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Factors affecting early-life intestinal microbiota development

Y. Vandenplas[a,*] yvan.vandenplas@uzbrussel.be, V. P. Carnielli[b], J. Ksiazyk[c], M Sanchez Luna[d], N. Migacheva[e], J.M. Mosselmans[f], J.C. Picaud[g], M. Possner[h], A.Singhal[i], M.Wabitsch[j]

[a]KidZ Health Castle, UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium

[b]Neonatal Pediatrics, Polytechnic University of Marche, Ancona, Italy

[c]The Children's Memorial Health Institute, Dept. of Paediatrics, Nutrition and Metabolic Diseases, Warsaw, Poland

[d]Neonatology Division. Complutense University. Research Institute University Hospital Gregorio Marañón, Madrid, Spain

[e]Department of Pediatrics, Samara State Medical University, Samara, Russia

[f]Citadelle for life, Brussels, Belgium

[g]Neonatology, Croix-Rousse Hospital, Lyon and CarMen Unit, INSERM U1060, INRA U197, Claude Bernard University, 69100 Lyon 1, France

[h]Nestlé Nutrition Institute, 60528 Frankfurt/Main, Germany

[i]Childhood Nutrition Research Centre, Great Ormond Street, UCL, Institute of Child Health, London, United Kingdom

[j]Ulm University Hospital, Department of Paediatrics and Adolescent Medicine, Division of Paediatric Endocrinology and Diabetes, Centre for Hormonal Disorders in Children and Adolescents, Ulm, Germany

[*]Corresponding author: Prof. Dr. Yvan Vandenplas. Kidz Health Castle, UZ Brussel, Vrije Unversiteit Brussel, Laarbeeklaan, 101, 1090, Brussels, Belgium. Tel: +32 (0)24775794; Fax +32 (0)24775783

Abstract

Objectives: To review the published evidence on early-life intestinal microbiota development, the different factors influencing its development prenatally, at birth and post-natally.

Results: A growing body of evidence indicates that the intrauterine environment is not sterile as once presumed, but that maternal-foetal transmission of microbiota occurs during pregnancy. The genetic background of the infant may also strongly influence microbial colonization of the gastrointestinal (GI) tract. The consecutive order of bacteria with which the GI tract is colonized will influence the outcome of community assembly and the

ecological success of individual colonizers. The composition and development of infant gut microbiota can be influenced by many prenatal factors such as maternal diet, obesity, smoking and use of antibiotics during pregnancy. Mode of delivery is generally accepted as a major factor determining the initial colonisation, which persists for months, if not for years. Breastfeeding, mainly because of its high content of unique oligosaccharides, stimulates the most balanced microbiome development for the infant. Feeding is, in general, another important factor determining intestinal colonization. Compared with breastfed infants, formula-fed infants have an increased richness of species. Initial clinical studies show that infant formulas supplemented with specific human milk oligosaccharides (HMO) -2´-FL alone or in combination with LNnT, structurally identical to those in breast milk-, increase the proportion of infants with a high bifidobacteria dominated gut microbiota typical of that observed in breastfed infants, lead to plasma immune marker profiles similar to those of breast-fed infants, and to lower morbidity and antibiotics use. Further clinical studies with the same, others or more HMOs are needed to confirm these clinical effects.

Conclusions: A growing number of studies have reported on how the composition and development of the microbiota during early life will affect risk factors related to health up to and during adulthood. If exclusive breastfeeding is not possible, the composition of infant formula should be adapted to stimulate the development of a bifidobacteria dominated gut microbiota typical of that observed in breastfed infants. The main components in breast milk that stimulate the growth of specific bifidobacteria are HMOs.

Key words: breast feeding; formula feeding; human milk oligosaccharide; microbiota; bifidobacteria

Introduction

Joshua Lederberg in 2001 originally coined the term microbiome when discussing the importance of the microorganisms inhabiting the human body for health and disease. Knowledge on the microbiome's composition, development and effects on health and disease evolves daily. In eubiosis, the microbiome is in a healthy balanced status. Bacteria communities live in the gastro-intestinal (GI) tract in a complex ecosystem, composed of over 1000 species [1]. The adult GI tract was initially estimated to harbour 10^{14} bacteria, 10 times more cells than the human body. However, a more recent calculation estimates there to be 10^{13} bacteria, which is equivalent to the number of human cells [2]. The GI tract of an adult contains roughly 1.0 to 1.5 kg of bacteria [1]. A growing number of studies have reported on

how the composition and development of the microbiota during early life will affect risk factors related to health up to and during adulthood [3].

This paper reviews the recent literature on the development and influencing factors of GI colonization. Traditionally, the GI tract was considered to be sterile at birth. However, studies of infant meconium using molecular techniques suggest that bacteria are present in the foetal gut prior to birth [4, 5, 6]. These findings have led many scientists to challenge the "sterile womb paradigm" and to propose that microbiome acquisition instead begins in utero [5]. This hypothesis remains controversial to this day. The developing gut microbiome undergoes three distinct phases of microbiota composition: a developmental phase up to the age of 14 months, a transitional phase between 15 and 30 months, and a stable phase between 31 and 46 months [7]. GI colonization is a dynamic process: while the presence of most bacteria increases during life, some of them decrease as well; this is most obvious for staphylococci and clostridium perfringens [6]. A growing number of studies have reported on how the early human gut microbiota composition/development may affect risk factors related to adult health conditions [3].

Colonization of the foetus´ gut

A growing body of evidence indicates that the intrauterine environment is not sterile as once presumed, but that maternal-foetal transmission of microbiota occurs during pregnancy [8]. Animal studies have shown that prenatal transmission of microbes to the foetus is possible, and physiological changes observed in pregnant mothers indicate that in utero transfer is also likely in humans [9]. The maternal intrauterine microbiome environment is likely to influence the development of the foetus that continues after birth [10]. For example, preterm birth is often the result of an intrauterine dysbiosis or infection [10]. The placental membrane microbiome is altered in spontaneous preterm birth with and without chorioamnionitis [11]. Studies of infant meconium suggest that bacteria are present in the foetal gut prior to birth, showing that GI colonization could occur prenatally. A gut microbiota associated with an increased risk of developing necrotizing enterocolitis (NEC) can be identified in meconium samples: Clostridium perfringens continues to be associated with NEC from the first meconium until just before NEC onset [12]. In contrast, in post-meconium, increased numbers of staphylococci were negatively associated with NEC. These findings suggest causality [12].

Some scientists argue that the evidence in support of the "in utero colonization hypothesis" is extremely weak [5]. They claim that the hypothesis is almost entirely based on studies that

lacked appropriate controls for contamination and failed to provide evidence of bacterial viability [5]. The strongest evidence against the existence of microbiomes in the foetal environment, stems from the ability to derive germ-free animals via C-sections and subsequently raise the offspring in a sterile environment [5]. Studies targeting colonization of the foetal gut in utero will be paramount to furthering our understanding of the early life gut microbiome [4]. If in utero transfer of maternal microbes to the foetus occurs in humans, the maternal microbiome during pregnancy could be a target for modification aiming to optimize this process to support transfer of beneficial microbes and suppress the transfer of harmful or pathogenic bacteria to the infant [4].

Prenatal influence

The composition and development of infant gut microbiota can be influenced by many prenatal factors such as maternal diet, obesity, smoking and use of antibiotics during pregnancy. Several studies confirmed that maternal high-fat diet during gestation reduced the diversity of offspring intestinal microbiota in juvenile animals at 1 year of age [13], and persistently shapes the juvenile gut microbiome [14]. Infants fecal microbial composition is correlated with body weight and weight gain of their mothers during pregnancy [15]. The significant effects of maternal obesity on the composition of the gut microbiome of offspring have been shown [16]. In addition, there is recent evidence of causative role of maternal obesity-associated infant dysbiosis in childhood obesity [17].

Infant gut microbial colonization is shaped by the maternal microbiota and altered by maternal antibiotic treatment during pregnancy [18], which may have a dramatic effect on the neonatal immune development [19]. Gestational diabetes mellitus can alter the microbiota of both pregnant women and neonates at birth, which sheds light on another form of inheritance and highlights the importance of understanding the formation of the early-life microbiome [20].

Recent data also indicate distinct composition of gut microbiota of infants born to smoking mothers and possible impact of maternal smoking on the child overweight later on [21].

What happens at birth?

Mode of delivery is generally accepted as a major factor determining the initial colonisation, which persists for months, if not for years [22, 23, 24]. Bacteroides, especially Bacteroides

fragilis, are more predominant after vaginal delivery [7]. Bacteroides are also associated with increased gut microbiome diversity and faster maturation, regardless of the birth mode [7]. Infants born by elective caesarean delivery have a particularly low bacterial richness and diversity of their GI microbiome [25] at the age of 4 months. Elective versus emergency caesarean section, and intrapartum antibiotics, both result in a different microbiota of the GI tract, but also of the mother's milk [26]. The difference in microbiota development between elective and emergency caesarean section may be related to the difference in progesterone levels, since progesterone promotes Bifidobacterium growth during late pregnancy [27].

The administration of intrapartum antibiotics during caesarean and vaginal delivery is associated with infant gut microbiota dysbiosis [28]. Microbiota differences were especially evident following intrapartum antibiotic prophylaxis with emergency C section, with some changes such as increased Clostridiales and decreased Bacteroidaceae, persisting up to the age of 12 months, particularly in formula fed infants [28].

After birth

Analysis of faecal samples collected at 4 months of age from a subset of term infants from the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort showed a high variability in the profiles of faecal microbiota, generally dominated by Actinobacteria (mainly the genus Bifidobacterium), and Firmicutes, with diverse representation from numerous genera [29]. At the time of sampling, 10 (42%) of the infants were exclusively breastfed, 5 (21%) were partially breastfed (supplemented with formula), and 9 (38%) were not breastfed. Compared with breastfed infants, formula-fed infants had increased richness of species, with overrepresentation of Clostridium difficile [25]. Environmental factors including geographical location and household exposure such as siblings and furry pets also affected the faecal microbiota [7]. In controlled conditions, history of colonization has a major impact on the development of the immune system [29]. Most often, the dominant strains of the mother's microbiome are transmitted to the infant, but occasionally it is the secondary strains that colonize the infant's gut [30]. The genetic background of the infant may also strongly influence microbial colonization of the GI tract. A measurable effect of colonization history on gut microbiota assembly was demonstrated in a model in which host and environmental factors were strictly controlled, illuminating a potential cause for the high levels of unexplained individuality in host-associated microbial communities [31]. Thus, the

consecutive order of bacteria with which the GI tract is colonized will influence the outcome of community assembly and the ecological success of individual colonizers [32].

Early microbiome and immune system

Healthy infants harbour intestinal bacteria that protect against food allergy [33]. Human milk oligosaccharides (HMOs) provide a main substrate to help shape the infant's gut microbiota and affect the maturation of the intestinal mucosal immune system [34]. The GI microbiome differs between allergic and non-allergic infants and children, and these differences are present before symptoms develop [35]. Early infancy is a window during which gut microbiota may shape conditions for allergy outcomes during infancy and childhood. Neonatal gut microbiome dysbiosis is likely to promote CD4+ T cell dysfunction associated with childhood atopy [36]. Infants who received human milk with low Lacto-N-fucopentaose (LNFP) III concentrations (< 60μM) were more likely to develop cow's milk allergy when compared to high LNFP III-containing milk (odds ratio 6.7, 95% CI 2.0-22) [34]. Intestinal bacteria are critical for regulating allergic responses to dietary antigens and this suggests that interventions that modulate bacterial communities may be therapeutically relevant for prevention of food allergy [33]. Gut microbiome composition at age 3 to 6 months is associated with milk allergy resolution by age 8 years, with enrichment of Clostridia and Firmicutes in the infant gut microbiome of subjects whose milk allergy resolved [37]. Metagenome functional prediction supported decreased fatty acid metabolism in the gut microbiome of subjects whose milk allergy resolved [37]. Bacterial taxa within Clostridia and Firmicutes could be studied as probiotic candidates for milk allergy therapy [37]. The analysis of short-chain fatty acids (SCFA) levels in faecal samples of one-year-old children from a birth cohort showed that high levels of butyrate and propionate are associated with protection against atopy [38]. Children with the highest levels of butyrate and propionate (≥95th percentile) in faeces at the age of one year had significantly less atopic sensitization and were less likely to have asthma between 3 and 6 years [38]. Strategies to increase short chain fatty acid levels, as happens with HMOs, could be a new dietary preventive option for allergic diseases in children [38].

Feeding

Feeding is, of course, another important factor determining intestinal colonization. By stimulating the development of Bifidobacteria species (B. breve and B. bifidum) [7],

exclusive or partial breast feeding, is the most significant factor associated with microbiome structure. This effect is related to the presence of human milk oligosaccharides [39], the third largest solid component of mother's milk, which are known to have a bifidogenic effect on the infant's microbiome. These oligosaccharides are unique to human milk. They promote the growth of specific bifidobacteria, supporting an early Bifidobacteria-dominated gut microbiome [39]. Over 200 different oligosaccharides have been identified in human milk [40]. The structure of some HMOs resembles that of epithelial pathogen receptors, enabling them to serve as a decoy receptor to prevent pathogen binding and enhance pathogen clearance [39].

Breastmilk is also known to contain some probiotic bacteria, although the clinical significance of breast milk microbiota is currently poorly understood. The composition of the breast milk microbiota is influenced by mode of delivery and the administration of intrapartum antibiotics [26], with mode of delivery having the greater effect. Caesarean section delivery has an independent effect on breast milk microbiota composition [26] as does the environment. For example, data from Gambia shows that mothers nursing in the wet season (July to October) produced significantly less oligosaccharides compared to those nursing in the dry season (November to June) [41]. These results suggest that specific types and structures of HMOs are sensitive to environmental conditions, protective of morbidity, predictive of growth, and correlated with specific microbiota [41].

HMO considerations

Breastfeeding, mainly because of its high content in unique oligosaccharides, stimulates the most balanced microbiome development for the infant. HMO profiles are highly variable; total HMO concentrations vary 3.7-fold and individual HMOs vary 20- to >100-fold [42]. The HMO content varies between mothers in composition and amount, over the duration of lactation, and even during one feeding [43]. Most HMO concentrations are lower in milk collected later in lactation, although some, including DSLNT and 3'-sialyllactose, are higher [42]. Which particular HMO structures are secreted with the milk is mostly genetically determined [44]. The most extreme interpersonal variations are found with respect to HMO fucosylation and are based on the woman's Secretor status [35]. Worldwide, about 75-80% of mothers are secretors [45]. Secretor mothers have higher total HMO concentrations than non-Secretors (median approximately 10 vs 5 g/L total HMOs) [46]. The secretor (*Se*) gene encodes for fucosyltransferase-2 (*FUT2*), which is necessary for the synthesis of 2´-

fucosyllactose (2′-FL) and other Fucosyl-HMOs [47]. The milk of secretor (*Se*+) women is, therefore, characterized by an abundance of α1-2-fucosylated HMOs, especially 2′-FL [43]. The absence of 2´-FL and other Fucosyl-HMOs explains the lower total amount of HMOs in "non-secretor" women´s milk [39]. However, all individual HMOs except disialylacto-N-tetraose (DSLNT) differ by Secretor status [42]. Independent of Secretor status and lactation stage, seasonal and geographic variation were observed for several HMOs [42]. Parity, ethnicity, and breastfeeding exclusivity also emerged as independent factors associated with some HMOs, whereas diet quality and mode of delivery did not [42].

HMO in preterm milk

A recent study compared the HMO composition of milk of mothers of very preterm infants (< 32 weeks of gestational age, < 1500 g of birthweight) and milk of mothers of term infants [48]. At equivalent postpartum age (lactation stages), the concentrations of most HMOs were comparable, suggesting that birth represents a trigger reprogramming HMO trajectory during lactation. However, subtle differences were observed probably due to unachieved enzyme expression. α1,2-fucosyl HMO concentrations were reduced in preterm milk during the first month of lactation. The concentrations of a number of sialylated HMOs, on the contrary, were elevated in preterm milk, in particular 3´sialyllactose (3´SL) and disialyllacto-*N*-tetraose (DSLNT). It is speculated that the higher concentration of 3´SL may help preterms' brain development and the higher concentration of DSLNT may contribute to the prevention of necrotizing enterocolitis (NEC).

As in term milk, most HMOs in preterm milk, with the exception of 3'FL, show a decrease in concentration with post-partum age. At equivalent post-menstrual age, the concentrations of a number of HMOs were therefore significantly different in preterm compared to term milk with preterm infants consuming more 3´FL, but lower concentrations of most other HMOs than term infants [48]. This may contribute to the fact that bifidobacteria are only present at low abundance in preterm infants. This current study, however, was small, and the observations need corroboration in larger cohorts [48].

The differences between the gut microbiota of preterm and term infants, however, are not only due to the different HMO concentrations in breast milk, but mainly to organ immaturity, frequent use of antibiotics and hospital stay in neonatal intensive care units [49]. Aside from the low abundance of Bifidobacteria and Bacteroides, a low diversity with increased

colonization of potentially pathogenic bacteria from the gram-negative family Enterobacteriaceae of the Proteobacteria phylum is characteristic for the gut microbiota of preterm infants [49].

Maternal secretor status is associated with breast milk microbiota composition and is maintained during the first 4 weeks [50]. Specific associations between milk microbiota, HMO and secretor status were observed, although the potential biological impact on the neonate remains unknown [50]. The effect of secretor versus non-secretor status on the GI microbiome was found to be much larger in infants born after C section than in vaginally born infants [51].

There is some evidence that health outcomes of exclusively breastfed infants of non-secretor mothers are inferior to those born to secretor mothers [52]. It is mainly the amount of certain oligosaccharides in mother's milk that stimulates the development of bifidobacteria in the intestinal microbiome. As mentioned above, milk of secretors contains a high percentage of 2′-fucosyllactose (2′-FL), whereas the milk of non-secretors contains no or only minimal amounts of 2′-FL [53]. The number of bifidobacteria in the GI microbiome of breastfed infants of non-secretor women is lower than in that of children of secretor women [54]. Breast milk with high 2‘-FL levels also provides infants with better protection against specific diarrheal diseases and could reduce the risk of eczema in caesarean section infants with increased allergy risk [53, 55]. The order of colonization with strains has a major effect on immune function. [29].

Microbiome in formula-fed infants

Compared to human milk, oligosaccharide concentrations in cow’s milk are 100-1000-times lower [39]. In fact, these unique complex carbohydrate structures in human milk are virtually absent in cow’s milk or any other farmed animal milk, and their variety is much lower [40]. The GI microbiome of infants fed with cow's milk-based formula that is not supplemented with probiotics or oligosaccharides contains much fewer bifidobacteria than that of a breastfed infant. Therefore, cow’s milk based infant formula should be adapted to stimulate the development of a bifidobacteria-dominated gut microbiome. The supplementation of the missing bacteria as probiotics, more specifically bifidobacteria, initially seemed the most logical choice. However, a placebo-controlled intervention study demonstrated that bifidobacterial supplementation of infant formula does not substantially affect proportions of

bifidobacterial sequences during the first year of life [56]. Such an intervention is therefore not likely to compensate for differences in microbiota composition observed between breast- and formula feeding [56].The addition of prebiotics, of human or non-human origin, has the advantage of stimulating the growth and development of bifidobacteria strains already present in the microbiome of the host [57, 58, 59]. Galacto-oligosaccharides (GOS, enzymatically synthesized from galactose), fructo-oligosaccharides (FOS, extracted as inulin from chicory/other plant sources), and pectin-derived acidic oligosaccharides (pAOS, extracted from citrus fruit or cellulose) are the most frequently used prebiotics of non-human origin. GOS and FOS were shown to stimulate the development of a bifidobacteria-dominated gut microbiome. After a 6-week intervention period, the percentage of bifidobacteria of the total bacterial load determined by PCR was 90% in the breastfed infants; in infants fed the formula with GOS and FOS it was only 73.4%) [60]. Interestingly, a prolonged effect has been demonstrated: at the age of 12 months, the GI microbiome of infants fed supplemented formula up to the age of six months was still much richer in bifidobacteria than that of infants fed un-supplemented formula [61].

HMO benefits

However, oligosaccharides such as the GOS and FOS currently added to infant formula are not found in human breast milk (FOS) or found only in minimal amounts (GOS). They are structurally different from HMOs and therefore unlikely to be functionally equivalent [62]. For example, GOS and FOS contain fructose, but HMOs do not [63]. Fucose and sialic acid are also only present in HMOs [63]. HMOs have a high microbiome specificity, GOS a low one and FOS a weak one as shown by an in vitro study with several Enterobacteriaceae strains associated with necrotizing enterocolitis in mice and infants [64]. All tested Enterobacteriaceae showed no detectable growth when 2′-fucosyllactose (2´-FL), 6-siallylactose (6′-SL), or lacto-N-neotetraose (LNnT) was provided as the sole carbon source. Several *Enterobacteriacea* strains, including pathogens, on the other hand, grew well on GOS.

It has now become possible to synthesize a few of the more than 200 oligosaccharides present in mother's milk [65].Although scientists have known of the existence of these human milk oligosaccharides (H MO) for over 75 years, it has only recently become possible to manufacture some of them [65, 66]: mainly 2'-fucosyllactose (2FL), but also others such as LNnT and 3'FL. 2-FL quantity in breast milk is positively associated with the development of

bifidobacteria in the GI microbiome [54]. The addition of 2′-FL to infant formula was shown to lead to plasma immune marker profiles similar to those of breast-fed infants [67]. The addition of 2′-FL and LnNT to infant formula was shown to globally shift bacterial diversity of the gut microbiome at three months closer to that of breastfed infants [68]. The addition of 2′-FL and LnNT significantly increased the proportion of formula-fed infants with a high *Bifidobacteria* dominated gut microbiota typical of that observed in breastfed infants [68,69]. A randomized controlled trial with infant formula supplemented with 2′FL and LNnT showed that this formula was safe, well tolerated and supported age-appropriate growth [70]. While the number of bifidobacteria increased, the number of potential pathogenic bacteria decreased [71]. Additionally, secondary outcome findings of lower morbidity, particularly bronchitis and decreased use of antibiotics, were reported in infants fed the supplemented formula [72]. The addition of these HMOs increased the faecal content of butyrate and propionate [69], which were shown to decrease the risk of developing atopic disease [71].

Postbiotics are bioactive compounds produced by food-grade microorganisms during fermentation processes. Postbiotics have been added to infant formula in combination with FOS/GOS, resulting in increased Bifidobacterium sp. and decreased Clostridium difficile occurrence in the infants' stools [72]. The supplementation of infant formula with a symbiotic mixture of GOS/FOS + B breve M-16V resulted in a bifidobacteria-dominant microbiome comparable to the microbiome in vaginally born breastfed infants in infants born by C section. The same formula with GOS/FOS alone failed to do so in infants born by C section [73]. Both formulas were well tolerated and resulted in adequate growth [74]. This study, however, lacked a group fed a formula supplemented only with the probiotic bacteria B breve M-16V without GOS/FOS. This is why the study does not allow for a conclusion on whether the formula is a symbiotic formula, i.e. whether the supplemented mixture of B breve M-16V and GOS/FOS has a better effect than B breve M-16V alone. Similar effects on the GI microbiome were demonstrated for the addition of a symbiotic of bovine milk-derived oligosaccharides (BMOS) and B. animalis subsp. lactis CNCM I-3446 [75]. This study too lacked control groups fed a formula with oligosaccharides or BMOS only. This study also therefore does not allow for a conclusion on whether the combined supplementation of the formula with BMOS and bifidobacteria has a better effect than formulas with only one of the components.

Antibiotic administration

Antibiotic administration is another major factor that interferes with the composition of the GI microbiome. Summarizing the literature, it can be postulated that the earlier in life and the more frequent antibiotics are administered to infants, the greater their impact on the microbiome. Antibiotics will destroy large parts of the microbiome, inducing a loss of health-promoting bacteria as well as a reduced expression of antibacterials and IgG and therefore increase the susceptibility to infections [76]. Administration of antibiotics early in life alters the balanced development of the microbiome [77]. These alterations can be transient but also persistent [77]. Resistance of some gut microbes to the antibiotic may also occur and these resistant genes can possibly be transferred to pathogens. Inflammatory cytokines will increase, insulin sensitivity will be altered and the metabolism of short chain fatty acids and bile acids is modulated. The immune homeostasis will be challenged, disrupting the T-reg/Th balance [76]. Therefore, antibiotic administration increases the risk of developing immune mediated diseases such as cow's milk protein allergy, diabetes and asthma [76]. Antibiotics given early in life also increase the risk for infectious conditions such as otitis media, obesity and inflammatory bowel disease [78]. The younger, the more frequent, and the larger spectrum of antibiotics administered, the stronger the association with overweight status [78]. But antibiotics are not alone in interfering with the microbiome, other medications such as, for example proton pump inhibitors, also have similar effects.

All health care providers and paediatricians should restrict as much as possible the administration of any medication that could alter the microbiome of an infant during the first months of life, when the immune system is developing rapidly.

In conclusion

The early life gut microbiome plays an important role in the development of the immune system and metabolism, which may affect the risk of chronic diseases such as allergies, obesity and other chronic immune and metabolic diseases in later life. Bifidobacteria should be predominant in the GI microbiome of an infant. Many factors such as mode of delivery, medication and feeding influence GI microbiome development. The use of antibiotics antenatally, perinatally and in the infant, as well as caesarean section birth, disrupt the establishment of a bifidobacteria-dominated gut microbiota and therefore their use should be strictly controlled. Breastfeeding is the preferred infant feeding. If exclusive breastfeeding is not possible, the composition of cow's milk based infant formula should be adapted to stimulate the development of bifidobacteria using, for example, a formula containing probiotics and/or prebiotic oligosaccharides. However, many oligosaccharides currently added

to infant formula are structurally different from HMOs and therefore most likely not to be functionally equivalent. However, cow´s milk-based formulas supplemented with 2´-FL or 2´-FL + LNnT, structurally identical to those in breast milk, are now available. Initial clinical studies show that infant formulas supplemented with these HMOs increase the proportion of infants with a high *Bifidobacteria* dominated gut microbiota typical of that observed in breastfed infants, lead to plasma immune marker profiles similar to those of breast-fed infants, and to lower morbidity and antibiotics use. Further clinical studies with the same, others or more HMOs are needed to confirm these clinical effects.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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V. Carnielli has participated as an advisory board member for Nestle Nutrition Institute

J. Ksiazyk was a speaker for Danone, Nutricia, Nestle, Fresenius Kabi and participated in the meetings of Nestle Nutrition Institute

M Sanchez Luna has participated as a clinical investigator, and/or advisory board member, and/or consultant, and/or speaker for Abbvie, Dräger, Nestle Nutrition Institute, Linde Healthcare.

N. Migacheva declare no conflict of interest.

J.M. Mosselmans is an Advisory Board moderator for Nestle Nutrition Institute

J.C. Picaud participated as a clinical investigator, and/or advisory board member, and/or speaker for Nestle Nutrition Institute, Modilac France, Bledina France and Nestlé Health Science.

M.Possner is employed by Nestlé Nutrition Institute

A. Singhal has participated as a clinical investigator, and/or advisory board member, and/or speaker for Abbott Nutrition, Wyeth Nutrition, Nestle Health Science, Nestle Nutrition Institute, Danone and Phillips.

M. Wabitsch has participated as advisory board member for Nestle Nutrition Institute