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Invited Review

# Intestinal Microbiota During Infancy and Its Implications for Obesity

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**ABSTRACT**

Obesity is a worldwide epidemic, threatening both industrialized and developing countries, and is accompanied by a dramatic increase in obesity-related disorders, including type 2 diabetes mellitus, hypertension, cardiovascular diseases, and nonalcoholic fatty liver disease. Recent studies have shown that the gut microbial community (microbiota) is an environmental factor that regulates obesity by increasing energy harvest from the diet and by regulating peripheral metabolism. However, there are no data on how obesogenic microbiotas are established and whether this process is determined during infancy. The sterile fetus is born into a microbial world and is immediately colonized by numerous species originating from the surrounding ecosystems, especially the maternal vaginal and fecal microflora. This initial microbiota develops into a complex ecosystem in a predictable fashion determined by internal (eg, oxygen depletion) and external (eg, mode of birth, impact of environment, diet, hospitalization, application of antibiotics) factors. We discuss how the gut microbiota regulates obesity and how environmental factors that affect the establishment of the gut microbiota during infancy may contribute to obesity later in life. ***JPGN 48:249–256, 2009.*** **Key Words:** Obesity—Gut microbiota—Infancy. 

The adult human intestine contains an order of magnitude more bacteria than we have human cells in our bodies (1,2). There is sparse colonization in the proximal intestine, but the number of bacteria increases along the length of the gut to approximately $10^8$ bacteria per gram content in the distal ileum and $10^{11}$ per gram in the colon (3). The microbial ecosystem has coevolved with the host and provides us with metabolic features that we have not had to evolve ourselves, such as vitamin K production, metabolism of otherwise indigestible carbohydrates, and xenobiotic metabolism (4). However, not all microbial functions are beneficial: it has recently been proposed that the gut microbiota is an environmental factor that regulates obesity (5–7). Because the gut microbiota is acquired at birth and modulated by several environmental factors both during infancy and later in life, the properties of the flora should be viewed as dynamic. Similarly to invertebrates, adult humans have a relatively stable core microbiota and a proportion that varies within individuals in response to environmental factors (8–10). In this review, we discuss some potential mechanisms that underlie microbially induced obesity and how different selective pressures during the development of a gut microbiota may lead to an obesogenous microbiota later in life.

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## MICROBIALLY INDUCED OBESITY: ENERGY HARVEST

The incidence of obesity has increased exponentially during the past 3 decades and thus cannot be explained solely by genetic factors. Could the gut microbial community contribute to the obesity epidemic? Although most mouse gut species are unique, the mouse and human microbiotas are similar at the division level, with Firmicutes and Bacteroidetes dominating (8,11,12). Thus, mouse models may be useful to study the role of the gut microbiota in obesity. Recent studies have shown that obese ($Lep^{ob/ob}$) mice have dramatically higher levels of Firmicutes and lower levels of Bacteroidetes than do their

lean littermates (12). Moreover, metagenomic studies indicate that the distal gut microbiome in these obese mice is enriched with genes involved in energy harvest (6). It is important to note that the obese phenotype seems to be a transmissible trait: transplantation of an obese microbiota to germ-free mice results in increased adiposity compared with transplantation of a lean microbiota (6). In a recent study, Ley et al (8) found that obese humans exhibit a shift in the microbial composition similar to that found in obese mice. Furthermore, the microbial composition is normalized after weight reduction, independently of weight reduction method (ie, carbohydrate- or fat-restricted diet) (8). Duncan et al (13) recently confirmed that obese humans have reduced numbers of specific Firmicute groups after consuming a low-carbohydrate diet.

Feeding mice a high-calorie Western diet is a common model of diet-induced obesity. Mice fed the Western diet for 8 weeks display increased levels of Firmicutes and a reciprocal decrease in Bacteroidetes (14). In contrast to genetically obese mice (12), the increase in Firmicutes is not divisionwide but restricted to the Mollicute class (14). Shifting these mice to a fat- or carbohydrate-restricted diet with fewer calories reduces the number of Mollicutes (14).

Collectively, these data suggest that obesity and the caloric content of the diet may alter the structure of the gut microbial community. However, large epidemiological studies are required to verify that obesity is associated with an altered microbiota in humans. New techniques such as bar-coded pyrosequencing are likely to facilitate these studies (15,16).

Thus, obesity may alter the gut microbial structure, but can the gut microbiota directly affect the development of obesity? In support of this hypothesis, we demonstrated that conventionally raised mice have significantly more body fat than do their germ-free counterparts and colonization of germ-free mice with a normal gut microbiota induces hepatic lipogenesis and increases lipid storage in adipocytes (5). Thus, differences in the metabolic capacity of an individual's gut microbiota may be important in the pathogenesis of obesity. Pathways illustrating how the gut microbiota can affect obesity are summarized in Figure 1. The concept that obesity has a microbial component may have important therapeutic implications. Moreover, is it possible that human physiology is significantly influenced by minor components of the microbiota or low-level colonizers established early in life?

## MICROBIALLY INDUCED OBESITY: PERIPHERAL METABOLISM

In addition to its effects on energy harvest, the gut microbiota modifies metabolism in peripheral organs. Colonization of germ-free mice increases serum levels of glucose and short-chain fatty acids, which induce

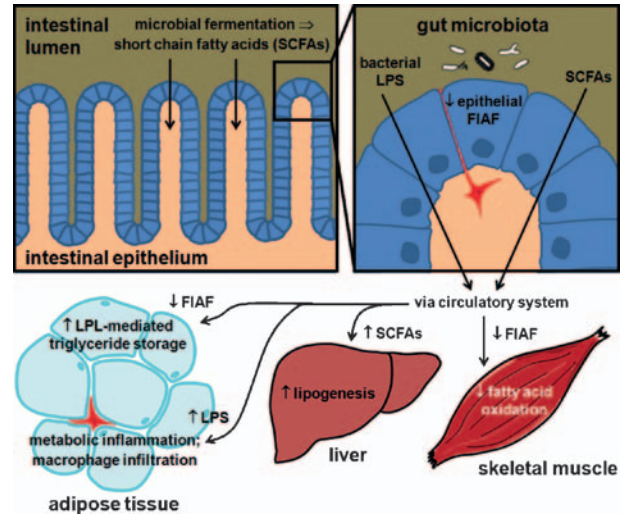


**FIG. 1.** The gut microbiota regulates obesity through several metabolic pathways. Short-chain fatty acids (SCFAs), which are produced during bacterial fermentation of complex polysaccharides in the gut, are rapidly absorbed by the epithelium and transported to the liver via the portal circulation, where they are converted to triglycerides through lipogenesis. Colonization of the mammalian gut reduces expression of intestinal Angptl4/Fiaf, which regulates adipose lipoprotein lipase (LPL) activity and fatty acid oxidation in skeletal muscle. Bacterial endotoxin (lipopolysaccharide [LPS]) induces metabolic inflammation in adipose tissue.

hepatic lipogenesis (5). The elevated triglyceride production is associated with increased adiposity and decreased glucose tolerance (5). The relative energy surplus in colonized mice leads to decreases in adenosine monophosphate–activated protein kinase (AMPK) activity in both skeletal muscle and liver (7). AMPK is a heterotrimeric enzyme that has been conserved throughout evolution (17). It functions as a ''fuel gauge'' to monitor cellular energy status and is activated in response to an increased intracellular ratio of AMP to adenosine triphosphate (ATP) (17). AMPK is activated by phosphorylation of the α-subunit, leading to suppression of ATP-consuming anabolic pathways and induction of ATP-generating catabolic pathways (eg, fatty acid oxidation) (17). Accordingly, increased AMPK activity in germ-free mice translates into increased fatty acid oxidation in skeletal muscle and liver (7). Thus, the presence of a gut microbiota will shift the preferred energy substrate toward dietary carbohydrates, whereas the absence of gut microbes will make the individual more dependent on dietary lipids.

We have identified gut-derived angiopoietin-like protein 4, also known as fasting-induced adipocyte factor (Angptl4/Fiaf), as an important regulator of host lipid metabolism. Angptl4/Fiaf is primarily expressed in liver, placenta, and adipose tissue (18–20), but the intestinal epithelium is another significant source (5). In mice,

intestinal expression of Angptl4/Fiaf increases dramatically at birth, and its expression peaks 2 days after birth (21). This may indicate that the fetus receives Angptl4/Fiaf from the placenta and that endogenous production must be induced immediately after birth. Furthermore, the microbiota directly regulates Angptl4/Fiaf: colonization of germ-free mice leads to significant diminution of Angptl4/Fiaf expression (5). Angptl4/Fiaf is an important regulator of lipid metabolism in both mice and humans (5,7,22). Nonsynonymous variants in *ANGPTL4/FIAF* are more prevalent in humans with triglyceride levels in the lowest quartile than in individuals with levels in the highest quartile, indicating that Angptl4/Fiaf also functions as an important regulator of lipid metabolism in humans (22). One variant (E40K), present in approximately 3% of Americans of European descent, is associated with significantly lower plasma levels of triglyceride and higher levels of high-density lipoprotein cholesterol (22).

## METABOLIC FUNCTION OF ANGPTL4/FIAF

Angptl4/Fiaf was originally identified as a regulator of lipoprotein lipase (LPL) (5,23), a key enzyme involved in fatty acid release from triglyceride-rich lipoproteins (24). Increased adipocyte LPL activity leads to increased cellular uptake of fatty acids and triglyceride accumulation (24). Interestingly, an absence of gut microbes correlates with decreased LPL activity (5). By using genetic proof-of-principle experiments, we found that germ-free *Angptl4/Fiaf*-deficient mice exhibit increased LPL activity, which correlates with increased body fat accumulation (5,7). Although LPL is the rate-limiting enzyme for the import and subsequent storage of triglyceride-derived fatty acids in adipocytes, genetically engineered mice that express LPL only in their myocytes gain weight normally and have a normal body mass composition. Instead of importing triglycerides from the circulation, they increase de novo fatty acid synthesis in adipose tissue (25). These findings indicate that Angptl4/Fiaf may have additional functions in mediating the lean phenotype seen in germ-free animals. We and others recently demonstrated that Angptl4/Fiaf also regulates fatty acid oxidation in both muscle and adipose tissue, probably by acting on an as yet unidentified receptor (7,26). Combined, these findings indicate that Angptl4/Fiaf may be a suitable drug target to regulate host lipid metabolism and that alterations in the microbial flora may regulate peripheral metabolism in an individual by altering intestinal Angptl4/Fiaf expression.

## MICROBIALLY INDUCED OBESITY: ENTEROENDOCRINE CELLS

The gut communicates with controllers of energy balance in the brain by means of neural and endocrine pathways. Signals reflecting energy stores, recent nutritional state, and other parameters are integrated in the central nervous system, particularly in the hypothalamus, to coordinate energy intake and expenditure (27). Gut hormones are produced by specialized enteroendocrine cells that are scattered along the gastrointestinal tract from the stomach to the colon. There are at least 15 different types of enteroendocrine cells. Although they account for only 1% of the cells in the intestinal mucosa, they constitute the largest population of hormone-producing cells in the body (28,29). Unlike many other endocrine cell types, enteroendocrine cells actively self-renew throughout the life of an animal and turn over every 3 or 4 days (30,31). The gut microbiota seems to regulate enteroendocrine cells and to influence the release of several gut hormones (32). Germ-free rats exhibit increased amounts of gastrin- and serotonin-immunoreactive cells in the gastric mucosa (32). In addition, germ-free rats have a higher concentration of somatostatin in the distal small intestine and a higher level of glucagon in the plasma (32).

The incretin hormones, which include glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide, are secreted from enteroendocrine cells in response to nutrient intake (33). They stimulate insulin release from the pancreas, and they account for approximately half of the total insulin response after a meal (33). In addition, GLP-1 slows gastric emptying, promotes satiety and weight loss, and enhances β cell efficiency and neogenesis (34–38). In rodents, oligofructose (OFS) treatment increases the proportion of bifidobacteria in the distal intestine, leading to increased fermentation and decreased food intake, fat mass, and hepatic steatosis (39,40). In addition, OFS exerts antidiabetic effects in streptozotocin-treated rats and high-fat-fed mice (41,42). Interestingly, OFS feeding in rats results in double the number of L cells in the proximal colon (43), suggesting a role for the gut microbes in the regulation of enteroendocrine cell proliferation. In humans, OFS administration is reported to increase both colonic fermentation and GLP-1 levels after a mixed meal (44). Furthermore, OFS has been shown to promote satiety in healthy humans (45). Thus, OFS treatment may constitute a novel therapeutic strategy in humans by altering the gut flora.

Bariatric surgery remains the most effective treatment for obesity (46), and gut hormones are implicated in the reduction of appetite and weight after a Roux-en-Y gastric bypass (RYGP) (47,48). Levels of GLP-1 and peptide YY increase as early as 2 days after gastric bypass, and the levels correlate with increased satiety (47,48). In contrast to RYGP, gastric banding does not lead to altered gut hormone production or appetite (47). The mechanism by which RYGP causes increased gut hormone production is not clear, but it is associated with increased bacterial colonization of the stomach (49). It is possible that RYGP leads to an altered microbial composition that regulates enteroendocrine function.

## MICROBIALLY INDUCED OBESITY: THE INNATE IMMUNE SYSTEM

Obesity is associated with a low-grade chronic inflammation (also known as metabolic inflammation), which has been implicated in the development of the metabolic syndrome and insulin resistance (50). In addition, obese individuals have elevated levels of serum free fatty acids (FFAs), which activate proinflammatory pathways (51–54). However, until recently it was unknown how FFAs activate intracellular inflammatory signaling pathways in different target tissues. Recently, Shi et al (55) found that FFAs activate Toll-like receptor 4 (TLR4) signaling in adipocytes and macrophages. These findings suggest that obesity leads to increased levels of FFAs, which activate the innate immune system via TLRs and induce metabolic inflammation. Moreover, direct adipocyte-specific activation of NF-κB in transgenic mice results in increased inflammatory cytokine production and diabetes, which further demonstrates the detrimental effects of metabolic inflammation (56). Thus, the gut microbiota may regulate metabolic inflammation both directly by producing proinflammatory mediators (eg, endotoxin), and indirectly by regulating host lipid metabolism.

Obese mice have significantly increased paracellular permeability within the gastrointestinal tract, which is accompanied by a dramatic redistribution of tight junction proteins (57). The obese phenotype is associated with elevated circulating endotoxin levels, most likely originating from the gut microbial community, in both mice and humans (57–59). A recent publication provided further support of a link between elevated endotoxin levels and metabolic disease: Creely et al (58) demonstrated that fasting insulin levels significantly correlate with serum endotoxin levels. It should be noted that these endotoxin levels are in the physiological range and are 10 to 50 times lower than values seen in septicemia or other infections (60). Furthermore, treatment of patients with type 2 diabetes mellitus with rosiglitazone significantly decreases serum glucose, insulin, and endotoxin levels (58). To directly test whether metabolic endotoxemia affects metabolic parameters, Cani et al (59) implanted osmotic pumps releasing endotoxin subcutaneously, and observed increased whole-body, liver, and adipose tissue weight gain in addition to fasting glycemia and insulinemia. Moreover, metabolic endotoxemia is associated with increased macrophage infiltration into the adipose tissue and increased levels of circulating proinflammatory cytokines and triglycerides (59). The increase in inflammatory tone results in liver, but not whole-body, insulin resistance. Interestingly, CD14-deficient mice are protected from metabolic disease after both endotoxin infusion and high-fat feeding (59). These findings demonstrate that signaling through the endotoxin receptor complex (CD14/TLR4) regulates insulin sensitivity and the onset of diabetes and obesity, and they further suggest that factors that alter intestinal barrier function may be important in regulating the development of the metabolic syndrome.

High-fat feeding significantly alters the gut microbial composition, in part by reducing the numbers of bifidobacteria, which have many physiologically positive effects, including improved mucosal barrier function (61). Mice fed a high-fat diet supplemented with oligofructose have restored quantities of bifidobacteria and decreased endotoxemia (61). These observations suggest that increased levels of bifidobacteria may decrease intestinal permeability and lower the circulating levels of endotoxin. Furthermore, the increase in bifidobacteria correlates with improved glucose tolerance, glucose-induced insulin secretion, lower body weight gain, and decreased production of inflammatory mediators (61).

In addition to regulating anxiety and depression, childhood psychosocial factors and long-term stress also increase the risk for intestinal disorders (62). In rodents, neonatal maternal separation is a well-characterized model of stress that has been used in a wide range of studies. Recently, it was shown that daily separation of the pups from their mothers during the neonatal period results in increased intestinal permeability at adulthood (63). It is possible that stress during the neonatal period may result in higher levels of serum endotoxin during adulthood and increased risk for the development of the metabolic syndrome. The administration of probiotic *Lactobacillus* to pups that have been subjected to maternal separation dramatically improves gut dysfunction (64). Collectively, these findings indicate that intentional manipulation of the intestinal flora, even in neonates, may improve gut function and lower permeability, which may translate into lower serum endotoxin levels and protection against the development of metabolic disease.

## ESTABLISHMENT AND DEVELOPMENT OF NORMAL MICROFLORA IN EARLY INFANCY

The human fetus is sterile in utero and is colonized by microbes during its passage through the birth canal. Immediately after birth, the baby is exposed to numerous bacteria from the environment (eg, skin, mouth, mother's milk). This initial microbiota is relatively unstable and changes dramatically during the initial period of life (65–67). Culture-based studies show that infants are initially colonized by facultative anaerobes such as enterobacteria and Gram-positive cocci, which are thought to create a reduced environment favorable for the establishment of obligate anaerobes, including *Bacteroides*, *Bifidobacterium*, and *Clostridium* (Fig. 2) (65,68).

By using culture-independent microarray studies during the first year of life, Palmer et al (69) recently confirmed the general developing pattern of the human

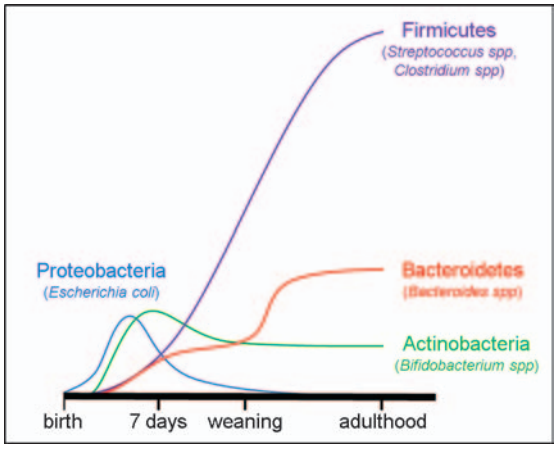


**FIG. 2.** Colonization pattern of the developing human gut. The initial microbiota after birth is dominated by facultative anaerobes. After weaning, the microbiota develops into a stable community dominated by bacteria belonging to the Firmicutes, Bacteroidetes, and Actinobacteria divisions (65,68).

infant intestinal microbiota. The major discrepancy between their study and those of others was an underrepresentation of bifidobacteria, which could have been due to their small sample size (n = 14) or to geographic and/or demographic differences. As expected, they demonstrated a large variation in microbial composition between individuals during the first months of life, which can be explained by the fact that the infants are continuously exposed to, and recolonized by, bacteria from the environment (69). Moreover, similar microbial signatures could be seen in the early infant and in swabs from breast milk and the vagina, suggesting that the gut microbiota is inherited maternally (8,69a). Studies have reported that an ''adult'' microbiota begins to develop in babies at 1 to 2 years of age (68–72). The transition to the ''adult'' flora seems to follow the transition to solid foods (69) and is in good agreement with studies in mice showing that gut microbial ecology is dramatically affected during weaning (65). Weaning also coincides with a dramatic shift in the metabolic capacity of the small intestine as the high-fat milk-based diet is replaced by a carbohydrate-rich diet. This shift also affects the metabolism of gut bacteria as determined by global gene expression profiling in gnotobiotic mice: *Bacteroides thetaiotaomicron* begins to use plant-derived instead of host-derived polysaccharides (66). Below we review how some extrinsic factors may shape the gut microbiota during infancy and how they influence the onset of obesity later in life.

## DELIVERY METHOD AFFECTS THE GUT MICROBIAL COMPOSITION

Numerous factors determine the microbial composition during early infancy (mode of delivery, infant feeding, hospitalization, prematurity, and antibiotic use) (73). The fecal microbiota of infants born by cesarean section differs significantly from the microbiota of those born vaginally (73,74). During a natural birth, microbes from the mother's birth canal and feces rapidly colonize infants (75), and the same *Escherichia coli* serotypes have been found in babies' mouths immediately after birth as in their mothers' feces (76,77). By contrast, babies who are delivered by cesarean section are colonized by microbes from environmental isolates from the mother, the air, and other infants, transferred by the nursing staff (76,78,79).

It has been suggested that early colonizers of the gastrointestinal tract ''train'' the immune system, mediating beneficial relations between bacteria and mammalian hosts during mutualism (67). Thus, in addition to different inocula, differential activation of the immune system as a result of birth mode may also shape the microbiota. A recent study showed that vaginally delivered mice exhibit an immediate mucosal activation of TLR4 and the innate immune system, which is absent in pups delivered by cesarean section (80). These findings indicate that the epithelial lining of the gut rapidly develops tolerance to the intestinal microbial community, which is a prerequisite for the lifelong host–microbial symbiosis.

Fecal samples from the KOALA Birth Cohort Study in the Netherlands demonstrated that infants born through cesarean section have lower numbers of bifidobacteria and *Bacteroides* spp and are more often colonized by *C difficile* in comparison with vaginally born infants (73,81). Because bifidobacteria and *Bacteroides* spp seem to be protective against the development of obesity (8,12,61), the mode of birth could affect the development of obesity later in life. Furthermore, it is possible that probiotic treatment of infants may have favorable effects later in life. Studies are required to confirm these hypotheses.

## ENVIRONMENTAL FACTORS THAT SHAPE THE MICROBIOTA

A recent study in Rhesus macaques showed that moderate disturbance (daily exposure to an acoustic startle paradigm for 6 weeks either early or late in the 24-week gestation) during pregnancy leading to elevation of mean plasma cortisol levels was sufficient to alter the intestinal microflora in the newborn infant (82). During the first 24 weeks of life, monkeys from stressed pregnancies had slightly lower numbers of *Lactobacillus* spp than did infants from undisturbed pregnancies. Bifidobacteria counts were not significantly affected by early gestational stress but were lower in infants from late stress pregnancies than in control infants.

Both adults and neonates are regularly exposed to microorganisms via their diet, but neonates are affected differently because they lack stable climax communities. Breast milk from healthy mothers may contain up to

$10^9$ microbes per liter (83), dominated by *Staphylococcus* spp, *Streptococcus* spp, *Corynebacterium* spp, *Lacobacilllus* spp, *Micrococcus* spp, *Propionibacterium* spp, and *Bifidobacterium* spp. These commensal bacteria can be found on the skin and in the milk ducts of the breast (84,85). The microbiota of infants fed only breast milk becomes dominated by bifidobacteria during the first week, and there is a concomitant decrease in members of the Enterobacteriaceae family (86,87). By contrast, the microbiota of formula-fed infants becomes more diverse, with a longer presence and higher counts of members of the Enterobacteriaceae and *Enterococcus* families (71,86,88). Compared with breast-fed infants, formula-fed infants at 1 month of age were found to be more often colonized with *E coli*, *C difficile*, *Bacteroides* spp, and *Streptococcus* spp (75,89,90). Inasmuch as breast-feeding has a major influence on the composition of the infantile intestinal microflora, by providing both beneficial bacteria and prebiotic growth factors (91), it may also affect the propensity toward the development of obesity during adolescence or later in life (92–95). However, new studies that carefully monitor infant feeding and correlate it with changes in the gut flora are required to support this hypothesis.

Excessive use of antibiotics will rapidly alter the gut microbial structure, and the use of antibiotics in infants is associated with decreased numbers of obligate anaerobes (eg, members of bifidobacteria and *Bacteroides*) (73). After antibiotic treatment, there is a slow regrowth of bifidobacteria, whereas *Bacteroides* spp are not usually reestablished. Because both of these microbes may be antiobesogenic, these findings suggest that antibiotic reduction of *Bifidobacterium* and *Bacteroides* may lead to an increased risk for the development of obesity.

## OUTLOOK

The composition and temporal pattern of the gut microbial community varies widely during the first year of life, supporting a broader definition of healthy colonization than has been previously recognized (69). Environmental exposure plays a major role in determining the distinctive characteristics of the microbial community in each baby. Thus, it is of utmost importance that prospective studies are performed to determine how different environmental factors (eg, mode of delivery, food, antibiotic treatment) affect microbial colonization of the gut during early infancy and the subsequent formation of stable communities, and whether these factors affect the onset of obesity.

An altered microbiota could be just one of many factors that influence the development of obesity. Thus, several questions need to be addressed: Is the gut microbiota altered in obese individuals in large population-based studies and in twins discordant for obesity? Does the gut microbiota regulate visceral obesity or obesity in general? Can gut bacteria or their products directly influence appetite and satiety? How does antibiotic treatment during childhood affect microbial composition in relation to obesity later in life? Genetically modified mouse models in combination with well-designed clinical studies are required to further define how different microbially induced mechanisms interact to promote obesity. Increased knowledge of the mechanisms involved may identify new therapeutic targets to treat obesity and its related diseases.

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