Probiotics in human milk and probiotic supplementation in infant nutrition: a workshop report

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Abstract
Probiotics in human milk are a very recent field of research, as the existence of the human milk microbiome was discovered only about a decade ago. Current research is focusing on bacterial diversity and the influence of the maternal environment as well as the mode of delivery on human milk microbiota, the pathways of bacterial transfer to milk ducts, possible benefits of specific bacterial strains for the treatment of mastitis in mothers, and disease prevention in children. Recent advances in the assessment of early host–microbe interactions suggest that early colonisation may have an impact on later health. This review article summarises a scientific workshop on probiotics in human milk and their implications for infant health as well as future perspectives for infant feeding.

Key words: Human milk microbiota: Enteromammary pathway: Probiotic supplementation: Prevention of infections: Allergies: Colic: Obesity

After completion of the Human Genome Project in 2003, research has focused on the human microbiome, defined as ‘the ecological community of commensal, symbiotic, and pathogenic microorganisms that share our body space’(1). There seems to be an intricate symbiosis with microbes (mainly bacteria) supporting the immune system, metabolism and many other functions, making every human being a unique ecosystem. Several human microbiome projects are currently investigating the microbes colonising the human body, and a focal point of interest is the microbiota acquired in early life.

Bacteria in human milk
Bacterial composition of human milk
The human body contains 10–100 times more bacterial cells than body cells. The human microbiome is thought to be involved in many important functions such as metabolism, immune function and even neuromodulation(2,3). Humans cannot survive without microbiota. Nevertheless, most of the knowledge about the functions of the human microbiome is very recent, and many of the interactions between the human body and the bacterial microbiota are still not well understood.

In spite of the bacterial richness in the human body, breast milk was considered to be free of bacteria until about a decade ago when lactic acid bacteria were first described in human milk hygienically collected from healthy women(4). Following this discovery, more than 200 different species (belonging to fifty different genera) have been described in human milk(5) with great individual variations (also depending on which methods of analysis were used) (6–8). Breast milk today is recognised as a source of commensal and potentially probiotic bacteria, including staphylococci, streptococci, corynebacteria, lactic acid bacteria and bifidobacteria(4,7,9–12), able to act as pioneer bacteria in the crucial stage of initial neonatal gut colonisation(13). With a measured viable bacterial density in the range of 2–4 log colony-forming units/ml, resulting in an estimated daily ingestion of 5–7 log cells, it is not surprising that the neonatal gut microbiota reflects the bacterial composition of breast milk(14,15). Parallel, culture-independent molecular methods have revealed the presence of DNA belonging to major gut-associated obligate anaerobic bacterial taxa in breast milk, members of the Bacteroidetes phylum (i.e. Bacteroides) and the Clostridia class(5,8,16–22).

Globally, all these studies confirm the existence of site-specific human milk microbiota and microbiome(5,13,16). Using culture-independent methods based on pyrosequencing of the 16S ribosomal RNA gene, Hunt et al.(5) showed

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a relatively stable bacterial DNA composition over time in milk samples collected from sixteen women at three time points during 4 weeks, with nine observational taxonomic units/ genera being identified in every sample and Staphylococcus, Streptococcus and Serratia being the most abundant genera. Cabrera-Rubio et al. (18) investigated the microbiome of human milk using culture-independent methods in eighteen mothers over a period of 6 months, starting immediately after delivery. They reported a relatively high bacterial DNA diversity and also found that the bacterial DNA composition changed over time: the most common genera in the colostrum were Weissella and Leuconostoc (both lactic acid bacteria from the order lactobacillales) followed by Staphylococcus, Streptococcus and Lactococcus. In milk samples collected at 1 and 6 months, lactic acid bacteria were still most abundant, but the abundance of typical inhabitants of the oral cavity such as Veillonella, Leptotrichia and Prevotella and members of the TM7 phylum increased significantly. Furthermore, milk samples collected from mothers who gave birth by non-elective caesarean delivery displayed a bacterial DNA composition more similar to that of samples collected from mothers who gave birth by vaginal delivery than to that of samples collected from mothers who gave birth by elective caesarean delivery, indicating that there may be factors such as stress and hormonal secretions during delivery that influence the bacterial composition of milk. The report suggests that although the microbiota contained DNA of many bacterial species and genera, the composition of the milk microbiome was not identical to that of any mucosal, faecal or skin samples.

Sinkiewicz & Nordström (25) found that the composition of the human milk microbiota also seems to be influenced by geographical factors. Using culture-based methods, they investigated the occurrence of lactobacilli in breast milk samples collected from different parts of the world. In general, there seem to be higher numbers of lactobacilli and bifidobacteria in samples collected from rural areas than in those collected from urban areas. The geographical variations show that the human milk microbiota is adapted to the mother's environment and lifestyle, preparing the infant for the specific conditions that he or she will be born into.

The research results also suggest that in spite of great inter-individual variations in bacterial species, there is a 'core microbiome': that is, there are certain bacterial species with DNA that seems to be present in most or all human milk samples (5,15,18). The variations in bacterial DNA diversity (the 'variable microbiome') may be explained by external factors such as nutrition, host physiology and immune system, as well as other environmental and lifestyle factors. General differences in the bacterial strains could also originate from the methods used to determine the bacteria in the first place (8,38).

**Bacterial transfer to mother's milk**

The pathways by which bacteria reach breast milk have been discovered only very recently: initially, it was assumed that human milk becomes contaminated by bacteria from the infant's mouth and the mother's skin (24). However, only little backflow of milk into the mammary glands was observed in ultrasound examinations (25). Another assumption was that infants acquire most of their intestinal microbiota through contact with the vaginal epithelia at birth. However, there are more similarities between human milk and infant gut microbiota than between human milk and vaginal exudate. Martin et al. (12) analysed bacteria isolated from breast milk and infant faecal samples collected from twenty mother–infant pairs and detected the same strains of bacteria that were present in the mothers' milk to be present in the infants' faecal samples. These results suggest that at least some bacteria are transferred from the mother's milk to the infant and that breast-feeding contributes to this process and the gut colonisation of the infant.

This discovery was preceded by numerous publications reporting the discovery of bacteria in many different parts of the body, even in those that have previously been thought to be sterile. The European Union-funded PROSAFE project, for example, was set up to establish a relevant collection of probiotic and human lactic acid bacteria (26). A total of 907 strains were collected and lactic acid bacteria were found in almost every body tissue and fluid, even in the blood of healthy people and in the cerebrospinal fluid. It seems that bacteria are already transferred to the fetus through umbilical blood and that there is a considerable flux of bacteria from the mother's gut to the mammary glands beginning in late pregnancy.

In 2001, first results suggesting that dendritic cells in the lamina propria can send dendrites into the gut lumen via tight junctions and trap bacteria and then transport them back to the lamina propria and through the blood to distant organs, allowing them to cross the mesenteric lymph node barrier, were reported (27,28). These studies led Martin et al. (15) to hypothesise that maternal bacteria could translocate through the intestinal epithelial barrier and migrate to the mammary glands via an endogenous cellular route (entero-mammary pathway). Later, Perez et al. (16) examined the intracellular transport of bacteria from the maternal intestine to the mammary glands through the circulation in healthy mothers. They found common bacterial DNA signatures in milk and maternal peripheral blood mononuclear cells as well as in maternal and infant faeces. The results suggest that intestinally derived bacterial components may be transported to the lactating breast within mononuclear cells. Subsequent culture-independent studies of human milk also suggested a vertical bacterial transfer via breast milk (7,16,19,21,29). However, culture-independent methods do not allow strain-level identification of bacteria. Therefore, culture-dependent techniques were essential to assess a potential transfer of bacterial strains from the mother to the infant. Using strain-level discrimination, recent studies have demonstrated the transfer of bifidobacteria from the maternal gut to the neonatal gut (30–32), transfer of orally administered Lactobacillus spp. from the maternal gut to breast milk (2;35–35), transfer of bifidobacteria, lactobacilli and staphylococci from breast milk to the neonatal gut (12,36), and sharing of several butyrate-producing members of clostridia between maternal faeces and breast milk (22).
Thus, recent data support the hypothesis of bacterial transfer from the mother to the infant via an enteromammary pathway. This could influence the current understanding of neonatal gut development and provide future opportunities for manipulating an aberrant microbiota (Fig. 1).

**Development of the human mammary microbiota**

The bacteria in milk ducts appear in the last trimester of pregnancy\(^{(13)}\). This seems to be driven by hormonal signalling and, at the same time, significant changes in the intestinal microbiota of the mother. The fetus exerts increasing pressure on the mesenteric vessels, and there is an increased bacterial translocation from the mother’s gut to the blood stream and the mammary glands. The mammary milk ducts fill with pre-colostrum\(^{(13)}\). The concentration of bacteria reaches a maximum during peripartum and then slowly decreases during the nursing period. During the weaning period, there is a sharp decrease in bacterial counts as a result of the apoptosis process responsible for the involution of the mammary glands and, also, of the decrease in lactose levels in the mammary environment. After weaning, no bacteria can be detected in the mammary glands under physiological conditions\(^{(13)}\).

**Role of milk microbiota in the prevention of infections**

The differential composition of bacterial communities in the mammary glands and in human milk is associated with maternal and infant health. Bacteria are involved in the production of bioactive substances such as polyamines, vitamins, peptides, mucins and SCFA\(^{(37)}\). Lactic acid bacteria consume oxygen in the gut, thereby generating an anaerobic environment necessary for bifidobacteria and later for several intestinal bacterial strains after weaning. The milk microbiota also contributes to the maturation of the immune system and is involved in the competitive exclusion of pathogens.

One example for the competitive exclusion of pathogens is *Staphylococcus* in human milk: although staphylococci are usually considered to be pathogens, several *Staphylococcus* species, e.g. *Staphylococcus epidermidis*, appear to be a part of the commensal microbiota of breast-fed infants. In fact, *S. epidermidis* is even present and common in amniotic fluid and seems to have its natural habitat not only in the skin but also in the digestive and urogenital tracts. In a study comparing the bacterial diversity of milk and faecal samples collected from breast-fed and formula-fed infants, Jimenéz et al.\(^{(36)}\) reported that *S. epidermidis* was the predominant species in the milk and faecal samples of breast-fed infants, but less prevalent in the faecal samples of formula-fed infants. Generally, the staphylococcal isolates obtained from the milk and faecal samples of breast-fed infants had less number of virulence determinants and were sensitive to most of the antibiotics tested\(^{(20)}\).

A study carried out by Park et al.\(^{(38)}\) showed that mice nasally pre-colonised with *S. epidermidis* became more resistant to colonisation with methicillin-resistant *Staphylococcus aureus* (MRSA), suggesting that the application of commensal bacteria could be a more effective strategy than the treatment with antibiotics to prevent MRSA colonisation.

Competitive pathogen exclusion by commensal bacteria may be one of the reasons why skin contact and breast-feeding of premature/low-birth-weight infants, the so-called Kangaroo mother care method, help to reduce the risk of nosocomial infections\(^{(39)}\). Possibly, pre-colonisation of infants with parental staphylococci helps to prevent infections with virulent staphylococcal strains from the hospital environment by mechanisms such as competitive exclusion.

An important factor in the possible protective role of bacterial transfer from the intestine to the mammary glands...
and to the fetus is the competition of bacteria with HIV: HIV rapidly attach to dendritic cells, to be presented to T lymphocytes in the vicinity, thereby spreading the infection to the whole body. Some of the bacteria compete with HIV for the same receptor, being preferentially taken up by the dendritic cells and preventing attachment and transport of HIV. This has special implications for regions with a very high prevalence of HIV: lactic acid bacteria have also been reported to bind to viruses and to facilitate their inactivation(40).

Infections of the mammary gland: dysbiosis as a cause?

In spite of being part of the commensal milk microbiota, staphylococci and streptococci are also frequently found in the breast milk of women suffering from mastitis. Mastitis is defined as an inflammation of more than one lobule of the mammary gland, occurring in up to one-third of lactating mothers(43). According to the WHO, Staphylococcus aureus is often an aetiological agent of acute mastitis (95%), while coagulase-negative staphylococci, such as S. epidermidis, and viridans streptococci seem to be the dominant species responsible for subacute, chronic and recurrent mastitis(55,44,43).

Several factors may contribute to the microbiological imbalance facilitating the overgrowth of normal components of the microbiota in milk ducts. The complex ductal system of lactating mammary glands may, in such dysbiosis cases, favour the growth of S. aureus and S. epidermidis(44). In addition, human milk contains large amounts of lactose and oligosaccharides. Staphylococci and streptococci are efficient lactose/galactose utilisers(45,46) and thus find optimal growth conditions in this environment. On top of this, mammary polymorphonuclear neutrophil recruitment is decreased in the first 3 months postpartum, so that there may not be sufficient numbers of these leucocytes for the control of mastitis-causing bacteria.

Interestingly, antibiotics may also be a risk factor for developing mastitis: women who received antibiotics in the last trimester of pregnancy and peripartum have a 25-fold risk of developing mastitis during lactation compared with women who did not take antibiotics. Possibly, antibiotics eradicate the non-resistant bacteria in the mammary glands and milk ducts, sparing resistant and virulent strains and leaving the breast unprotected from other bacteria. This may be the reason why antibiotic therapy is not effective in some cases of mastitis(35). Antibiotic therapy may also break the resilience of normal breast milk and milk duct microbiota, increasing the risk of further deviations.

When bacteria such as staphylococci and streptococci are under stress, they can actively form highly organised and dense bacterial communities called biofilms on epithelia. The biofilms develop protective coats, providing resistance to antibiotics and the host’s immune response and allowing undisturbed bacterial multiplication(47).

In view of the ability of probiotic bacteria to displace pathogens, researchers investigated whether Lactobacillus isolated from breast milk could be an alternative treatment option for infectious mastitis and found that lactic acid bacteria isolated from human milk had the potential to prevent breast infections(35). More recently, placebo and antibiotic treatment of infectious mastitis has been compared with treatment with oral Lactobacillus salivarius CECT5713 and Lactobacillus gasseri CECT5714 and/or Lactobacillus fermentum CECT5716(55). On day 14 of this study, no clinical signs of mastitis were observed in women who were assigned to the lactobacilli group, whereas clinical signs persisted in the control group receiving antibiotic throughout the study. These results indicate that some lactobacilli can be used as an effective alternative to antibiotics for the treatment of infectious mastitis (Fig. 2).

Development of the intestinal microbiota in infants

As has been described above, infants receive their ‘original inoculum’ of bacteria early in life, beginning prenatally with the transfer of bacteria through umbilical blood and continuing with the transfer of bacteria via contact with the vaginal and intestinal microbiota at birth (depending on the mode of delivery) and through skin contact and mother’s milk during breast-feeding. Colonisation of the intestine of infants may be essential for the maturation of the gut-associated lymphoid tissue, homeostasis of the intestinal epithelium, and developmental regulation of the intestinal physiology(29).

Palmer et al. (48) followed the intestinal microbiota of fourteen healthy, full-term infants (including one pair of twins) from birth to 12 months of age. Although the composition and temporal patterns of the microbiota varied widely from baby to baby, the individual features of each baby’s microbial community often remained recognisable for months. The strikingly parallel temporal patterns of the twins suggested that incidental environmental exposures play a major role in the determination of the distinctive characteristics of the microbiota in each baby. The intestinal microbiota of the infants began developing towards an adult profile 5d after birth. By the end of the first year of life, the idiosyncratic microbial ecosystems in each baby had converged towards a profile characteristic of the adult gastrointestinal tract. The interaction and transfer of microbiota from the mother to the...
infant during the perinatal period have been reviewed by Rautava et al.\(^\text{49}\).

A more recent study carried out by Grzeskowiak et al.\(^\text{50}\) has compared the gut microbiota of 6-month-old infants living in rural Malawi (n 44) with that of infants of the same age living in urban Finland (n 31), both breast-fed and receiving an age-appropriate diet typical for each area. They found significant differences in the intestinal microbiota of infants from both countries, with higher proportions of bifidobacteria and bacteroides/prevotella group bacteria being found in Malawian infants and only Clostridium perfringens as well as S. aureus being detected in Finnish infants. These results demonstrate that – similar to human milk – the intestinal microbiota of infants is adapted to their specific environment.

**Research on the influence of probiotics on health**

As environmental factors seem to play a major role in the development of the intestinal microbiota, this may also mean that it could be possible to influence the microbial composition to achieve beneficial effects for an individual’s health.

There is no ‘one size fits all’ model for the microbial intervention: considering the huge inter-individual, temporal and geographical variation, a healthy standard is almost impossible to define. In any case, the core microbiota of healthy mothers and infants should serve as a model for probiotic products.

The official definition of a probiotic by the FAO and WHO today is ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’. The European Food Safety Agency also requires the health effects and safety of the microbial preparation to be proven and that strains be identified clearly and deposited in public culture collections. Before a bacterial strain can actually be termed ‘probiotic’ according to the European Food Safety Agency definition, adequate preclinical as well as clinical studies have to be performed to prove health benefits that are as good as or better than standard prevention or treatments for a particular condition or disease. In addition, the manufacturing procedures have to be standardised and comply with ‘Good Manufacturing Practice’ guidelines\(^\text{51}\).

To evaluate evidence on the health effects of probiotics in infants, meta-analyses and systematic reviews should focus on studies with clearly structured clinical questions. The studies should be adequately designed and the outcome measures should be clearly defined and validated.

**Supplementation with probiotics**

**Choosing the right probiotic**

A multitude of factors have to be considered in the quest for the ‘right’ probiotic for nursing mothers and infants. First, an adequate strain – or several adequate strains – must be found. The next step is to confirm the effect of this strain in well-designed prospective, randomised studies in human target populations. Numerous studies investigating the effects of a multitude of probiotics on different outcome parameters were carried out in breast-fed as well as in formula-fed infants of different ages, but the majority of these studies were neither very well designed nor comparable with regard to the observed populations, the bacterial strains, the type and duration of treatment, or the outcome variables. Consequently, current recommendations regarding probiotic supplementation are cautious and justifiably demand more well-designed and well-controlled studies\(^\text{52,55}\).

**Infant formula supplemented with probiotics**

The Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) conducted a systematic review of published evidence related to the safety and health effects of the administration of formula supplemented with probiotics and/or prebiotics compared with unsupplemented formula\(^\text{52}\). The studies included in the review investigated growth parameters, gastrointestinal infections, respiratory symptoms, antibiotic use, colic, crying and irritability, allergy, stool frequency and stool consistency, as well as several non-clinical parameters (e.g. faecal lactobacilli and stool pH).

On the basis of this review, available scientific data suggest that the administration of currently evaluated probiotic- and/or prebiotic-supplemented formula to healthy infants does not raise safety concerns with regard to growth and adverse effects. At present, there are insufficient data to recommend the routine use of probiotic- and/or prebiotic-supplemented formula.

**Probiotics and infectious diseases**

Significantly reduced incidence rates of gastrointestinal infections and upper respiratory tract infections have been found in infants receiving formula supplemented with L. fermentum CECT 7516 by two recently published studies\(^\text{54,55}\) that had not been included in the ESPGHAN review.

The double-blind, randomised controlled study of Maldonado et al.\(^\text{55}\) comparing follow-on formula supplemented with L. fermentum plus galacto-oligosaccharides with the same formula containing only galacto-oligosaccharides was conducted in 215 infants between 6 and 12 months of age. Infants receiving the formula supplemented with L. fermentum exhibited 46% reduction in the incidence rate of gastrointestinal infections \((P=0.032)\), 27% reduction in the incidence rate of upper respiratory tract infections \((P=0.026)\), and 30% reduction in the total number of infections \((P=0.003)\) at the end of the study period compared with infants who had received the formula containing only galacto-oligosaccharides.

The second randomised controlled study conducted by Gil-Campos et al.\(^\text{55}\) investigated the safety and tolerability of an infant formula supplemented with L. fermentum v. a non-supplemented formula in 126 infants of 1–6 months of age. No significant differences in weight gain or tolerability were found between the two groups. However, the incidence rate of gastrointestinal infections in infants of the control
group was three times higher than that in infants of the probiotic group (P=0.018).

**Probiotics and allergies**

The composition of the intestinal microbiota may be associated with the development of humoral immunity in infants\(^{(56)}\). As humoral immunity is involved in allergic reactions, it was postulated that there may be an association between the composition of the intestinal microbiota and the occurrence of allergies. The results of recent research suggest that the intestinal microbiota of children with allergies is different from that of non-allergic children.

In a prospective study comparing the development of the intestinal microbiota of infants in Estonia and Sweden during the first 2 years of life, using culture-dependent methods, Björksten et al.\(^{(57)}\) discovered that the microbiota of allergic children already exhibited differences when compared with that of non-allergic children during the first year of life. Allergic infants had higher counts of clostridia and S. aureus but fewer enterococci and bifidobacteria compared with non-allergic children, indicating that differences in the composition of the gut microbiota between infants who will and infants who will not develop allergy may be demonstrable before the development of any clinical manifestations of atopy. As the observations were made in two countries with different standards of living, the findings could indicate a role for the intestinal microbiota in the development of and protection from allergy.

More recently, Nylund et al.\(^{(58)}\) have investigated the possible association of prenatal maternal probiotic supplementation and the development of atopic eczema in a double-blind, placebo-controlled study. Interestingly, the effect of the probiotic supplementation was only minor, but the children developed significantly different microbiota profiles. At 18 months, healthy children had 3-fold greater amounts of members of the Bacteroidetes phylum (P=0.018); on the other hand, children suffering from eczema had increased numbers of Clostridium cluster IV and XIVa members, which are typically abundant in adults. This may indicate that an adult-type microbiota in early childhood could be associated with eczema later in life. An important factor was also observed in the diversity of bacteria: breast-fed infants had slightly lower diversity in early life than formula-fed infants, which may describe the early colonisation of infants during breast-feeding.

In spite of the potential immunomodulatory effects of microbiota and the differences in the intestinal microbiota of allergic and non-allergic children, systematic reviews and current recommendations did not obtain consistent results regarding the protective effect of probiotic supplementation with regard to childhood allergies.

A systematic Cochrane review\(^{(59)}\) published in 2008 evaluated nine studies on the role of probiotics in the prevention of atopic dermatitis in infants (Table 1). Of the nine studies, six found significantly reduced rates of atopic dermatitis with probiotic supplementation in comparison with no supplementation; three of the studies did not find significant differences in the infant’s microbiota.
differences between the probiotic and placebo groups\(^{(60)}\). The World Allergy Organization Position Paper on the Clinical Use of Probiotics in Paediatric Allergy came to similar conclusions, namely that no single probiotic supplement or class of supplements has been demonstrated to be sufficient to influence the course of any allergic disease\(^{(53)}\).

**Probiotics and colic/crying**

Colic and crying are important outcome variables in many studies on infant nutrition. Up to 40% of infants suffer from colic – a condition characterised by repeated, prolonged episodes of inconsolable crying\(^{(61)}\). Possibly, children with colic symptoms have an imbalance in the intestinal microbiota: analyses of faecal samples found higher counts of coliform bacteria and lower counts of lactobacilli in infants with colic symptoms compared with children not suffering from colic\(^{(52)}\). On the other hand, probiotics have been shown to influence intestinal motility and sensory neurons as well as contractile activity of the intestine and to exert anti-inflammatory effects\(^{(63, 64)}\).

An Italian randomised controlled study carried out in breast-fed colicky infants showed significant reduction in crying after supplementation with *Lactobacillus reuteri* DSM 17938 compared with placebo\(^{(65, 66)}\). The results of this trial were confirmed by another similar study carried out by Szajewska et al.\(^{(61)}\). Concluding from these results, exclusively or predominantly breast-fed infants with infantile colic could benefit from the administration of *L. reuteri* DSM 17938. There are also recent studies suggesting that the administration of *Lactobacillus rhamnosus* may be associated with decreased crying in young infants and compositional fusing in early infancy\(^{(67, 68)}\).

**Probiotics and obesity**

In 2005, Ley et al.\(^{(69)}\) found that the intestinal microbiota of genetically obese (ob/ob) mice was significantly different from that of genetically lean and wild-type mice, which were all fed the same high-polysaccharide diet. Regardless of kinship, the ob/ob mice exhibited a 50% reduction in the abundance of members of the Bacteroidetes phylum and a proportional increase in that of members of the Firmicutes phylum, indicating that there may be an association between gut microbiota and obesity.

Studies in human subjects indicate that there may be a similar association between the composition of the gut microbiota and obesity in humans. Before diet therapy, obese people had fewer members of the Bacteroidetes phylum \((P<0.001)\) and more members of the Firmicutes phylum \((P=0.002)\) compared with lean controls. Over time, the relative abundance of members of the Bacteroidetes phylum increased \((P<0.001)\) and that of members of the Firmicutes phylum decreased \((P=0.002)\), irrespective of diet type, with bacterial diversity remaining constant over time. Interestingly, the increased abundance of members of the Bacteroidetes phylum correlated with the percentage loss of body weight and not with changes in dietary energy content\(^{(70)}\).

The differences in bacterial colonisation between normal-weight and obese mothers were even evident in milk samples: a study in 2012 investigating the milk microbiota of obese and normal-weight women directly postpartum (colostrum), at 1 month and after 6 months\(^{(38)}\) found that the milk samples collected from obese mothers tended to contain a different and less diverse bacterial community compared with those collected from normal-weight mothers. As there seems to be an association between BMI and the composition of the intestinal microbiota, the bacteria in mother’s milk possibly reflect these differences.

Koren et al.\(^{(71)}\) characterised faecal bacteria of ninety-one pregnant women with varying pre-pregnancy BMI and gestational diabetes status and their infants. Similarities between infant and maternal microbiota increased with children’s age. The gut microbiota of mothers changed dramatically from the first to the third trimester, with a vast expansion of the bacterial diversity between mothers, an overall increase in the abundance of Proteobacteria and Actinobacteria, and reduced bacterial richness. When transferred to germ-free mice, third-trimester microbiota induced greater adiposity and insulin resistance compared with the first-trimester microbiota, indicating that host–microbe interactions could affect host metabolism.

Evidence that probiotics may be able to influence metabolism and body weight development was provided by researchers in Finland who conducted a double-blind, randomised, placebo-controlled study to evaluate the influence of perinatal probiotic intervention on childhood growth patterns and overweight development during a 10-year follow-up in 159 women. Study participants were randomised to receive daily doses of either \(10^{10}\) colony-forming units of *L. rhamnosus* GG, ATCC 53103 or placebo, beginning 4 weeks before expected delivery until 6 months postpartum. The perinatal probiotic intervention appeared to moderate the initial phase of excessive weight gain (prenatally until 2 years of age), especially among children who later became overweight, but not the second phase of excessive weight gain, with the impact being most pronounced at the age of 4 years\(^{(72)}\). Further epidemiological and clinical trials with precise data on early growth patterns and on confounding factors influencing weight development will be needed to confirm these results.

**Conclusions**

The human milk microbiome is a very recent field of research. The presence of non-pathogenic microbes in human milk was only acknowledged about 10 years ago. Since then, numerous studies have been performed to determine the source of bacteria in the mammary glands and the effects of the human milk microbiota on maternal and infant health. Human milk receives bacteria from a multitude of sources, including the mother’s intestine. Recent studies have shown that dendritic cells may be able to transport bacteria directly from the mother’s gut to the mammary glands, providing infants with a bacterial inoculum specifically adapted to the environment and nutrition of the mother.

As imbalances in the composition of the mammary and intestinal microbiota may be responsible for a number of
problems such as maternal mastitis as well as diarrhoeal diseases, infant colic or atopic dermatitis in children, and even overweight, research is focusing on the influence of bacterial supplements on infant and maternal health.

There is evidence that some lactobacilli can be used as an effective alternative to antibiotics for the treatment of infectious mastitis and that early skin contact and breast-feeding significantly reduce the risk of nosocomial infection/sepsis in low-birth-weight infants.

Clinical research has shown that supplementation of pregnant women and infants with specific bacterial strains is safe and well tolerated. Randomised controlled studies indicate that supplementation of infants with specific lactobacilli may be associated with a reduced risk of non-specific gastrointestinal infections or a reduction of colic and crying. An assumed long-term effect of perinatal probiotic supplementation on the BMI of children is currently under discussion. No clear conclusions are possible with regard to the preventive effects of probiotics on the development of atopic dermatitis so far.

Further adequately designed, prospective, randomised, double-blind, controlled studies based on structured clinical questions with regard to the investigated populations, the type and duration of intervention, the type of comparison and the outcome variables will be needed to prove the health benefits of specific bacterial strains for infants.

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