REVIEW

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Prebiotic supplementation supports immunity & growth-development in preterm infants: a review and expert perspectives

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ABSTRACT

Preterm birth still causes significant mortality and morbidity in newborns, despite modern advancements in preterm care. The gut microbiota is the most significant microbial colonization in the human body and plays a significant role in supporting a healthy body. Preterm infants are susceptible to gut dysbiosis, which is thought to contribute to the many adverse outcomes of prematurity, such as necrotizing enterocolitis (NEC), late-onset sepsis (LOS), and developmental delays. Human breast milk is considered the ideal nutrition source for newborns and can help create ideal microbiota in preterm infants. Breast milk contains human milk oligosaccharides (HMO), prebiotics that promote beneficial bacteria growth, absent in cow's milk. Supplementation of non-milk oligosaccharides such as long-chain fructo-oligosaccharides (lcFOS) and small-chain galacto-oligosaccharides (scGOS) in formula milk for preterm infants is found to be beneficial with a favourable safety profile. This review discusses gut microbiota in preterm neonates, its role in developing immune systems, growth and development, and the benefits of prebiotic supplementation.

Keywords: preterm birth, prematurity, prebiotic, immunity, growth, development.
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INTRODUCTION

Preterm birth is a significant cause of mortality and morbidity in children, accounting for 35% of all newborn deaths.[1] Despite notable advancements in treatment and technology to support preterm neonates, the care of preterm neonates poses a unique set of challenges for the clinician. The gut microbiota, known to play essential roles in human health and is the largest microbiota of the human body, is prone to alterations and imbalances in preterm infants due to various reasons such as immature organs, antibiotics use, and invasive medical interventions.[2] Microbial colonization of the gut starts at birth – some even argue it starts in utero – and is essential to the development of the gastrointestinal system and influences the immune system's development.[3] The gut microbiota composition fluctuates substantially in the first three years of life, highly influenced by external factors such as lifestyle and antibiotic usage.[4] The gut microbiota of term, vaginally delivered, breastfed infants are considered ideal, and variations in the mode of delivery, choice of nutrition, and medical interventions can have a marked impact on the gut microbiota. Prebiotic supplementation is thought to have a positive impact on the gut microbiota of infants who are delivered via Caesarean section (C-section), born preterm, and formula-fed, as a substitute for human milk oligosaccharides (HMO), the third most prevalent component in human milk, which is absent in cow's milk.[5] This review and its concluding remarks discuss gut microbiota in preterm neonates and how it relates to the immune system and the role of prebiotic supplementation.

NUTRITION FOR THE PRETERM INFANT

Preterm birth is classified by the World Health Organization (WHO) based on gestational age as extremely preterm (<28 weeks gestation), very preterm (28-32 weeks), and moderate to late preterm (32-37 weeks).[6] Extremely preterm birth causes high mortality rates, with 64% mortality in infants born at 22-24 weeks, and survivors have a higher chance of neurodevelopment abnormalities.[7]

Provision of adequate, high-quality nutrition is imperative in the treatment of preterm infants. During gestation, the brain grows remarkably, and premature birth cuts short the brain's growth. As the preterm infant is cared for in the NICU, the nutrients given to the infant must be able to support extra-uterine brain development to achieve optimal neurodevelopmental outcomes.[8] Postnatal undernutrition is an independent predictor of chronic lung disease.[9] Increased time to achieve full enteral feeds and prolonged duration of intravenous nutrition are consequences of feeding intolerance due to intestinal dysmotility and affect subsequent growth and development.[10] Recent evidence shows enhanced nutrition in the first days of life is essential and can improve neurodevelopmental outcomes. Adequate

nutritional support can significantly improve growth outcomes in preterm infants, and faster weight gain is associated with more favourable neurodevelopmental outcomes.[11] Enteral protein, fat, and energy intakes were related to more cerebellar, basal ganglia, and thalamic volume and prolonged intravenous nutrition were associated with smaller overall brain volume.[12] Emerging evidence suggests that early and higher protein and energy intake are beneficial for preterm infants, as they are associated with a faster head circumference and improved cognitive outcomes.[13]

GUT MICROBIOTA IN THE PRETERM INFANT

The gut is home to diverse microbiota, known to have essential roles in human health. There is some debate on when gut colonization starts; the prenatal environment is considered sterile, supporting the common belief that microbial colonization starts at birth,[3] but several studies challenged the paradigm, arguing that evidence has been found for a placental microbiome.[14–16] Other studies support microbiota in the amniotic fluid,15 but claims of the prenatal environment being unsterile are still debated.[17–19]

The gut microbiota provides many benefits to the developing mucosal barrier and helps modulate the risk of inflammation and diseases. The composition of the gut microbiota can strengthen the gut mucosa and degrade it if colonization of pathogenic bacteria dominates over beneficial commensal bacteria. Commensal bacteria contribute to the regulation of tight junction proteins, while dysbiotic bacteria may compromise intestinal integrity. Decreased gastrointestinal motility in the preterm infant and the underdevelopment of the GIT can lead to permeable GIT barrier and pathogenic colonization, causing dysbiosis and chronic inflammation.[20]

Literature suggests that term vaginally delivered and breastfed infants are most likely to have a healthy GIT microbiota.[2] Many preterm babies are shown to have dysbiosis with a dominance of Gammaproteobacteria, a class of Gram-negative bacteria, including *Enterobacteriaceae, Vibrionaceae,* and *Pseudomonadaceae*, and this dysbiosis is postulated to contribute to adverse outcomes of prematurity such as NEC, LOS, and developmental delay.[21] Preterm infants are exposed to many factors that negatively impact healthy gut microbiota, including a higher rate of C-sections,[22] use of antibiotics,[23] admissions to the neonatal intensive care unit (NICU),[24] parenteral and/or enteral nutrition via nasogastric tubes,[25] and a higher rate of respiratory support need.[26] Gut microbiota of preterm infants are found to have a higher number of pathogens and a lower bacterial load,[27] dominated with Firmicutes (*Staphylococcus, Enterococcus*), Proteobacteria (*Enterobacteriaceae, Klebsiella*), and *Bacteroides*.[28] In contrast, term infants' gut microbiota is predominated by Actinobacteria (*Bifidobacterium*) and Firmicutes (*Staphylococcus, Streptococcus*). This difference in composition is seen even in preterm babies who were delivered vaginally, received no antibiotic therapy, and received breast milk.[29]

Infants who were vaginally delivered receive colonizing bacteria from their mother's vagina and intestines, most of which produce short-chain fatty acids that help inhibit pathogen colonization.[30] Conversely, infants delivered via C-sections will first be colonized by bacteria from their mothers' skin or their neonatal environment, with a general gut microbiota imbalance.[22] A study on Japanese neonates found bacterium taxa that dominate vaginally delivered babies are Bacteroidales and Enterobacteriales. At the same time, Bacillales and Lactobacillales were hypothesized to be acquired from maternal skin and the hospital's environment, dominated in C-section babies.[31] This finding is supported by the KOALA Birth Cohort Study by Penders et al., which found C-section babies had lower amounts of *Bifidobacterium* and *Bacteroides*, with a higher number of *Clostridium difficile*.[29] C-section babies were presumed to miss *Bifidobacterium* and *Bacteroides* acquisition from the maternal birth canal, causing delayed colonization by beneficial bacteria.[32] This difference in microbiota is found to persist for 2 to 7 years after birth.[33,34]

Human breast milk is considered the ideal nutrition source for newborns due to the macro and micro-nutrients that work synergistically with the gut's normal flora to control inflammation and prevent infection.[35] Breast milk also contributes to positive microbial colonization due to pre-and probiotics. Prebiotics promote the growth of beneficial bacteria, therefore contributing to the health of the host. The third most common component of breast milk is human milk oligosaccharides (HMO),[36] prebiotics that promotes Bifidobacteria, Bacteroides, and Lactobacillus.[37] Studies on preterm infants found breast milk associated with better feeding tolerance, lower risk of NEC, and allergy.[2] Live microbes are also found in breast milk, which may influence the gut colonization process in newborns. Microbes in breast milk are thought to translocate from the maternal intestine to lymph nodes before moving to mammary glands.[38]

Due to their susceptibility to infection, antibiotics use is higher in preterm infants than their term counterparts. The use of antibiotic therapy is known to increase the number of potential pathogens and decrease the number of commensal bacteria with delayed Bifidobacteria colonization in the gut.[39,40]

GUT MICROBIOTA AND THE PRETERM INFANT'S IMMUNE SYSTEM

Immediately after birth, the preterm neonate is exposed to many antigens that their immune system needs to respond to their birthing conditions. The response to these antigens plays an influential role in the subsequent development of their immune systems.[20] When infants are born preterm, intestinal motility, villus and crypt anatomy, and feeding reflexes are not yet fully developed, negatively impacting the establishment of proper nutrition absorption and functional immune system.[41] In neonatal immunity, maternal antibodies play a significant role. Newborns lack functional plasma cells in the first months of life. Thus they rely on maternal IgG for the first three months. The amount of transferred maternal IgG increases with gestational age. Therefore preterm neonates have decreased

maternal IgG, resulting in disturbances in opsonization, and consequently, phagocytosis.[1]

Another way for mothers to provide passive immunity is through breast milk, which contains secretory IgA (sIgA).[42] sIgA is known to play a significant role in shaping gut microbiota and supporting the symbiotic relationship the microbiota with the host.[43] At birth, newborns lack sIgA, they rely entirely on breast milk to receive maternal sIgA, further emphasizing the important role of breast milk as the ideal source of nutrition.[42]

In general, newborns have intact innate immune systems but lack mature B and T cells.[41] The composition of leukocytes from preterm neonates is considered inferior with lower cell counts than term infants, leading to reduced bacterial elimination, suboptimal endothelial adhesive rolling, and decreased pattern recognition receptor (PRR) functions.[44] The number of neutrophils and monocytes in preterm infants is reduced due to low granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), and this relative neutropenia and monocytopenia play significant roles in preterm neonates' infection-fighting ability.[45,46] Their neutrophils have reduced neutrophil extracellular traps (NETs) production and are less effective in migrating to infection sites due to a reduction in adhesion molecules.[47,48] Preterm infants also have reduced complement activity due to C1, C4, and factor B deficiencies compared to term infants, which are essential in complement activation through both classical and alternative pathways.[49]

B and T lymphocytes, involved in adaptive immunity, are known to mature after term birth. In general, all newborns lack mature B and T cells, which lead to the lack of immunoglobulin production.[1] However, preterm neonates have lower absolute numbers of circulating lymphocytes.[50] During pregnancy, the immune system is skewed towards a Th2 phenotype, thought to be a preventive strategy against fetal rejection by the mother's body; high Th1 cytokine production is associated with a higher risk for spontaneous abortion.[1] The prevalence of CD4 T cells in the Th2 phenotype may influence preterm infants' susceptibility to infections because there is the lower synthesis of pro-inflammatory cytokines such as TNF-a and IFN-y.[51]

Gut microbiota is thought to play a role in the pathogenesis of many diseases, including antibiotic-associated diarrhea, NEC, atopic eczema, infectious diarrhea, inflammatory bowel disease, diabetes, allergies, and psoriasis.[52]

Late-onset sepsis (LOS), defined as sepsis after 72 hours of life, is a severe cause of morbidity and mortality in preterm infants.[53] Despite unclear pathogenesis, studies suggest gut microbiota is a crucial risk factor for LOS. Preterm infants with LOS are shown to have lower microbiota diversity, and bacterial-host interactions are thought to modulate gut and systemic immune responses, contributing to LOS pathogenesis.[54] In a study comparing preterm infants with LOS and healthy preterm infants as control, *Bifidobacterium* dominant microbial communities were specific to infants in the control group, as well as *Bifidobacterium*-associated prebiotic oligosaccharides such as raffinose.[53]

HOW THE GUT MICROBIOTA INFLUENCES GROWTH AND DEVELOPMENT

Brains of preterm infants are highly susceptible to inflammatory processes, which consequently affects neurodevelopmental outcomes.[55] Much of the normal brain development and maturation in utero happens from 20 weeks gestation, depriving preterm infants of a period of critical development.[56] Preterm infants have overall reduced brain size and reduced size of brain regions such as frontotemporal regions and the hippocampus, which persist into adolescence and adulthood.[57–59] Preterm infants have an increased risk for neurological complications such as cerebral palsy, attention-deficit/hyperactivity disorder, and reduced academic performance.[56] A study on preterm infants evaluated at 3-4 years of age found children born preterm had specific deficits in visuospatial processing, attention, and spatial working memory compared to age-matched peers who were born term.[60] Visual perception and auditory development are also found to be altered in preterm infants.[6] Preterm infants are at high risk of delays in motor development, as premature exposure to gravity and extra-uterine environment affects musculoskeletal and nervous system development.[61] Disability at 30 months of age is found to be predictive of outcomes at six years. However, preterm infants who go on through toddlerhood with minimal disabilities may still experience subtle motor problems in their school years, leading to more inferior academic achievements and reduced social leisure activities.[62,63] Cognitive abilities may be negatively affected by prematurity, as shown by studies reporting more inferior academic achievements and a higher prevalence of learning difficulties in children who were born preterm.[6] Speech development and communication skills are also negatively affected, especially expressive language.[64] Behavioral problems, including attention deficit hyperactivity disorder (ADHD), are found to be more prevalent in very preterm infants, which can influence classroom performance and social capabilities.[6]

Nutrients provided to the preterm infant play essential roles in ensuring optimal brain development after birth. Some nutrients, which support primary neuronal metabolism, are thought to have a notable impact on brain development between 24 and 44 weeks post-conception, including protein, glucose, iron, zinc, copper, iodine, folate, and choline.8 Preterm birth adds risk to rapidly developing systems, such as the hippocampus (learning, memory), cerebellum (balance, cognition, motor integration), and myelin; disruptions to these systems in early life may lead to behavioral manifestations.[65,66] Other morbidities to which preterm infants are more susceptible, such as infections and invasive procedures, also affect the body's ability to absorb and utilize certain nutrients; for example, during sepsis, amino acids are used for gluconeogenesis and are not available to perform other functions such as building tissue.[8] Inflammatory diseases and infections such as NEC and LOS are associated with adverse neurological outcomes. A link between pro-inflammatory molecules that are bacteria-derived with

systemic inflammation, which results in brain injury, was observed, suggesting a link between microbial dysbiosis and neurodevelopmental abnormalities.[56]

A landmark paper by Sudo et al. showed germ-free mice had exaggerated stress responses compared to mice with normal gut flora, and oral administration of *Bifidobacterium infantis* reversed the exaggerated responses to normal, indicating the importance of gut microbiota in the development of behavior.[67] Furthermore, the gut microbiota is found to have crucial roles in the maturation of microglial cells, which are tissue-resident macrophages of the brain. In mice, depletion of gut flora disturbs microglia development and function. This disturbance could be reversed by administering short-chain fatty acids, suggesting gut microbes have a notable influence on microglia function.[68]

In the early months of life, in the lactation-only period, the developmental capacity of the brain is remarkable, and it may be postulated that this growth is supported by components of breast milk, such as the glycans that are found to influence the infant GIT microbiota.[69] Concerning growth, studies have shown that gut microbiota indirectly affects the somatotropic axis, influencing growth phenotypes.[70] Studies on germ-free mice show germ-free mice's weight and body length gains during lactation were slower than conventionally raised animals, and levels of IGF-1, which mediates the effects of growth hormone (GH), were decreased.[70] Studies on animals with antibiotic-induced growth deficits show that the administration of short-chain fatty acids can restore production and activity of IGF-1 and restore growth, suggesting microbial activities in the GIT play a regulatory role in the somatotropic axis growth in life's early years.[71]

Products of bacterial fermentation, such as short-chain fatty acids, have influenced metabolism, including weight gain. Acetate and butyrate play roles in regulating satiety, and reduced weight gain was associated with high Bacteroides and low acetogens and methanogens.[69] This suggests the possible role of gut microbiota in infant weight gain and growth. Furthermore, the gut microbiota has positive associations with gut motility, feeding tolerance, and faster achievement of full enteral feeds, contributing to optimal growth in preterm infants.

The connection between the gut and the brain, frequently called the gut-brain axis, is a topic of intense scientific investigation. Despite the efforts, the exact mechanisms of this connection remain to be fully elucidated. Several proposed mechanisms include mediation by the vagus nerve, whose afferent terminals are found underneath the gut epithelium, possibly mediating communication between the gut microbiota and the central nervous system.[56] The enteric nervous system that innervates microvilli can be activated by gut microbes, and those signals can be transmitted to the brain via the vagus nerve.[72] The current evidence suggests crosstalk between the brain and the gut microbiota, and more studies are needed to investigate the mechanistic foundations of this connection properly.

PREBIOTIC SUPPLEMENTATION FOR PRETERM INFANTS

Gibson et al. first defined prebiotic in 1995 as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health".[73] In 2004, Gibson described prebiotics as resistant to gastric acidity, are fermented by intestinal microflora, and selectively stimulate the growth and/or activity of intestinal bacteria that contribute to health.[74] HMOs, prebiotics present in human milk, selectively amplify bacterial populations and have direct immunomodulatory effect; HMOs reduce leukocyte rolling, activation, and transmigration, postulated to protect breastfed neonates against inflammatory diseases.[75] Benefits of HMOs are not limited to the GIT; when consumed breastmilk passes through the laryngopharyngeal region, HMOs were shown to have inhibitory effects to pathogen attachment to pharyngeal or buccal epithelial cells.[75]

Healthy colonic microbiota, supported by HMOs, produce short-chain fatty acids and lactate, which can modulate cytokine production, contribute to the integrity of the gut barrier, and improve gut motility.[75] Furthermore, oligosaccharides can protect the gut from pathogenic bacteria through binding with the pathogens' adhesion-related virulence factors, disabling them from directly adhering to the gut's epithelial surface.[76]

It is well established that breast milk is the gold standard for preterm infants nutrition. Thus the formulations of infant formula strive to mimic the composition of breast milk. Galacto-and fructo-oligosaccharides that are manufactured to function similarly to HMOs have been used as prebiotic supplements in infant formula to mimic natural HMOs. They have been shown to have positive immune system development benefits,[75] may induce Bifidobacterium-dominant faecal microbiota similar to breastfed infants.[77] However, these formulas have not been able to emulate breastfed infants' microbiota completely.[2,54,78]

Various studies described an increase in bifidobacteria counts in preterm and term neonates given oligosaccharides-supplemented formula and better stool frequency, consistency, and pH.[79,80] Studies by Indrio et al. and Modi et al. shows prebiotics given to preterm neonates improved feeding tolerance, and this improvement is seen in preterm neonates born at 28- and 26-week gestation.[81,82]

A recent meta-analysis shows prebiotic supplementation for preterm infants can decrease sepsis incidence, reduce the time needed to full enteral feeding, cut lengths of hospital stays, and increase stool frequency (similar to stool frequency of breastfed infants).[54,83] This beneficial effect was seen when prebiotics supplementation was given for more than 28 days. However, no significant decrease in risk ratio is seen in NEC incidence, supporting other studies' findings that the role of prebiotics in NEC is limited.[54,75] Several studies elucidated the possible mechanisms behind prebiotics' positive impact on preterm infants' health, such as pathogenic bacteria colonization prevention, intestinal motility and intestinal permeability improvements, and inhibition of pathogen adherence to the epithelial surface.[84–86]

Two papers that assessed the same cohort of preterm infants at different time points (the first year of life and at two years corrected age) described neurodevelopmental benefits of prebiotic

supplementation. They found no significant difference in neurodevelopmental outcomes between the control group and infants supplemented with small-chain galacto-oligosaccharides (scGOS), long-chain fructo-oligosaccharides (lcFOS), and pectin-derived acidic oligosaccharides (pAOS). However, they found a decreased incidence of neonatal severe infections in the supplemented group. They concluded that neurodevelopmental outcomes are affected by neonatal infections and lower bifidobacteria counts. Thus microbiota still potentially play a role in neurodevelopment.[55,87]

The overall safety profile of prebiotic supplementation is favorable, because adverse effects are rarely reported. A meta-analysis of 24 randomized clinical trials (RCTs) described that 23 of the 24 RCTs analyzed reported good tolerance for prebiotic supplementation.[88] Only one RCT reported more diarrhea, irritability, and eczema in the infants who received prebiotic supplementation.[89]

CONCLUSIONS AND EXPERT OPINIONS

Gut microbiota influences many aspects of a preterm infant's life and is prone to imbalances, leading to dysbiosis. Factors like mode of delivery and choice of nutrition significantly affect the gut microbiota. Prebiotic supplementation is shown to have a positive impact on preterm infants with a good safety profile. We conclude, based on the available literature, that prebiotics can be given to preterm infants in the form of prebiotic-supplemented formula to help with feeding intolerance, gut motility, stool frequency, development of the immune system, and long-term growth and development, but more evidence is needed to support its role in preventing NEC.

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Author Contribution

All authors contributed equally in preparing the manuscript for publication.

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