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

Ann Nutr Metab 2013;63(suppl 2):17–26

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

Altered dietary intake modifies the microbial composition of the gut, which in turn influences nutrient accessibility for the host and potentially boosts weight gain. For further details, please refer to the article. LPS = Lipopolysaccharide; SAFA = saturated fatty acid.

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

Collado MC, Laitinen K, Salminen S, Isolauri E: Maternal weight and excessive weight gain during pregnancy modify the immunomodulatory potential of breast milk. *Pediatr Res* 2012;72: 77–85.

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

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Key Messages

- Considering that the perinatal microbial contact and initial colonization guide the immune and metabolic programming of an infant, interventions aiming to prevent obesity and its metabolic consequences later in life should be initiated as early as during the perinatal period.
- The contribution of the gut microbiota to the development of overweight and obesity-related low-grade inflammation is becoming clear, although the causality remains to be proved in humans.
- Further work is required to establish how microbiota may interact with the host immune response in the context of obesity and obesity-related disorders and thus to identify specific bacterial communities and mechanisms applicable in preventive and therapeutic interventions in weight management.

Key Words

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

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.

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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 satisfac-

tory. 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 colo-

nization 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 *Bifidobacteria* 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 pre-pregnancy 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 *Leuconostocaceae* 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 β 2 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

Milk from obese mothers tends to contain a different and less diverse bacterial community compared with milk from normal-weight mothers.

Fig. 1. Gut microbiota filling the gap between energy intake and weight gain. Increased energy intake alters the composition and diversity of gut microbiota. Obesogenic microbiota increases energy harvest and storage. Saturated fatty acids (SAFAs) provide metabolic changes in tissues. The gut barrier guides nutrient intake and lessens/enhances inflammatory response induced by aberrant microbiota. LPS is a potent inflammatory mediator causally linked to adiposity and insulin resistance. The ultimate goals in attempts to reverse obesity are: optimal diet quality and quantity, balanced gut microbiota composition and well-functioning intact gut barrier.

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 expo-

sure 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 adipose 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, LPSs trigger 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 de-

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.

velopment 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 abovementioned 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 microbial 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 pre-pregnancy 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.

Disclosure Statement

The authors declare no conflicts of interest. The writing of this article was supported by Nestlé Nutrition Institute.

References

- 1 Branca F, Nikogosian H, Lobstein T (eds): The Challenge of Obesity in the WHO European Region and the Strategies for Response. Copenhagen, WHO Regional Office for Europe, 2007.
- 2 Monasta L, Batty GD, Macaluso A, et al: Interventions for the prevention of overweight and obesity in preschool children: a systematic review of randomised controlled trials. *Obes Rev* 2011;12:e107–e118.
- 3 Monasta L, Batty GD, Cattaneo A, et al: Early-life determinants of overweight and obesity: a review of systematic reviews. *Obes Rev* 2010;11:695–708.
- 4 Ley RE, Hamady M, Lozupone C, et al: Evolution of mammals and their gut microbes. *Science* 2008;320:1647–1651.
- 5 Wu GD, Chen J, Hoffmann C, et al: Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011;334:105–108.
- 6 Kelly P: Nutrition, intestinal defence and the microbiome. *Proc Nutr Soc* 2010;69:261–268.
- 7 Kau AL, Ahern PP, Griffin NW, et al: Human nutrition, the gut microbiome and the immune system. *Nature* 2011;474:327–336.
- 8 Brandtzaeg P: Development and basic mechanisms of human gut immunity. *Nutr Rev* 1998;56:S5–S8.
- 9 Hooper LV, Gordon JI: Commensal host-bacterial relationships in the gut. *Science* 2001;292:1115–1118.
- 10 Jost T, Lacroix C, Braegger CP, Chassard C: New insights in gut microbiota establishment in healthy breast fed neonates. *PLoS One* 2012;7:e44595.
- 11 Kurokawa K, Itoh T, Kuwahara T, et al: Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. *DNA Res* 2007;14:169–181.

- 12 O'Toole PW, Claesson MJ: Gut microbiota: changes throughout the lifespan from infancy to elderly. *Int Dairy J* 2010;20:281–291.
- 13 Mackie RI, Sghir A, Gaskins HR: Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr* 1999; 69:1035S–1045S.
- 14 Palmer C, Bik EM, DiGiulio DB, et al: Development of the human infant intestinal microbiota. *PLoS Biol* 2007;5:e177.
- 15 Koenig JE, Spor A, Scalfone N, et al: Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci USA* 2011;108(suppl 1):4578–4585.
- 16 Satokari R, Grönroos T, Laitinen K, et al: *Bifidobacterium* and *Lactobacillus* DNA in the human placenta. *Lett Appl Microbiol* 2009; 48:8–12.
- 17 Perez PF, Doré J, Leclerc M, et al: Bacterial imprinting of the neonatal immune system: lessons from maternal cells? *Pediatrics* 2007; 119:e724–e732.
- 18 Jiménez E, Fernández L, Marín ML, et al: Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Curr Microbiol* 2005;51: 270–274.
- 19 Rautava S, Collado MC, Salminen S, Isolauri E: Probiotics modulate host-microbe interaction in the placenta and fetal gut: a randomized, double-blind, placebo-controlled trial. *Neonatology* 2012;102:178–184.
- 20 Jiménez E, Marín ML, Martín R, et al: Is meconium from healthy newborns actually sterile? *Res Microbiol* 2008;159:187–193.
- 21 Mshvildadze M, Neu J, Shuster J, et al: Intestinal microbial ecology in premature infants assessed with non-culture-based techniques. *J Pediatr* 2010;156:20–25.
- 22 Gueimonde M, Laitinen K, Salminen S, Isolauri E: Breast-milk: a source of bifidobacteria for infant gut development and maturation? *Neonatology* 2007;92:64–66.
- 23 Jost T, Lacroix C, Braegger C, Chassard C: Assessment of bacterial diversity in breast milk using culture-dependent and culture-independent approaches. *Br J Nutr* 2013, E-pub ahead of print.
- 24 Favier CF, Vaughan EE, De Vos WM, Akkermans AD: Molecular monitoring of succession of bacterial communities in human neonates. *Appl Environ Microbiol* 2002;68:219–226.
- 25 Roger LC, Costabile A, Holland DT, et al: Examination of faecal *Bifidobacterium* populations in breast- and formula-fed infants during the first 18 months of life. *Microbiology* 2010;156:3329–3341.
- 26 Cabrera-Rubio R, Collado MC, Laitinen K, et al: The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. *Am J Clin Nutr* 2012;96:544–551.
- 27 Malamitsi-Puchner A, Protonotariou E, Boutsikou T, et al: The influence of the mode of delivery on circulating cytokine concentrations in the perinatal period. *Early Hum Dev* 2005;81:387–392.
- 28 Collado MC, Laitinen K, Salminen S, Isolauri E: Maternal weight and excessive weight gain during pregnancy modify the immunomodulatory potential of breast milk. *Pediatr Res* 2012;72:77–85.
- 29 Rescigno M, Urbano M, Valzasina B, et al: Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2001;2: 361–367.
- 30 Donnet-Hughes A, Perez PF, Doré J, et al: Potential role of the intestinal microbiota of the mother in neonatal immune education. *Proc Nutr Soc* 2010;69:407–415.
- 31 Riskin A, Almog M, Peri R, et al: Changes in immunomodulatory constituents of human milk in response to active infection in the nursing infant. *Pediatr Res* 2012;71:220–225.
- 32 Kalliomäki M, Kirjavainen P, Eerola E, et al: Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001; 107:129–134.
- 33 Collado MC, Donat E, Ribes-Koninckx C, et al: Specific duodenal and faecal bacterial groups associated with paediatric coeliac disease. *J Clin Pathol* 2009;62:264–269.
- 34 Mai V, Young CM, Ukhanova M, et al: Faecal microbiota in premature infants prior to necrotizing enterocolitis. *PLoS One* 2011; 6:e20647.
- 35 Mai V, Torrazza RM, Ukhanova M, et al: Distortions in development of intestinal microbiota associated with late onset sepsis in preterm infants. *PLoS One* 2013;8:e52876.
- 36 Kalliomäki M, Collado MC, Salminen S, Isolauri E: Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr* 2008;87:534–538.
- 37 Neu J, Rushing J: Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. *Clin Perinatol* 2011;38: 321–331.
- 38 Goldani HA, Bettiol H, Barbieri MA, et al: Cesarean delivery is associated with an increased risk of obesity in adulthood in a Brazilian birth cohort study. *Am J Clin Nutr* 2011;93:1344–1347.
- 39 Barros FC, Matijasevich A, Hallal PC, et al: Cesarean section and risk of obesity in childhood, adolescence, and early adulthood: evidence from 3 Brazilian birth cohorts. *Am J Clin Nutr* 2012;95:465–470.
- 40 Rautava S, Luoto R, Salminen S, Isolauri E: Microbial contact during pregnancy, intestinal colonization and human disease. *Nat Rev Gastroenterol Hepatol* 2012;9:565–576.
- 41 Bäckhed F, Ding H, Wang T, et al: The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004;101:15718–15723.
- 42 Cani PD, Amar J, Iglesias MA, et al: Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007;56:1761–1772.
- 43 Membrez M, Blancher F, Jaquet M, et al: Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. *FASEB J* 2008;22:2416–2426.
- 44 Schwirtz A, Taras D, Schäfer S, et al: Microbiota and SCFA in lean and overweight healthy subjects. *Epidemiology* 2009;18: 190–195.
- 45 Velagapudi VR, Hezaveh R, Reigstad CS, et al: The gut microbiota modulates host energy and lipid metabolism in mice. *J Lipid Res* 2010;51:1101–1112.
- 46 Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI: Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci USA* 2007;104:979–984.
- 47 Samuel BS, Shaito A, Motoike T, et al: Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci USA* 2008;105:16767–16772.
- 48 Hotamisligil GS: Inflammation and metabolic disorders. *Nature* 2006;444:860–867.
- 49 Cani PD, Amar J, Iglesias MA, et al: Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007;56:1761–1772.
- 50 Saltiel AR, Kahn CR: Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 2001;414:799–806.
- 51 Shi H, Kokoeva MV, Inouye K, et al: TLR4 links innate immunity and fatty acid induced insulin resistance. *J Clin Invest* 2006; 116:3015–3025.
- 52 Ley RE, Bäckhed F, Turnbaugh P, et al: Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* 2005;102:11070–11075.
- 53 Ley RE, Turnbaugh PJ, Klein S, Gordon JI: Microbial ecology: human gut microbes associated with obesity. *Nature* 2006;444: 1022–1023.
- 54 Turnbaugh PJ, Ley RE, Mahowald MA, et al: An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006;444:1027–1031.
- 55 Everard A, Belzer C, Geurts L, et al: Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci USA* 2013;110: 9066–9071.
- 56 Turnbaugh PJ, Hamady M, Yatsunenko T, et al: A core gut microbiome in obese and lean twins. *Nature* 2009;457:480–484.
- 57 Angelakis E, Armougom F, Million M, Raoult D: The relationship between gut microbiota and weight gain in humans. *Future Microbiol* 2012;7:91–109.
- 58 Ley RE: Obesity and the human microbiome. *Curr Opin Gastroenterol* 2010;26:5–11.
- 59 Santacruz A, Collado MC, García-Valdés L, et al: Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. *Br J Nutr* 2010;104:83–92.

- 60 Walker AW, Ince J, Duncan SH, et al: Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J* 2011;5:220–230.
- 61 Kadooka Y, Sato M, Imaizumi K, et al: Regulation of abdominal adiposity by probiotics (*Lactobacillus gasseri* SBT2055) in adults with obese tendencies in a randomized controlled trial. *Eur J Clin Nutr* 2010;64:636–643.
- 62 Delzenne NM, Neyrinck AM, Cani PD: Gut microbiota and metabolic disorders: how prebiotic can work? *Br J Nutr* 2013;109(suppl 2):S81–S85.
- 63 Liou AP, Paziuk M, Luevano JM Jr, et al: Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Sci Transl Med* 2013;5:178ra41.
- 64 Kong LC, Tap J, Aron-Wisnewsky J, et al: Gut microbiota after gastric bypass in human obesity: increased richness and associations of bacterial genera with adipose tissue genes. *Am J Clin Nutr* 2013;98:16–24.
- 65 Borody TJ, Paramsothy S, Agrawal G: Fecal microbiota transplantation: indications, methods, evidence, and future directions. *Curr Gastroenterol Rep* 2013;15:337.
- 66 Vrieze A, Van Nood E, Holleman F, et al: Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012;143:913–916.
- 67 Chan RS, Woo J: Prevention of overweight and obesity: how effective is the current public health approach. *Int J Environ Res Public Health* 2010;7:765–783.
- 68 Barker DJ: The origins of the developmental origins theory. *J Intern Med* 2007;261:412–417.
- 69 Kaplan JL, Shi HN, Walker WA: The role of microbes in developmental immunologic programming. *Pediatr Res* 2011;69:465–472.
- 70 Collado MC, Isolauri E, Laitinen K, Salminen S: Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am J Clin Nutr* 2008;88:894–899.
- 71 Collado MC, Isolauri E, Laitinen K, Salminen S: Effect of mother's weight on infant's microbiota acquisition, composition, and activity during early infancy: a prospective follow-up study initiated in early pregnancy. *Am J Clin Nutr* 2010;92:1023–1030.
- 72 Lawlor DA, Lichtenstein P, Fraser A, Långström N: Does maternal weight gain in pregnancy have long-term effects on offspring adiposity? A sibling study in a prospective cohort of 146,894 men from 136,050 families. *Am J Clin Nutr* 2011;94:142–148.
- 73 Ajslev TA, Andersen CS, Gamborg M, et al: Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics. *Int J Obes (Lond)* 2011;35:522–529.
- 74 Laitinen K, Poussa T, Isolauri E, et al: Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial. *Br J Nutr* 2009;101:1679–1687.
- 75 Luoto R, Laitinen K, Nermes M, Isolauri E: Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study. *Br J Nutr* 2010;103:1792–1799.
- 76 Ilmonen J, Isolauri E, Poussa T, Laitinen K: Impact of dietary counselling and probiotic intervention on maternal anthropometric measurements during and after pregnancy: a randomized placebo-controlled trial. *Clin Nutr* 2011;30:156–164.
- 77 Gueimonde M, Sakata S, Kalliomäki M, et al: Effect of maternal consumption of *Lactobacillus* GG on transfer and establishment of fecal bifidobacterial microbiota in neonates. *J Pediatr Gastroenterol Nutr* 2006;42:166–170.
- 78 Luoto R, Kalliomäki M, Laitinen K, Isolauri E: The impact of perinatal probiotic intervention on the development of overweight and obesity: follow-up study from birth to 10 years. *Int J Obes (Lond)* 2010;34:1531–1537.