spp.</td>
<td>↑ Lactobacillus spp.</td>
<td>16025386</td>
<td></td>
</tr>
<tr>
<td><strong>B. longum</strong></td>
<td>$1 \times 10^9$</td>
<td>4 weeks</td>
<td>36</td>
<td>heavy smokers</td>
<td>no change</td>
<td>↑ Lactobacillus spp.</td>
<td>↑ Lactobacillus spp.</td>
<td>12450890</td>
<td></td>
</tr>
<tr>
<td><strong>L. plantarum</strong></td>
<td>$1 \times 10^9$</td>
<td>4 weeks</td>
<td>16</td>
<td>irritable bowel syndrome</td>
<td>no change</td>
<td>↑ Lactobacillus spp.</td>
<td>↑ Lactobacillus spp.</td>
<td>19601815</td>
<td></td>
</tr>
</tbody>
</table>

CFU = Colony forming units; conc. = concentration; DGGE = denaturing gradient gel electrophoresis; NA = not available; NASH = nonalcoholic steatohepatitis; QOL = quality of life; qPCR = quantitative polymerase chain reaction; REP-PCR = repetitive sequence-based polymerase chain reaction; T-RELP = terminal restriction fragment length polymorphism.
Probiotics, Prebiotics and Symbiotics

Probiotics are defined as live microorganisms which confer health benefits to the host when taken in adequate quantities. These are discussed by Versalovic in this issue. Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of beneficial colonic bacteria. A combination of probiotics and prebiotics is termed symbiotics. Probiotics are strain specific and require sufficient dosages and time to exert efficient effects. Various kinds of probiotics have been tested clinically as potential therapeutic agents for both localized and systemic diseases. A recent review of the effects of probiotics on health and disease is shown in table 2. The effects of probiotics in local gastrointestinal diseases are generally positive, although there is usually a lack of evidence that the effects were gut microbiota mediated. While still largely unknown, the effects of probiotics in systemic diseases are more variable.

Antibiotics

It is well documented that antibiotic treatments cause aberrancies in the host microbiota. Though it is generally believed that such changes are normalized within weeks of cessation of antibiotics, recent evidence challenges this notion [68]. For example, significant reduction in diversity of Bacteroides persisted up to 2 years following 7 days of Clindamycin administration [68]. In the context of dysbiosis, antibiotics thus can be viewed as a double-edged sword. They are effective in eradicating pathogens but also non-specifically reduce microbial diversity enabling opportunistic bacteria to colonize the newly hospitable niches in the gut ecosystem. Such is the case of Clostridium difficile, an opportunistic pathogen which emerged in the 1970s in patients treated with Clindamycin [69]. Another example of antibiotics’ conflicting nature in dysbiosis is their effect on IBD. For instance, the use of ciprofloxacin has been shown clinically to modestly improve symptoms and remission rates of patients with CD [70]; however, antibiotic exposure in childhood has been associated with development of IBD in later years [71]. In a clinical setting, this raises important concern regarding the appropriate use or avoidance of antibiotics. It is important to develop more specific antimicrobial or concurrent therapies to restore or minimize disturbances to the normal microbiota.

Fecal Transplantation

One promising approach for relieving dysbiosis-associated diseases is the re-establishment of normal microbiota via transplantation of a healthy donor’s stool into a symptomatic host, called fecal transplantation (FT) (fig. 4). In clinical settings, FT has emerged as a much more effective and safer procedure than standard antibiotics treatment in the immediate and lasting resolution of recurrent C. difficile. Currently, this procedure suffers from a lack of standardization; however, its success rate being above 95% [72] and seemingly lack of adverse effects has led experts to investigate its use in treatment of other chronic illnesses such as IBD [73] and metabolic syndrome [74]. As our understanding of the essential role that host microbiota plays in disease and immunity increases, the use of microbiota manipulation therapies becomes more sensible. For example, one possible future venue for FT is the use of a patient’s own stored healthy stool to restore their intestinal microbiota following antibiotic treatment or disease onset. Due to its inexpensive nature, FT might be particularly favorable in populations where expensive treatments are not easily accessible.

Conclusions

Interactions between different dietary factors and gut microbes may lead to dysbiosis that exerts distinct immune responses in the host, resulting in higher susceptibility to various gastrointestinal and systemic diseases. Restoration and maintenance of a healthy gut microbiota may be an effective, inexpensive and safe remedy to these diseases.

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The production of a variety of neuroactive signaling molecules by bacterial components of the microbiome provides additional evidence that the gut microbiome may generate signals with remote effects in different organ systems, including the central nervous system.

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The Human Microbiome and Probiotics: Implications for Pediatrics
by James Versalovic

Key insights
Knowledge of the composition and function of the human microbiome at multiple body sites including the gut, skin and airways contributes to our understanding of the mechanisms of probiosis. In turn, an enhanced understanding of the effects of probiotics on the microbiome should facilitate selection of optimal probiotic strains for the treatment of specific diseases.

Current knowledge
The human microbiome is composed of bacteria, viruses (including bacteriophages), fungi, archaea and protozoa. Each body site has its own distinct microbiome, with a unique microbial composition that presumably reflects the differences in tissue structure and function. For decades, it has been widely accepted that the ingestion of probiotic strains has beneficial effects. The work of the Human Microbiome Project has provided a wealth of data on the taxonomic profiles of over 5,000 bacterial strains from 18 different body sites. A major challenge is to quantify the relative abundance of the different strains in health and disease, before we can harness the full potential of probiotics.

Practical implications
Knowledge of the intestinal microbiome provides an opportunity for understanding the roles of the microbial populations in other parts of the body. The compositional differences between the gut microbiomes of infants who develop necrotizing enterocolitis (NEC) versus healthy infants underscore the need to tip the balance in favor of beneficial strains. Recent studies have demonstrated that probiotics or symbiotics can have a direct impact on gut microbial composition and profoundly affect the clinical outcomes for infants with NEC. The successful application of probiotics for NEC has been extended to the treatment of other intestinal disorders and provides a paradigm for the therapy of other conditions such as asthma.

Recommended reading
The Human Microbiome and Probiotics: Implications for Pediatrics

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Key Messages
- The composition and function of the microbiome can be changed by probiotics.
- The composition of the human microbiome at each body site is distinct, and different probiotics are likely needed for diseases at different body sites.
- Microbial deficiencies may be treated by probiotics as a strategy for microbial supplementation and promotion of microbial diversity.
- Probiotics may change the ability of the microbiome to produce nutrients and bioactive compounds by de novo biosynthesis or by luminal conversion.

Key Words
Human microbiome · Probiotics · Bacteria · Antibiotics · Microbes · Metagenomics · Beneficial microbes · Dysbiosis

Abstract
Steady advances in our knowledge of the composition and function of the human microbiome at multiple body sites including the gut, skin and airways will likely contribute to our understanding of mechanisms of probiotic action by beneficial microbes. Microbe:microbe and microbe:human interactions are important considerations as we select probiotics for pediatric patients in the future. Although our knowledge about the composition of the microbiome is progressing rapidly, many gaps exist about the functional capacity and metabolic machinery of the human microbiome. Based on a limited amount of data, probiotics appear capable of altering the composition and function of the microbiome. Probiotics may be part of dietary strategies that combine ways to enhance microbiome function with nutrients that may be converted to active compounds promoting human health. Probiotics have yielded beneficial effects in numerous studies in the context of different diseases in pediatric gastroenterology. These disease states include necrotizing enterocolitis, antibiotic-associated diarrhea and colitis, acute gastroenteritis and irritable bowel syndrome. In the skin and airways, it is unclear if probiotics can affect the function of the microbiome to reduce the impact of diseases such as asthma and atopic dermatitis. An enhanced understanding of the effects of probiotics on the microbiome should facilitate selection of optimal probiotic strains for specific diseases in the future.

Pediatrics, the Microbiome and Probiotics
The practice of pediatrics, as in other medical specialties, has viewed microorganisms with a defensive posture and considered each bacterium, fungus or virus as a potential infectious agent. The prevailing ‘infectious diseases/antimicrobial’ strategic world view in medicine be-
came a predominant view since the first 3 decades of the twentieth century. As infectious agents and infectious diseases were being characterized a century ago, the beginning of antimicrobial agent discovery and consideration of antibiotics as novel treatments began to take shape. In fact, phage therapy as an antimicrobial strategy in medicine was being considered during the second decade of the 1900s (reviewed by Pirisi [1] and Keen [2]), and arsphenamine (Salvarsan) and its less toxic derivative (Neosalvarsan), discovered by Ehrlich and Hata in 1910, were being applied to treat different infections. In 1928 (reprinted by Fleming [3]), Sir Alexander Fleming’s discoveries led to the identification of antibacterial compounds produced by specific fungi and laid the foundation for the antimicrobial era. This prevailing world view in medicine dominated the landscape of pediatrics until the first decade of this century, when the attitudes towards commensal and beneficial microbes began to change profoundly.

Beneficial microbes and more specifically probiotics were described initially by Nobel laureate Elie Metchnikoff [4] in 1907/1908, when he described the potential benefits of consumption of large quantities of microbes to improve longevity and human health. Unfortunately, this viewpoint was effectively subordinated to the view that microbes must be considered as potentially infectious agents, and most attention in the medical profession including pediatrics turned to vaccine development and antibiotic production. The term ‘probiotic’ was first credited to Lilly and Stillwell [5] who proposed this term in the context of microbes producing substances that promoted the growth of other microorganisms. Parker [6] was the first to use the term ‘probiotic’ to describe microorganisms (and substances) that have beneficial effects on a host animal. Outside of the dairy and fermented food industry, the probiotic concept remained largely dormant until the late 1980s. The British Professor R. Fuller [7] described the modern probiotic concept in a landmark review published in 1989 and proposed the importance of microbial viability to probiotic function. A formal definition of probiotics was formulated in 2001 by the advisory body of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), and this definition has been widely utilized during the past 12 years. This definition states that probiotics are ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’ [8].

The emergence of investigations concerning the nature and mechanisms of probiosis during the 1990s and the rapid coalescence of the human microbiome research community globally since 2005 [9] provided the foundation for the current era in metagenomics (the genomic analysis of microorganisms by direct extraction and cloning of DNA from an assemblage of microorganisms). Human microbiology includes canonical and opportunistic infectious agents, but this field has rapidly expanded to embrace the many commensal and beneficial microbes that may contribute to human health and disease prevention. The specialty of pediatrics has been swept into this new era of human microbiology and medicine as a result of numerous publications describing the composition of the microbiome in children and differences in the microbiome associated with diseases of childhood [10]. Alterations in microbial composition associated with human diseases have been described as examples of dysbiosis. Dysbiosis refers to differences in microbial populations that may reflect an abnormal ecological state contributing to pathology or the excess of pathogenic mechanisms within the human microbiome [see Chan et al. in this issue]. Functional components of the microbiome may be studied by determination of DNA sequences or genes present in the microbiome, but this information only reveals the metabolic capacity. RNA sequencing and metabolomics studies are necessary to determine which microbial genes are expressed and which metabolites may affect disease susceptibilities in children.

The human microbiome is composed of bacteria, viruses (including bacteriophages), fungi, archaea and prototzoa in declining order. Human-associated bacterial species comprise the vast majority of the human microbiome in terms of microbial DNA content and cell count. In fact, in one recent study, more than 99% of mapped DNA sequencing reads in healthy adults were bacterial sequences [11]. Human-associated bacterial communities are composed of four dominant phyla (Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria) and a number of minority phyla [12]. A recent study of the biogeography of the human microbiome identified 30 phyla in 18 different body sites in neonates and adults [13], and reports of cultured and uncultured bacteria estimates ap-

**Human-associated bacterial species comprise the vast majority of the human microbiome in terms of microbial DNA content and cell count.**
The composition of the microbiome is distinct at each body site or habitat, each body site has a distinct microbial composition and, presumably, differences in function translate to differences in physiology at each body site (e.g. skin and intestine) (fig. 1).

The relative abundance of probiotic genera and species in the healthy human microbiome is a relevant consideration, as well as whether microbial deficiencies in individual species could be readily corrected by administration of probiotics to children. Alternatively, do probiotics simply enhance the ability of other bacterial genera to proliferate and reduce the numbers of potentially harmful bacteria? Rational probiotic strategies could be developed that take advantage of predictable changes in microbial composition following probiotic therapy. For 2 decades, we had scientific evidence that ingestion of probiotics in human volunteers resulted in persistence of probiotic strains (lactobacilli) 11 days later in the small intestine with corresponding reductions in other bacterial genera [16]. The initial comprehensive summary of the Human Microbiome Project included 242 individuals with greater than 5,000 bacterial taxonomic profiles from 18 body sites in healthy adults [11, 12]. The Firmicutes and Bacteroidetes were the dominant phyla in the gastrointestinal tract, and Lactobacillus was a minority Firmicute genus in these individuals. The phylum Actinobacteria includes the genus Bifidobacterium which has been underrepresented in human microbiome studies due to technical challenges in DNA amplification/detection methods. Focused efforts to quantify the relative abundance of Bifidobacterium have determined that this genus is present as a minority genus in the colon.

**Human Gut Microbiome, Diet and Probiotics**

Human nutritional components and dietary patterns clearly impact microbial composition and presumably function in the intestine. Children consuming a high-fiber, plant-based diet in western Africa demonstrated a relative abundance of two bacterial genera, Prevotella and Xylanibacter, that may contribute to digestion of plant components and fiber in the diet. These two genera contain genes and pathways capable of metabolizing cellulose and xylan in the diet, and these genera are rare or absent in children consuming a westernized diet in southern Europe [17]. A more recent study also described major differences in the fecal microbiomes of children in the USA and Bangladesh [18]. Although fundamental long-term differences in the diet clearly seem to affect gut microbial composition, short-term (days) major changes in diet do not have a correspondingly major impact on gastrointestinal microbial composition [19]. It appears that major changes in the diet will require longer time periods (months to years) to profoundly shift the composition of the gut microbiome in human individuals. As health care providers consider probiotic strategies, it is important to also keep in mind that short-term consumption of probiotics does not appear to shift microbial composition, but studies in mouse models suggest that the major impact of probiotics in the short term may pertain to changes in microbial gene expression and metabolite production [20].

In addition to the effects of diet and probiotic consumption on the composition of the gastrointestinal microbiome, prebiotics or symbiotic combinations may also affect gut microbial composition. Gibson et al.’s [21] landmark paper in 1995 provided evidence for the prebiotic concept and the idea that indigestible oligosaccharides would provide a substrate for beneficial microbes in
the intestine. Human milk oligosaccharides (HMOs) have been characterized in recent years as a distinguishing feature from bovine milk, and these milk components appear to promote the proliferation and biological functions of probiotic genera such as Bifidobacterium spp. [22]. For several decades, it has been recognized that breast milk-fed infants demonstrated relative resistance to infectious gastroenteritis [23]. The supplementation of probiotics to infant diets yielded protection against diarrhea and rotaviral infection [24]. So, the concepts of probiotics, probiotics and effects on the resilience and diversity of the microbiome become intertwined, and a broader consideration of combinations of probiotics with nutritional strategies may result in predictable changes to the function of the microbiome. The ‘center stage’ importance of nutrition early in life and during childhood and adolescent development highlights the potential impact of probiotics on basic aspects of human nutrition and nutrient availability. Twin pairs in eastern Africa were differentially susceptible to the condition of undernutrition known as kwashiorkor, based on differences of the composition of their intestinal microbiomes [25]. Presumably, susceptibilities to the clinical phenotypes of undernutrition are affected by the metabolic capacity of the microbiome and the abilities of gut bacteria to convert foodstuffs into available nutrients for childhood development.

Gut bacteria synthesize and convert a variety of compounds that impact the physiology, immunity and presumably disease susceptibility or resistance of human individuals. Examples of de novo biosynthesis pathways in the gut microbiome and probiotics include the synthesis of B complex vitamins such as vitamin B_{12} (cobalamin) [26] and vitamin B_{1} (thiamine) [27]. Examples of luminal conversion include the conversion of plant lignins to enterolignins, vitamin K_{1} (phylloquinone) to vitamin K_{2} and analogs (menaquinones) [28], amino acid decarboxylation reactions generating biogenic amines (e.g. histidine/histamine, glutamate/GABA) [29, 30] and carbohydredietary fiber conversion to short chain fatty acids (SCFAs) [31]. With respect to amino acid metabolism, oral administration of probiotic Bifidobacterium species can stimulate peripheral blood-derived immune cells to produce greater amounts of the immunosuppressive cytokine interleukin-10 (IL-10) and the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO) [32]. IDO facilitates the conversion of the amino acid tryptophan to the metabolite kynurenine, and this metabolic conversion has been associated with virus- and tumor-induced immunosuppression. Kynurenine may suppress inflammation in the context of a proinflammatory microbiome or host condition. Conjugated linoleic acids are fatty acid derivatives produced by various probiotic species, generating compounds with anti-inflammatory and anticarcinogenic effects [33]. Probiotic Lactobacillus plantarum administration in dairy goats resulted in shifts in gut microbiobal composition and alterations in milk fat composition including greater amounts of polyunsaturated fatty acids such as linoleic acid [34]. In summary, the human microbiome contains an enormous capacity for converting nutrients and dietary substrates, and this functional capacity can be affected by administration of probiotic strains.

**The human microbiome contains an enormous capacity for converting nutrients and dietary substrates, and this functional capacity can be affected by administration of probiotic strains.**

**Microbiome, Probiotics and Pediatric Gastroenterology**

**Early Microbial Ecology in the Gut**

The microbial ecology of the human intestine provides an opportunity to understand the nature of microbial populations in the human body and how these populations influence gene expression patterns and the ultimate functionality of the microbiome. It is unclear whether the fetus is exposed to microbes or their metabolites or DNA, although investigators are actively exploring the relationship of the human microbiome to the in utero environment and mode of delivery [see Luoto et al. in this issue]. During infancy, the composition of the gut microbiome fluctuates rapidly [35] and reaches an adult-like equilibrium by 3 years of age in one study [36] (fig. 2).

**Necrotizing Enterocolitis**

The development of the intestinal microbiome in early life and its importance has been highlighted in the preterm neonate at risk for developing necrotizing enterocolitis (NEC) [see Walker in this issue]. The gut microbiome of infants who developed NEC was characterized by compositional differences such as increased abundance of γ-Proteobacteria [37], a common feature of the gut microbiome in disease states. Compositional differences in...
the fecal microbiota preceded the development of NEC. Possibly, probiotics or nutritional approaches could shift microbial composition towards a more disease-resistant gut microbiome. A diet composed entirely of human milk has been effective in reducing the incidence of NEC in premature infants, and donor human milk supplementation has been recommended as one strategy to prevent NEC [38, 39]. In addition to human milk supplementation, several probiotics have yielded success in the prevention of severe NEC and all-cause mortality in premature infants, including very-low-birth-weight infants [40, 41]. Data could not be extrapolated to extremely-low-birth-weight infants. Heterogeneity among the clinical trials in terms of design and probiotic strains prevents any firm recommendation regarding a specific probiotic strain for prevention of NEC [42]. An increased incidence of NEC has been associated with administration of histamine 2 receptor (H2R) antagonists as acid blockers to preterm infants [43, 44]. The histamine signaling pathway may provide an opportunity for targeted interventions of probiotics based on known mechanisms. Probiotics capable of converting the dietary amino acid L-histidine to histamine have been reported [29], and histamine may suppress inflammation by promoting H2R signaling in the intestinal mucosa.

Recurrent Clostridium difficile Infection and Acute Gastroenteritis

Disorders of microbial ecology may be caused by consumption of antimicrobial agents (antibiotics) and chemotherapy, so that iatrogenic infections may be corrected by probiotics. In the past decade, the incidence of pediatric Clostridium difficile-associated disease has steadily increased [45]. Gorbach et al. [46] demonstrated successful treatment of C. difficile disease using a single strain of human-derived Lactobacillus rhamnosus (LGG). These findings laid the foundation for the generalized acceptance of probiotics in the last decade of the twentieth century. This strain of interest became commonly known as the probiotic LGG and has been applied in numerous pediatric studies [47, 48]. Several studies in children have demonstrated that probiotics may be effective at suppressing antibiotic-associated diarrhea [49, 50], and probiotics may promote restoration of microbial diversity as one mechanism for amelioration of the disease phenotype [51]. Prior evidence showed that the human intestinal microbiome was restricted in terms of bacterial diversity in patients with recurrent C. difficile disease [52]. Presumably, a gut microbiome with limited diversity (an example of dysbiosis) creates a permissive environment for recurrent colitis due to C. difficile, and probiotics may be useful by promoting increased gut bacterial diversity. The success of fecal microbiota or intestinal microbiome transplantation in C. difficile-associated disease [53] provides additional evidence that restoration of sufficient bacterial diversity and functional capacity can effectively treat this disorder of microbial ecology.

The American Academy of Pediatrics (AAP) endorsed the application of probiotics for the prevention of antibiotic-associated diarrhea and the treatment of acute viral gastroenteritis in healthy children [54]. Although data are lacking with respect to changes in the intestinal microbiome in cases of acute bacterial or viral gastroenteritis in humans, probiotics have demonstrated their ability to shorten the course of disease and ameliorate symptoms in several studies spanning 2 decades [49, 55]. The addition of probiotics may stimulate the mucosal immune system and the microbiome’s own defense mechanisms, resulting in rapid pathogen clearance and mucosal healing.

Celiac Disease

Although the microbial composition of the small intestine does not appear to differ in patients with celiac disease, differences in the intestinal microbiome were detected in self-collected stool specimens obtained from patients with celiac disease [56]. Corresponding changes in the fecal microbiome and the fecal and urinary metabolome were reported in children with celiac disease compared to healthy controls [56]. Breast milk feeding with
its possible ‘feeder’ effects on beneficial microbes and HLA genotype were found to influence the relative susceptibilities to celiac disease in the PROFICEL study [57]. In a provocative report, the relative timing of gluten introduction in early childhood appeared to impact disease susceptibility, and the disease phenotypes were correlated with changes in the intestinal microbiome and metabolome [58]. An interesting aspect relevant to celiac disease is the presence of gluten-metabolizing bacterial genera such as *Rothia* spp. in the oral microbiome [59]. Gluten-metabolizing microbes in the oral or intestinal microbiomes may reduce the relative susceptibilities of individuals with a genetic predisposition to celiac disease due to HLA gene polymorphisms. Future probiotic strategies may include consideration of probiotics with gluten metabolism genes and the ability of probiotics to enhance the function of such gluten-metabolizing bacteria in the microbiome.

**Irritable Bowel Syndrome**

Differences in composition or dysbiosis of the gut microbiome in irritable bowel syndrome (IBS) were first reported with comprehensive DNA sequencing and array studies in 2011 [60, 61]. Disease signatures based on differences in bacterial composition were detected and distinguished patients with more frequent abdominal pain and more severe gastrointestinal disease phenotypes. Overlapping features included the enrichment of γ-Proteobacteria in children and adults with IBS, and an association of this group containing known enteric pathogens with increased pain symptoms. Subsequent studies confirmed and extended these findings regarding distinct compositional differences of the intestinal microbiome in IBS [62–64]. In children, a follow-up study described differences in the fecal microbiome such as relative deficiencies of the genera *Bifidobacterium* and *Verrucomicrobiunm* in children with IBS-diarrheal predominant subtype (IBS-D) [65]. Administration of *Bifidobacterium* spp. in adult patients with IBS reduced symptoms and highlighted the potential benefits of probiotic therapies in IBS with carefully selected probiotic strains [66, 67]. Reduced amounts of *Bifidobacterium* spp. in the intestinal microbiomes of children and adults with IBS point towards a rational basis for supplementation of ‘missing’ or deficient bacteria in disease conditions as a way to prevent or treat disease. The suggested importance of intestinal *Bifidobacteria* may explain the relative success of a *Bifidobacterium-Lactobacillus* combination strategy [68] versus a *Lactobacillus*-only probiotics strategy [69] in children with IBS.

A general consensus in the field is that many functional gastrointestinal disorders including IBS can best be understood as disorders of brain-gut interactions [70]. The brain-gut axis represents a bidirectional connection between the digestive and nervous systems with emerging importance to human biology and medicine [70]. Recent preclinical evidence suggests that changes in the composition and function of the mammalian gut microbiome can affect brain systems related to pain and affect regulation [71]. In rodents, the lack of gut microbes in germ-free mice [72, 73] and the modulation of gut microbial ecology by probiotics [74] and antibiotics [75] have been associated with changes in affective behavior, pain responses and gene expression in the brain. The production of a variety of neuroactive signaling molecules by bacterial components of the microbiome provides additional evidence that the gut microbiome may generate signals with remote effects in different organ systems, including the central nervous system. Recent preclinical data in rodents suggest that changes in the gut microbiota can be associated with changes in the expression of brain signaling systems and associated emotional behavior [72, 74], and oral administration of probiotics to healthy women demonstrated changes in interoceptive, affective and reward circuits in response to chronic probiotic ingestion [76]. In summary, probiotics may be useful for the prevention or treatment of functional gastrointestinal disorders like IBS by affecting the function of the gut microbiome or by altering brain function and pain perception centrally.

**Inflammatory Bowel Disease**

Differences in the composition of the intestinal microbiome have been reported in several studies of patients with Crohn’s disease and ulcerative colitis. Such differences include reduced proportions of the bacterial phyla Bacteroidetes and Firmicutes, relative deficiencies of the genus *Faecalibacterium* in ileal Crohn’s disease and expansion of the phylum Proteobacteria in patients with inflammatory bowel disease (IBD) [77–79]. Early successes with probiotics in the context of acute and chronic pouchitis [80, 81] have been followed with mixed results and...
disappointments in clinical trials of probiotics for the treatment of IBD [82]. The relative enrichment of Proteobacteria and specifically γ-Proteobacteria in recent studies emphasizes the potential importance of Gram-negative bacteria in adult and pediatric IBD. Specific components including γ-Proteobacteria were useful for identification of children with IBD; a specific example was the enrichment of the genus *Escherichia/Shigella* in children with ulcerative colitis [83]. These findings are relevant because the genus *Escherichia coli* has been the source of an established probiotic strain in humans, and microbiome research may help steer physicians towards optimal probiotic/disease combinations. Recent advances in terms of understanding functional metagenomics may point to the next generation of probiotics for adult and pediatric IBD. Microbial function was more often affected than microbial composition in a population of adult patients with Crohn’s disease and ulcerative colitis [79]. Major shifts in oxidative stress were identified in the adult IBD state, and relative reductions were identified in genes and pathways involved in carbohydrate metabolism and amino acid biosynthesis in IBD [79]. These differences in terms of metagenomic capacity may be important for the rational selection of probiotics supplying ‘missing’ functions or factors that interfere with disease-promoting pathways in the microbiome.

**Microbiome, Probiotics and Atopic Disease**

**Microbiome of the Human Skin and Atopy**

The human skin contains several dominant bacterial genera across different sites, including *Corynebacterium, Eubacterium, Propionibacterium, Staphylococcus* and *Streptococcus* [84], and one dominant fungal genus *Malassezia* [85]. Focused studies on specific body compartments have highlighted key features of colonization in healthy individuals. *Corynebacterium* was the most common bacterial genus in the anterior nares [86], and the human pathogen *Staphylococcus aureus* was present in a substantial proportion (36%) of healthy human subjects [87]. Relative differences in composition and function of bacterial communities on the human skin may explain different patterns of atopic diseases involving the skin and airways. In cases of atopic dermatitis, staphylococci and all patients had minimal exposure to antibiotics and microorganisms may help prevent or mitigate these atopic disease flares in the future. Unfortunately for patients, the identification of probiotic strains that bestow beneficial effects on the human skin has not been defined, and such applications in dermatology await further investigation. Past limited successes with oral probiotics and amelioration of atopic skin disease features in children [88, 89] have generated optimism for the potential roles of oral or topical probiotics in the treatment of atopy. However, this enthusiasm has been tempered by the realization that many gaps exist in our knowledge of the skin microbiome, probiotics and pediatric allergic diseases, and no single probiotic strain can be recommended at this time [90].

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Relative differences in composition and function of bacterial communities on the human skin may explain different patterns of atopic diseases involving the skin and airways.

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**Microbiome of the Airways: Asthma and Atopy**

Alterations in the human microbiome and pathogens have been implicated as possible causes of asthma and potential triggers of asthmatic episodes. In a study of healthy children and children with asthma, there were no significant shifts in bacterial phyla detected in the respiratory tract, and the predominant phyla in both groups included Bacteroidetes, Firmicutes and Proteobacteria [91]. Whereas healthy children were characterized by the genera *Prevotella, Streptococcus, Veillonella* and *Fusobacterium*, the genus *Haemophilus* was relatively abundant in the asthmatic group. Within the genus *Haemophilus*, the pathogenic species *Haemophilus influenzae* was previously implicated as a potential trigger of asthmatic episodes. A pediatric study from Ecuador yielded intriguing results with respect to the Airways microbiome; treatment of respiratory illnesses differs greatly in Ecuador from the standard of care in the United States. Oropharyngeal swabs were obtained from wheezing and healthy infants, and all patients had minimal exposure to antibiotics and no exposure to inhaled steroids [92]. The overall bacterial community in the study population (healthy and wheezing children) consisted primarily of the bacterial phyla Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes and Fusobacterium in order of predominance. The most common genera isolated were consistent with a prior study [91], with most bacteria belonging to *Streptococcus, Veillonella, Atopobium* and *Prevotella*. In the

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wheezing group, a greater frequency of the bacterial genera *Neisseria, Corynebacterium, Staphylococcus, Actinomyces* and *Haemophilus* were identified [92]. Possibly, these differences in the microbiome of the airways account for differences in the susceptibility to asthma or symptoms such as wheezing in the context of immune dysregulation. To reiterate, although future probiotic strategies may be applied by oral or inhaled administration, many gaps exist in our knowledge about the microbiome of the airways, effective probiotics and effects on asthma and allergic diseases [90].

**Summary and Future Directions**

Steady advances in our knowledge of the composition and function of the human microbiome at multiple body sites including the gut, skin and airways should contribute to our understanding of mechanisms of probiosis. Although our knowledge about microbial composition in *Homo sapiens* is progressing rapidly, many gaps exist in our knowledge about the functional capacity and metabolic machinery of the human microbiome. Although more studies are needed, probiotics appear capable of affecting the composition and function of the microbiome. Effects on function are likely to be more important in the short term (hours to days) following initial administration. Probiotics have yielded beneficial effects in numerous studies in the context of different disease states in pediatric gastroenterology. These disease states include NEC, antibiotic-associated diarrhea and colitis, acute gastroenteritis and IBS. An enhanced understanding of the effects of probiotics on the microbiome should facilitate selection of optimal probiotic strains for specific diseases.

Future directions include studies of effects of specific probiotic strains on the human microbiome. Such studies may include experiments evaluating changes in microbial composition using in vitro model systems, ‘humanized’ animal models containing human-associated bacteria, and clinical studies determining effects on human-associated bacterial communities following probiotics administration. Changes in composition could be extended to evaluation of changes in microbiome function and the related changes in specific metabolic pathways caused by individual probiotic strains. By understanding how probiotic strains alter specific functions of the human microbiome at different body sites, probiotic strain selection may be optimized for specific disease states (fig. 3). As we proceed into the era of metagenomic medicine, patients may be tested for their own microbial compositional and functional features so that probiotics may be customized and tailored to the disease state and the individual patient. The fusion of the microbiome with microbe-based therapies in medicine will advance the causes of holistic and personalized medicine.

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