The Biotics Family in Early Life

second edition

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Disclaimer
Any information provided herein regarding infant dietary supplementation is intended to serve an informative purpose only, and should not take the place of careful and appropriate clinical judgment. Guidelines and recommendations may vary between countries.

Glossary

2'-FL	2'-fucosyllactose
3'-GL	3'-galactosyllactose
AD	Atopic dermatitis
CMA	Cow's milk allergy
FAO	Food and Agriculture Organization of the United Nations
FOS	Fructo-oligosaccharides
GI	Gastrointestinal
GOS	Galacto-oligosaccharides
HiMOs	Human identical milk oligosaccharides
HMOs	Human milk oligosaccharides
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
Ig	Immunoglobulin
ISAPP	International Scientific Association for Probiotics and Prebiotics
lcFOS	Long chain fructo-oligosaccharides
NEC	Necrotizing enterocolitis
QPS	Qualified Presumption of Safety
SCFA	Short chain fatty acid
scGOS	Short chain galacto-oligosaccharides
Th	T helper (cell)
TLR	Toll-like receptor
WAO	World Allergy Organization
WHO	World Health Organization

Table of contents

Chapter 1

Introduction

The first 1000 days, from conception to around a child's second birthday, represent a critical period of growth and development that can shape a child's future health and wellbeing.[1,2] It is widely recognized that nutrition during early life can significantly impact growth as well as immediate and future health, and that breastfeeding and/or nutritional intervention during this critical window can help avert both infectious and non-communicable disease risk during childhood and later life (**Figure 1**).[1]

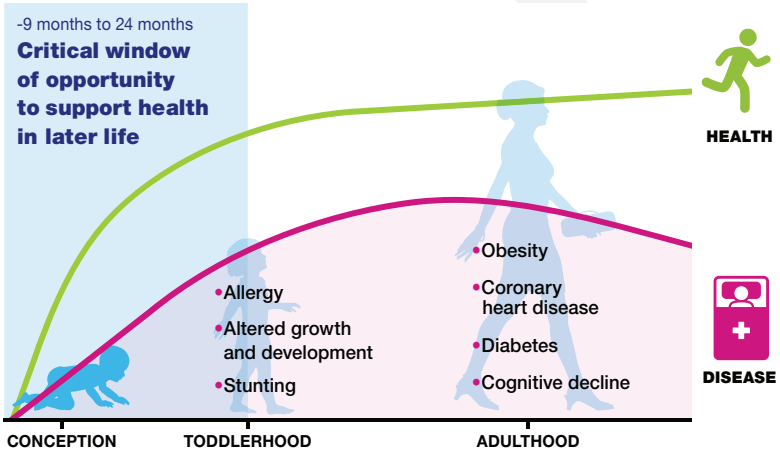

Figure 1. Nutrition during early life: critical window of opportunity

This Essential Knowledge Briefing series discusses various aspects of health in early life. The books are intended to be used as a practical guide for healthcare professionals working with infants and their families. **Book 1** highlighted the gut microbiota and its importance for infant and future health. **Book 2** focused on functional gastrointestinal disorders and digestive problems in pregnant women and infants. In **Book**

3, we discussed the impact of fetal and infant nutrition on growth.

In this updated fourth Essential Knowledge Briefing, we present more information on immunity, specifically with regard to the influence of the gut microbiota on immune function. Human milk is the gold standard for infant nutrition. Besides nutritional components, human milk contains many bioactive components (for example, human milk oligosaccharides [HMOs], (glyco-) proteins, (glyco-) lipids, long-chain polyunsaturated fatty acids, microRNA, leptin, insulin, and insulin-like growth factors [IGF]), as well as beneficial bacteria and immune cells.[3] These all play a key role in supporting the development of a healthy, balanced gut microbiota and immune system.[4,5]

In this book, we discuss these concepts, and how active modulation of the gut microbiota through the use of dietary 'biotics' in non-exclusively breastfed infants and those with dysbiosis may help optimize health outcomes and help to reduce the risk of disease in later life.

While all biotics (prebiotics, probiotics, synbiotics, postbiotics) have the ability to modulate the gut microbiota, their mechanisms of action differ. The type of biotic indicated will depend upon the individual infant and clinical circumstances.

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Chapter 2

The infant gut and immune system

The human intestine is more than simply a digestive, absorptive, and waste-eliminating organ. It is a highly sensory organ harboring a complex enteric nervous system that communicates with the brain; it also contains 70%–80% of the body's immune cells, and hosts a huge microbial ecosystem.[1] This ecosystem is collectively called the 'gut microbiota', and comprises an ecological community of commensal, symbiotic, and pathogenic microorganisms including bacteria, archaea, fungi, and viruses. The gut microbiota interacts in complex ways with the immune, metabolic, and nervous systems in the host, and helps protect the body from pathogenic and chemical insults through its ability to modulate the gut barrier and immune responses (see **Chapter 2**).[2-4]

As such, the gut represents the largest interface between the host and the external environment, and shows complex and highly integrated responses to environmental signals and changes in its luminal contents.[1]

The gut barrier: mucosal defense

The intestinal barrier is composed of the epithelium and underlying *lamina propria*, as well as extracellular mucus layers.[5] Collectively, these represent a physical and chemical barrier to protect the host from attack by potentially harmful microorganisms and other environmental threats.[5,6]

Between the epithelial cells, tight junction proteins form a continuous intercellular barrier that acts as a permeable seal,

to selectively regulate the trafficking of important macromolecules, and exclude toxins.[5,7]

Within the gut lumen and on the epithelial surface, 'commensal' (i.e. normal, healthy, resident) gut microbes appear to contribute to the development and strengthening of the gut mucosal barrier through various mechanisms, including promotion of epithelial cell maturation and tight junction integrity.[8]

The *lamina propria* acts as an important interface between the environment and the gut immune system, facilitating activation of an immune response if antigens or pathogens cross the epithelial layer.[5]

Gut microbiota composition and activity

At birth, an infant transitions from an environment with limited exposure to microbes in the amniotic fluid, to an environment with widespread and continuous exposure to air-, skin-, and surface-borne microbes.[9] The infant gut, with its nutrient-rich and temperature-stable environment, nurtures colonization by beneficial bacteria – including *Bifidobacteria, Lactobacillus*, and *Bacteroides* species – allowing the development of a unique 'gut microbiota' or 'microbiome'.[9,10] The 'gut microbiota' refers to the collection of microorganisms that colonise the gut whereas the 'gut microbiome' refers to the collection of microorganisms that colonise the gut and their genetic material.[11] Early environmental exposures and microbial colonization are

believed to 'set the scene' for the long-term health of the gut mucosa and immune system.[12]

Microbiota colonization and composition

Gut colonization and establishment of the microbiota is a dynamic step-wise process through the first three years of life and beyond.[13] *Bifidobacteria* are among the first beneficial microbes to colonize the gastrointestinal tract of a newborn infant, and are present as the predominant bacteria in the intestinal tract of breastfed infants.[14] These bacteria are usually transmitted to the infant via the mother and the surrounding environment.[15,16] Other common 'pioneer' microbes include those from the genera *Bacteroides, Clostridium,* and *Eubacterium*.[2,17]

Bifidobacteria produce antimicrobial substances such as short chain fatty acids (SCFAs) (e.g. acetate and lactate) through anerobic fermentation of HMOs. These acidic components help to inhibit the growth of several potentially pathogenic bacteria contributing to healthy colonization of the newborn.[18,19]

Gradually, as the infant matures, the gut microbiota diversifies through colonization with a variety of microbes, and reaches a stable, balanced microbial community around three years of age.[13]

Many factors appear to shape the development (composition and function) of the gut microbiota during early life, including

genetics, pregnancy factors, mode of delivery (cesarean vs. vaginal), gestational age, dietary exposures (human vs. formula milk), antibiotic use, other drug use (e.g. proton pump inhibitors and non-steroid anti-inflammatory drugs), and other early environmental exposures[2,10,20, 21] and even air pollution.[22]

Functions of the gut microbiota

The gut microbiota has a profound influence on the maturation and functional development of the intestinal immune system during the first 1000 days of life and beyond (including toddlers and preschoolers), playing a vital role in normal gut function and maintenance of health (**Figure 2**).[6,10]

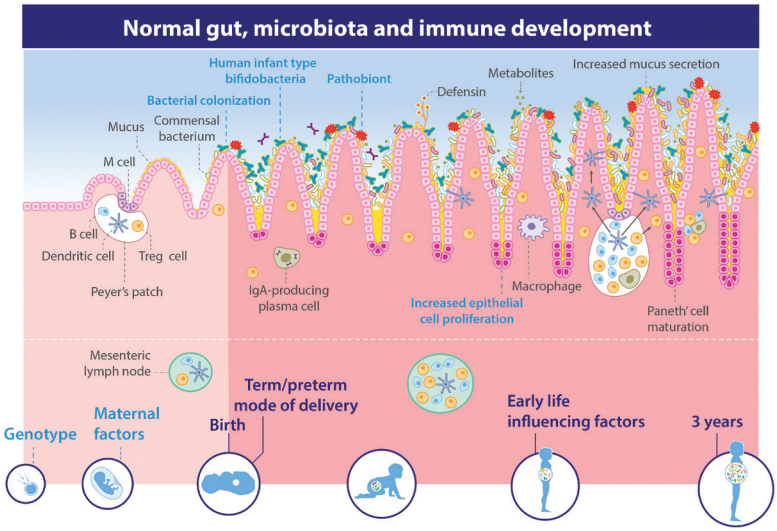


Figure 2. The first 1000 days of life: a crucial period for the development of immunity through the gut[10]

The microbiota plays a beneficial role for the host in a variety of ways, including nutritive, immunological, and nervous system benefits (**Figure 3**):[2-4,9,10,17, 23-25]

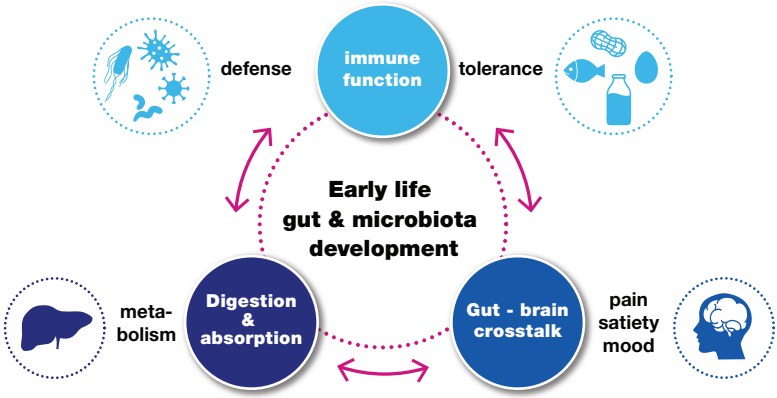


Figure 3. Vital role of the gastrointestinal tract and gut microbiota[9,10,20,21]

Facilitation of **efficient digestion** (e.g. fermentation of dietary fiber; pre-digestion of some nutrients), and **nutrient absorption**

- Maintenance of **intestinal homeostasis**
- **Stimulation of gut development**
- Maintenance of protective **epithelial barrier function**
- **Protection from pathogens** ('colonization resistance'), through competition for nutrients and adhesion sites, and production of antimicrobial peptides
- Development and functioning of the **mucosal immune system**
- **Modulation of immune and inflammatory responses**
- **Regulation of the enteric nervous system**
- Influences **neurodevelopment** (gut–brain 'crosstalk')

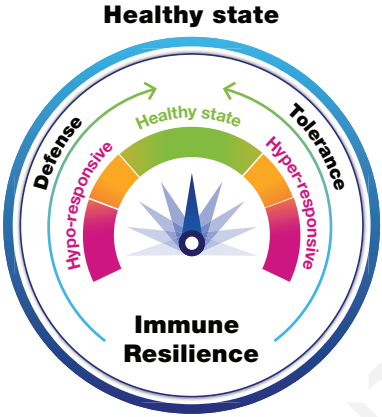
Evidence supporting the wide-reaching benefits of the gut microbiota in both short- and long-term human health and disease is rapidly expanding.[23,26]

Immune function and its link to the gut

The immune system collectively comprises the organs and physiological processes that provide protection for an individual against harmful infections and toxins. The mucosal immune system is the largest immune component in the body.[2] A complex interplay of innate and adaptive components takes place as the body responds to diverse environmental and microbial challenges in order to maintain homeostasis.[23]

Healthy immunity relies upon balance; to function properly, the immune system must be able to detect a pathogen or toxin, distinguish it from the body's own normal tissue, and provide the appropriate response.[27] Pathogens or damaged cells need to be destroyed, foreign elements that are beneficial need to be tolerated, and healthy cells must continue to be accepted (**Figure 4**).

'Resilience' means the ability of a system to withstand changes in its environment while still functioning properly. The term 'immune resilience' refers to an individual's ability to adapt to immunological challenges by regulating an appropriate immune response. In the short term, immune resilience has implications for food tolerance/allergy and infection; in the longer term, there are implications for the development of other non-communicable diseases, like autoimmune disorders.[27-29]



The immune response should be of an optimal strength:
not too weak (hypo-responsive) which will increase the risk of infections, or too strong (hyper-responsive) potentially resulting in allergy, chronic inflammation, or autoimmunity

Figure 4: Immune system balance: reaching or targeting resilience

Innate and adaptive immunity

Infants are at continual risk of infectious-related diseases.[30] It is vital that the body's gut-associated lymphoid tissues are able to provide effective and appropriate immune responses when necessary.[27,31]

As a first line of defense, the healthy development of the physically intact gut barrier and the commensal bacteria in the gut provide important protection against pathogens through, for example, promoting mucus production, lowering the pH of intestinal contents, secreting antimicrobial substances that inhibit the adhesion and growth of harmful bacteria, or by competing with invading organisms for binding sites and nutrients.[2]

Innate immune responses form a second line of defense. While commensal bacteria are non-invasive and do not

trigger inflammatory responses, other microorganisms such as pathogens and soluble toxins readily penetrate the epithelium, where they are immediately recognized by specialized cells and receptors that form the innate immune system, initiating a non-specific effector response.[2,31]

As a third line of defense, the adaptive (acquired) immune system involves functional properties of B and T lymphocytes, and their antigen-specific surface receptors. Adaptive immunity is characterized by a long-lasting 'immunological memory' after initial response to an antigen, leading to enhanced responses with subsequent exposures to the same antigen.[32] This reaction is mediated by secretory immunoglobulins (Ig) (antibodies), triggering a complex cascade of events resulting in destruction of the antigen.[2]

Tolerance is the normal physiologic response to innocuous (harmless) ingested antigens.[28] Early exposure to such potential antigens is necessary for immune system 'training' in early life, to promote appropriate effector responses and the development of oral tolerance.[33] A breakdown in, or overreaction of, the immunological response at the cellular and molecular level can lead to sensitization and allergic disease after antigen exposure, through inappropriate activation of the adaptive immune system.[29, 32]

The gut microbiota and immune function

Despite the ever-building volume of literature in the field of microbiomics (i.e. the study of communities of microbes in

the human body), the molecular mechanisms underlying the bi-directional interaction between the gut microbiota and the immune system – including allergy development – is not fully understood.[34]

However, it is becoming widely acknowledged that the establishment of an optimal microbial community after birth, and the maintenance of a balanced gut microbiota, can have a profound effect on the development of both the innate and adaptive immune systems.[2,4,27,35] A healthy gut microbiota is resilient and supports immune resilience by promoting the development of appropriate immunologic regulatory networks, both in the local intestinal immune system but also with regard to systemic responses. Aberrations in the composition of the microbiota, known as dysbiosis, can thus result in impaired or overactive immune responses.[23, 27]

An example of a common and early-onset immune disorder is allergic disease. The modern epidemic of allergic diseases points to vulnerability of the immune system to modern environmental change. Multifactorial depletion of microbes and gut microbiota imbalance (dysbiosis) can be a major underlying factor.

The gut–brain–immune axis

The gut is a highly sensory organ containing millions of neurons and 70%–80% of the body's immune cells.[1] Sensory neurons, endocrine cells, and immune cells enable signaling to modulate gut motility, tissue defense, vascular perfusion,

and functions of other organs; and signals are also sent to the central nervous system to influence feeding behavior.[1] In this way, the gut influences the brain, and in turn, the brain influences the gut by way of the 'gut–brain axis'.

Brain development appears to be partly modulated by the gut microbiota. The complex microbiota–gut–brain communication is driven by a variety of pathways including barrier function, hormonal and neural regulation, as well as via immune and metabolic pathways.[36] Bacterial metabolites such as SCFAs are able to cross the blood–brain barrier and can directly affect learning and memory. The blood–brain barrier plays a vital role in brain development by protecting the brain from external harm. In cases where the development of the gut microbiota is disrupted during the first 1000 days, brain development may be impacted, which can translate into complications that can last into adulthood.[36]

Dysbiosis and immunity

The concept of 'dysbiosis' refers to a state of imbalanced proportions of commensal, beneficial, and potentially harmful (opportunistic pathogens) and pathogenic microorganisms, largely due to environmental influences and exposures.[10]

As one important example, cesarean-born infants show delayed colonization by *Bifidobacterium* and *Bacteroides* and enrichment of other species, compared with those delivered by vaginal birth, due to lack of normal exposure to bacteria through the birthing canal **(Figure 5).**[37,38] Acquisition and

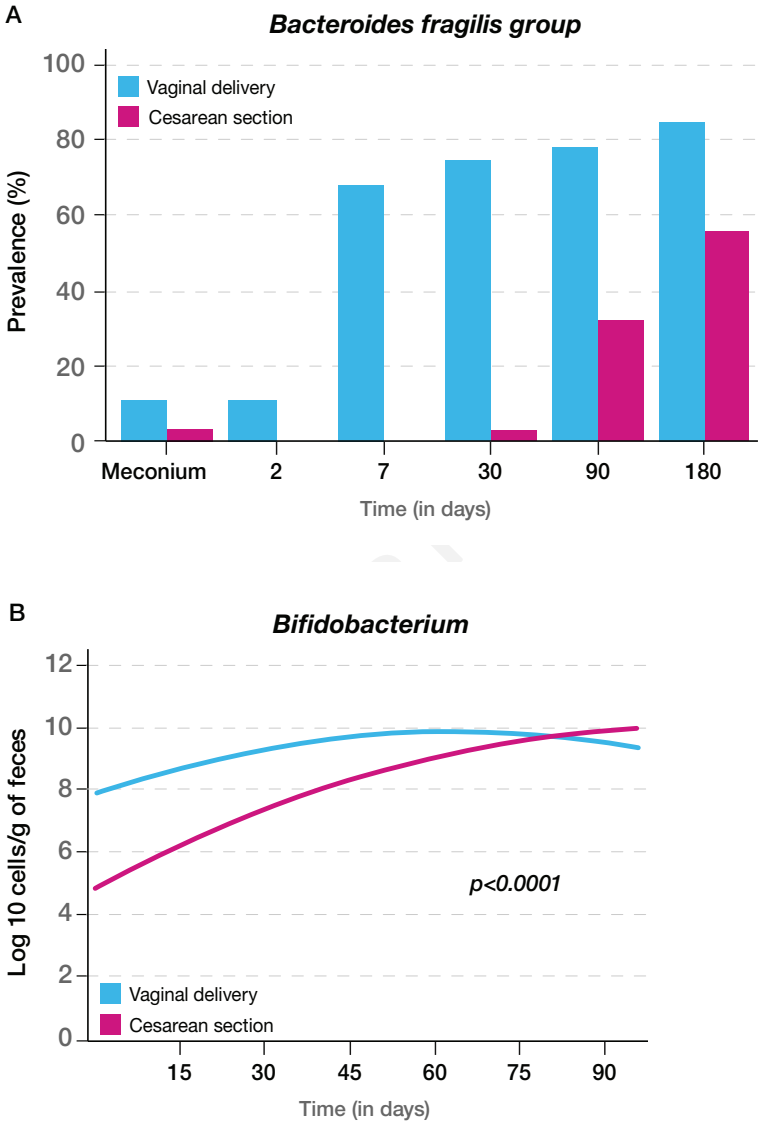


Figure 5. Prevalence of (A) *Bacteroides fragilis* and (B) bifidobacteria in fecal samples from infants born by cesarean versus vaginal delivery

colonization of commensal gut bacteria may also become delayed or disrupted in infants born prematurely.[9] This may be due to cesarean birth, and/or antibiotic use, use of other pharmaceuticals, exposure to hospital-acquired infections, delayed enteral feeding, or lack of human milk feeding.

Because the gut microbiota helps shape the immune system in early life[2] **(Figure 6),** dysbiosis can be associated with both short- and long-term consequences related to immunity. Several preclinical studies and clinical trials of pre- and/or probiotics have yielded promising results in the restoration of a normal microbiota composition[26] (see **Chapters 3-6**).

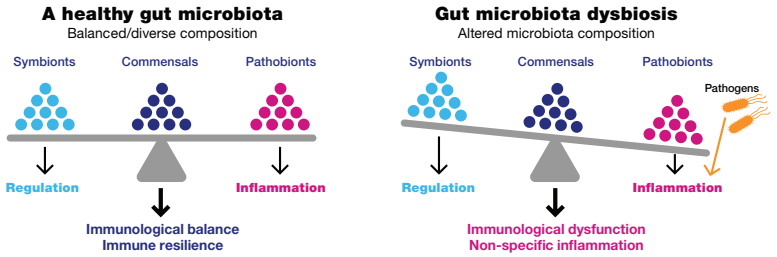


Figure 6. Dysbiosis and its potential immune consequences

For more information on the gut microbiota and the health implications of dysbiosis, please refer to **Significance of the Gut Microbiota and Nutrition for Development and Future Health**.*

*https://www.essentialknowledgebriefings.com/downloads/gut-health-in-early-life-significance-of-the-gut-microbiota-and-nutrition-for-development-and-future-health/

Immune benefits of human milk

The WHO recommends exclusive breastfeeding for the first six months of life and continued breastfeeding up to two years or longer, combined with the safe introduction of appropriate complementary feeding.[39] Breastfeeding provides a unique opportunity for ingestion of nutritional components and functional/bioactive agents to support gut maturation and optimal growth and development.[40]

Human milk composition is extremely complex, and naturally provides thousands of different nutritive and protective components that interact with each other in a unique way and are specifically tailored to the infant's needs. Human milk consists of 88% water and major components such as lactose (53-61 g/L), lipids (30-50 g/L), HMOs (up to 20 g/L) and proteins (8-10 g/L).[41] Immune cells, stem cells, bacteria, peptides, free amino acids, hormones, vitamins, minerals, nucleotides and other bioactive components are also present in human milk as minor components.[40] Human milk contains a variety of potentially health-promoting microbes and metabolites produced by and/or derived from these beneficial bacteria,[42] the most common being from the *Firmicutes* and *Actinobacteria* phyla.[38] There is high variability in the composition and number of bacteria in human milk among mothers, and in some cases even within mothers at different time points during the lactation period. It has been estimated that human milk contains between 10^3 and 10^6 bacterial cells/mL,[43,44] with some of this number being made up of non-viable bacteria.[43] Not only the bacteria in human milk, but

also their metabolites (e.g. cell wall components and various bacterial metabolites) are anticipated to stimulate a healthy gut microbiota, immune functioning, and gut development.[43]

In addition, human milk contains many immune cells and other bioactive components such as human milk oligosaccharides (HMOs). These play important roles in the development of a healthy immune system by supporting a balanced gut microbiota, and providing anti-infective and immune development stimulating properties.[43,45-47] Human milk is thus considered to provide the best immune training opportunity for an infant.[23,47,48] HMOs can be composed of five different monosaccharides: glucose, galactose, N-ethylglucosamine, fucose, and sialic acid.[49]

HMOs are one example of naturally occurring 'prebiotics' (see **Chapter 3**). They comprise a structurally diverse group of molecules with a size distribution of short chain (sc) HMOs and long chain (lc) HMOs.[50] HMOs have various functions and are known to exist in more than 1000 different structures[51], of which approximately 200 have been identified from human milk samples by chromatography and mass spectrometry.[52-54] While the biological functions of HMOs have not been fully elucidated, they remain a key focus of research interest.[55] In comparison, the synthesized human identical milk oligosaccharides (HiMOs) intended for addition to infant formula and follow-on milk are less complex and diverse.[56-58] HMO profiles are strongly influenced by maternal genetic predisposition and can be stratified into four different milk groups.[59-71] HMOs can be classified into three major types:

- Small (trisaccharides such as 2'-fucosyllactose (FL), 3-FL, 3'-sialyllactose (SL) or 6'-SL)) or large
- Acidic (sialylated) or neutral (non-sialylated)
- Fucosylated or non-fucosylated

According to their building blocks, three main HMO categories can be defined as:[62]

- Neutral or fucosylated HMOs, containing fucose at the terminal position, e.g (2'-FL) and lacto-difucotetraose (LDFT or DFL)
- Neutral N-containing or non-fucosylated HMOs, containing N-acetylglucosamine at the terminal end, e.g. lacto-N-tetraose (LNT)
- Acidic or sialylated HMOs, containing sialic acid at the terminal end, e.g. 2'-SL

Most HMOs escape digestion in the small intestine and progress to the colon where they are metabolized, acting as 'food' for the commensal gut bacteria. This results in production of beneficial components such as SCFAs and postbiotic type substances. HMOs thus play an important role in the 'feeding', nurture, and development of an infant's gut microbiota, intestinal barrier function, brain and immune system **(Figure 7).**[48, 55,63-70]

HMO profiles (structural diversity and abundance) are strongly influenced by maternal genetic predisposition and can be stratified into four different milk groups:[71-73]

Group 1: contains all types of fucosyl-oligosaccharides, with α1,2 to α1,3 and α1,4 linkages

Group 2: without α1,2 fucosyl-oligosaccharides, due to the lack of expression of the secretor gene

Group 3: identified by the absence of α1,4 fucosyl-oligosaccharides due to inactivity of the Lewis gene

Group 4: contains only α1,3 fucosyl-oligosaccharides due to the expression of the Lewis independent fucosyltranferase.

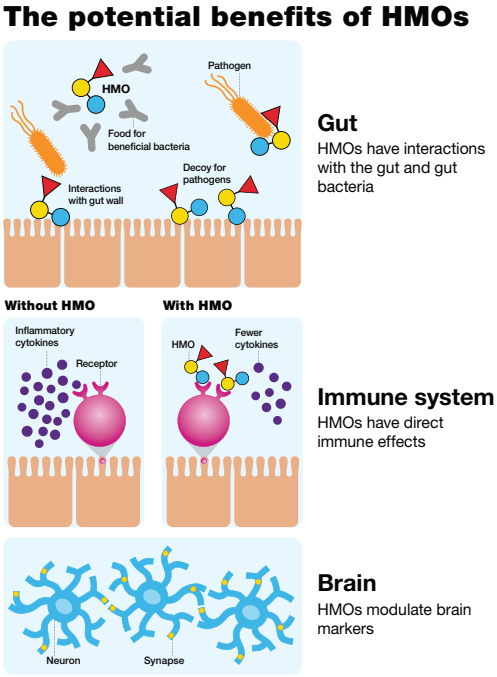


Figure 7. Human milk oligosaccharides and the gut–brain–immune axis[44,46–52]

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Chapter 3

The biotics family: Supporting immunity through the gut

What are biotics?

The term 'biotic' is derived from the Greek word *biōtikós*, meaning 'pertaining to life', and essentially refers to the biological ecosystem made up of living organisms together with their physical environment.[1] In the nutritional sense, biotics are a group of nutritionally active components that, when consumed, can confer a health benefit on the host.[1]

The study of the microbiota has recently spurred remarkable scientific, commercial, and public interest in the use of nutritional biotics to modulate the gut microbiota to support human health.[2] There is rapidly increasing awareness among healthcare professionals around the beneficial effects of prebiotics and probiotics in human health – particularly for infants and children.[3]

Prebiotics and probiotics may be used in combination, as 'synbiotics'. Finally, the latest member of the biotics family, postbiotics, refers to the preparation of inanimate microorganisms and/or their components that confers a health benefit on the host (**Figure 8**).[4]

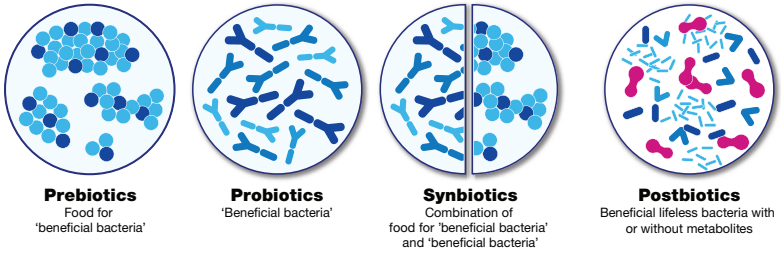

Figure 8. Prebiotics, probiotics, synbiotics, and postbiotics: Definitions and functions

Subsequent chapters discuss definitions, examples, benefits, and safety of each of the four members of the biotics family.

Postbiotic[4]	Preparation of inanimate microorganisms and/or their components that confers a health benefit on the host
Probiotic[5]	Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host
Prebiotic[6]	A substrate that is selectively utilized by host microorganisms conferring a health benefit
Synbiotic[7]	A mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host

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Chapter 4

Prebiotics

Definitions

In 2017, the International Scientific Association of Probiotics and Prebiotics (ISAPP) reviewed the definition and scope of prebiotics, and produced a consensus statement on the definition as illustrated below:

> *Prebiotics are substrates that are selectively utilized by host microorganisms conferring a health benefit*[1]
>
> ISAPP consensus statement, 2017

Note that the ISAPP consensus statement focuses on the importance of *selectivity*; as different prebiotics pass through the gastrointestinal tract to the colon, they are selectively fermented by specific health-promoting bacteria.[2-4]

The application of prebiotics for use in infant formula to influence clinical outcomes is expanding due to their good safety profile, ease of administration, and potential to influence the gut microbiota, both compositionally and functionally.[5]

Examples of prebiotics in infant formulas

It is established that HMOs play an integral role in the nurture and development of the infant gut microbiota.[4,6] Therefore, when breastfeeding is not possible, addition of specific oligosaccharide mixtures to infant formulas is one strategy used to help promote the growth of beneficial gut microbes, particularly *Bifidobacteria*.[4]

The most frequently used and well-studied oligosaccharides are galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS).[2,4] A specific combination of short chain (sc) GOS/long chain (lc) FOS in a 9:1 ratio in some infant formulas aims to mimic the structures, function and size of non-digestible oligosaccharides in human milk.[7-9] The beneficial effects of the prebiotic mixture scGOS/lcFOS (9:1) are supported by a large number of clinical and pre-clinical studies.

Other prebiotics that could potentially be used in infant formula include inulin and polydextrose.[2] To date, there are limited data describing specific benefits of these components.

Well-established prebiotics[10] (e.g. FOS and GOS) are distinctly different to HMOs in human milk, which have high complexity and a diverse set of biochemical, physiological and clinical properties.[11] Currently, progress in biosynthetic technology has allowed the production of selected HiMOs (e.g. 2′-FL and lacto-N-neotetraose; LNnT), which aims to bridge the gap between breast milk and formula milk. HiMOs molecules are synthetically produced *via* fermentation or by enzymatic synthesis. However, despite advancements in biosynthetic technology, development of complex and long-chain HiMOs remains a challenge.[12] In addition, there are stringent regulatory approval processes involved to allow the use of any and all HiMOs in infant formula especially in European Union novel food safety assessment. Therefore, at present, the infant formula industry is confined to a limited number of short-chain HiMOs which may reduce influence on infant

health outcomes. Indeed, the differences observed in clinical outcomes in breastfed infants compared with formula fed infants is likely due to the lack of diversity in HiMO structures, especially the absence of more complex and long-chain HiMOs, in infant formula.

2'-FL and LNnT – two of the most abundant HMOs in human milk – have widest use in infant formulas.[13,14] Both have been shown to promote *Bifidobacterium* growth in preclinical models.[14] More recently, other HiMOs have been added to some advanced infant formula and follow on milk including 3-FL, DFL, LNT, 3′-SL, and 6′-SL.[15-17]

2'-FL has been described to have beneficial effects on the immune system,[18] gut microbiota,[19-23] gut barrier,[24] and brain development in a preclinical model.[25] In addition, formula-fed infants supplemented with 2'-FL has been associated with lower inflammatory cytokine profiles (interleukin (IL) receptor antagonist (IL-1ra), IL-1a, IL-1b, IL-6, and tumor necrosis factor alpha (TNF-alpha), similar to that of breastfed infants.[26] In animal models, 2′-FL has also been reported to deliver a beneficial effect against NEC by decreasing pro-inflammatory markers and preserving the small intestinal mucosal architecture.[27]

2'-FL may be used with or without LNnT and with or without GOS. In a recent clinical study, supplementation of infant formula with both 2'-FL and LNnT was found to be safe and well-tolerated, and to result in lower parent-reported adverse events, namely

lower incidence of bronchitis and lower antibiotic use compared with unsupplemented control formula.[14]

3'-Galactosyllactose (GL) is a HMO trisaccharide that is found in higher levels in colostrum, and in lower levels in mature human milk. 3'-GL has been reported to have anti-inflammatory properties and to reduce IL-8 responses in ex vivo research, both of which are known to contribute to innate immune modulation.[28,29]

With more HiMOs becoming available, it's now possible for manufacturers to supplement infant formulas with more structurally diverse and complex blends for formula-fed infants. Several studies to date have shown that supplementing with specific blends of five HiMOs can modulate the gut microbiome.[15-17] One randomized controlled trial comparing an infant formula containing five HiMOs (2′-FL, 2′,3-di-fucosyllactose (DFL), LNT, 3′- SL, and 6′-SL) with a standard cow's milk-based infant formula (n=693) found that the specific blend of five HiMOs supports gut microbiome development by shifting the composition closer to that of breastfed infants with higher bifidobacteria, particularly *B. infantis*, and lower toxigenic *Clostridioides* difficile. The specific blend of five HiMOs also resulted in higher fecal SIgA responses suggesting an improved early-life intestinal immune response. [17]

While there are no safety concerns relating to the addition of HiMOs in infant formulas, the clinical benefits are still a topic of debate.[31] Therefore, infant formulas with added HiMOs are not routinely recommended above infant formulas without HiMOs.[31]

Known and possible benefits of prebiotics such as scGOS/lcFOS

Evidence for the health benefits of prebiotics is rapidly evolving[1,32,33] (**Figure 9**).

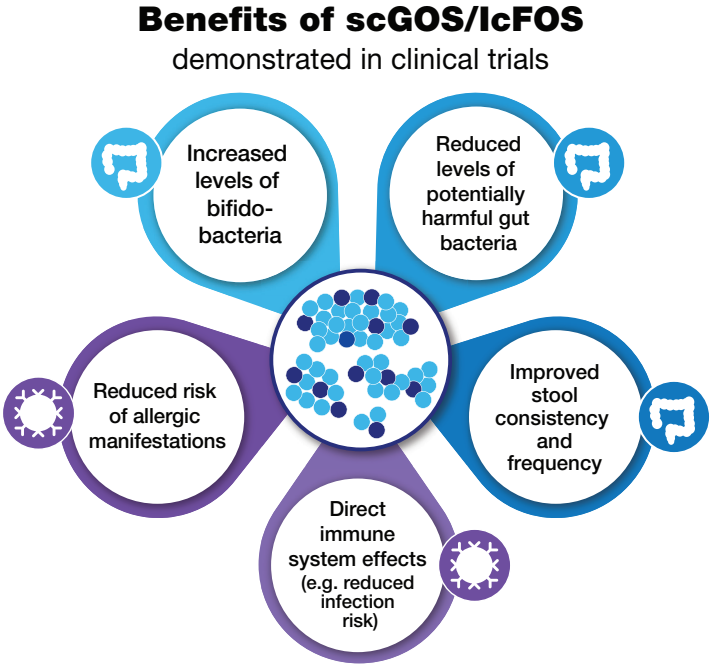

Figure 9. Benefits of scGOS/lcFOS prebiotic mixture demonstrated in clinical trials[4,7,20-35]

Specific benefits of prebiotics in infants and young children may include the following.

Modulation of the gut microbiota

Prebiotics have been shown to stimulate the growth and/or activity of important beneficial bacterial populations in the gut. For example, prebiotic supplementation of infant formulas

with scGOS/lcFOS has been shown to increase levels of fecal *Bifidobacteria* in a dose-dependent manner,[34-37] resulting in a microbiota composition closer to that of breastfed infants.[38,39]

In addition, this specific prebiotic mixture has been shown to reduce a wide array of clinically relevant pathogens.[40] This finding suggests that the bifidogenic effects of prebiotic oligosaccharides may help protect against infections.[36,38,40-43] Indeed, *Bifidobacteria* are known to selectively metabolize non-digestible oligosaccharides and HMOs, resulting in the production of SFCA and a low fecal pH that inhibits pathogens.[4,8,43]

A recent report reviewed 14 clinical studies and showed a trend toward increasing mean stool pH in healthy breastfed infants between 1926 and 2017, from pH 5.0 to pH 6.5. This trend mirrors the recent reported decreases in *Bifidobacterium* abundance and associated dysbiosis in infants in developed countries.[44] In addition, several pre- and perinatal factors are known to influence the gut microbiota and immune system during this critical window, including mode of delivery and use of medications.[5] This is pertinent given the rise in caesarean section deliveries and overuse of antibiotics.[45,46] Since human milk is the main factor driving development and function of the gut *via* a favourable gut microbiota composition, it is essential that infant formula mimics human milk as much as possible.

Immunomodulatory effects

Preclinical and clinical evidence indicates that non-digestible oligosaccharides may have direct immunomodulatory and anti-

inflammatory effects at the cellular level.[47] A large, randomized study showed a reduced risk of infection following consumption of young child formula supplemented with scGOS/lcFOS/n-3 long chain polyunsaturated fatty acids (LCPUFAs).[48]

Supplementation with scGOS/lcFOS has also been shown in some studies to reduce the incidence and duration of acute diarrhea and antibiotic use in infants.[32,49] In addition, a protective effect against upper respiratory tract infections has been observed in infants that were fed a formula supplemented with scGOS/lcFOS (**Figure 10**).[49,50] Furthermore, in a prospective, double-blind, randomized, placebo-controlled trial, infants at high risk of atopy showed a lower incidence of

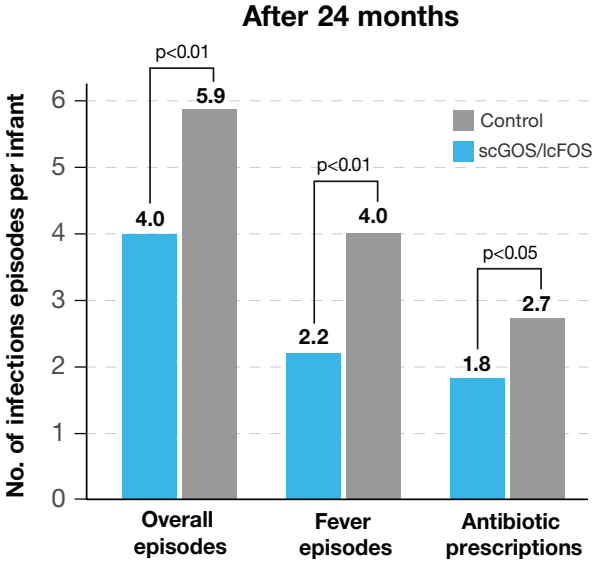

Figure 10. Significant reduction in infectious episodes between infants fed scGOS/lcFOS supplemented formula versus control formula. Data from Arslanoglu et al. *J Nutr.* 2008;138:1091–5[21]

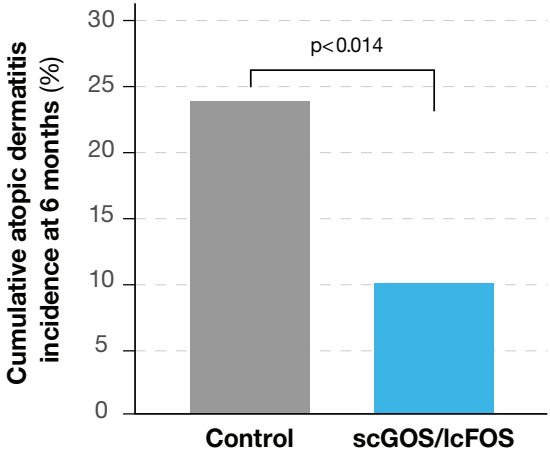

Figure 11. Decreased cumulative incidence of atopic dermatitis at 6 months in infants fed formula with scGOS/lcFOS versus formula with placebo. Adapted with permission from BMJ Publishing Group Limited. [*Arch Dis Child*. **Moro et al. 2006;91:814–9, copyright 2006**][31]

atopic dermatitis (**Figure 11**).[35] However, statistically significant reductions in infectious morbidity in prebiotic-supplemented infants have not consistently been demonstrated.[32, 51]

Improved gut motility and stool characteristics

Administration of prebiotics to infants has been shown in randomized trials to improve infant gut motility, gastric emptying, and stool softness (including in constipated infants) – mimicking the effects of human milk, and resulting in improved feeding tolerance.[8,43,52-54] However, some studies have also included partially hydrolyzed whey protein, making it difficult to draw firm conclusions regarding the effect of scGOS/lcFOS specifically.

A randomized controlled trial investigating clinical outcomes of an infant formula containing five HiMOs (2′-FL, 3-FL,

LNT, 3′-SL, 6′-SL) compared with a control milk-based formula (n=341) reported a higher frequency of soft stools compared with the control group. In addition, the five HiMO blend was well tolerated with no difference in adverse events between groups.[15] In another randomized controlled trial (n=366), the group consuming the same HiMOs blend produced a higher frequency of stools that were soft and yellow, and were more comparable to the group consuming human milk.[16]

Preclinical studies on HiMOs have demonstrated:

Anti-adhesive and anti-microbial effects[55-57]

- LNT was shown to cause the highest inhibition of B. Streptococcus growth (60-70%)
- LNT reduced the attachment and cytotoxicity of Entamoeba Histolytica
- A mixture of 3'-SL and 6'-SL showed maximum reduction in interactivity of human rotavirus when introduced during infection (73% reduction)

Brain development and cognition[58]

- A mixture of 3'-SL and 6'-SL may impact upon brain development and cognitive function (e.g. learning and memory)

Growth[59,60]

- High levels of 2'-FL are positively associated with child height and weight scores.

Immune development [29,61-64]

- The 3 galactosyllactoses (3'-GL, 4-GL, and 6-GL) attenuated NF-kB inflammatory signaling in human intestinal epithelial cells and in human immature intestine suggesting a strong anti-inflammatory effect.
- 3'-SL and 6'-SL have been shown to prevent infectivity of influenza viruses. 3'-SL alone showed anti-inflammatory activity.
- A three HiMO blend significantly correlated with CMA status
- HiMO profiles are associated with a lower risk of food sensitization, characterized by relatively high concentrations of fucosyl-disialyllacto-N-hexose (FDSLNH), lacto-N-fucopentaose (LNFP) II, LNnT, LNFP I, 3'-SL, difucosyl-lacto-N-hexaose (FLNH), lacto-N-hexaose (LNH), LNT, 2'-FL and disialyl-lacto-N-hexaose (DSLNH).
- A six HiMO blend conferred resistance against inflammatory-induced epithelial barrier dysfunction, although the removal of 2'-FL resulted in a significant reduction in the protective effect.

Safety of prebiotics

Prebiotic administration to infants is considered generally safe and well tolerated, with no concerns regarding age-appropriate growth or adverse effects.[8,54] One study showed non-inferior weight gain in infants fed oligosaccharide-containing formula, compared with standard formula;[54] another showed no significant difference in standard growth measurements

between infants fed prebiotic/probiotic-enriched formula versus standard formula or versus human milk.[65]

No serious adverse effects of prebiotic use have been reported; however, prebiotic intake is known to be associated with mild gastrointestinal side effects in some cases, such as bloating, flatulence, and diarrhea. This effect generally resolves in short time due to adaptation of the gut.[2,3]

A 2012 systematic review concluded, in line with an earlier statement by the Committee on Nutrition of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), that specific oral prebiotic supplementation may confer favorable effects in some infants, but that further research is warranted.[66]

Summary

Clinical and non-clinical effects

- Improved stool characteristics (softer stools[8,32,52,54]) (e.g. acidic oligosaccharides, scGOS, lcFOS, polydextrose, oligofructose-enriched inulin, bovine milk-derived oligosaccharides)
- Lower pH[4,8,43] (e.g. acidic oligosaccharides, scGOS, lcFOS, lactulose)
- SCFA pattern closer to breastfed infants[38,39] (e.g. GOS, FOS)
- sIgA levels similar to breastfed infants[67] (e.g. scGOS, lcFOS)
- Bifidogenic effects[43,35-37] (e.g. GOS, FOS)
- Protection against infections[36,40,42,43,48,68,69] (e.g. acidic oligosaccharides, scGOS, lcFOS, polydextrose, lactulose)
- Reduced incidence and duration of diarrhea episodes[32] (e.g. acidic oligosaccharides, scGOS, lcFOS, polydextrose, lactulose, oligofructose, inulin)
- Reduction of allergic manifestations[35,70] (e.g. scGOS, lcFOS)
- Possible improvement in colic symptoms[34] (e.g. GOS)

Preclinical effects

- Direct immune system effects[47] (e.g. scGOS, lcFOS)
- Promotion of intestinal barrier integrity[71] (e.g. GOS, FOS)
- Brain development[72] (e.g. 2'-FL and 3-FL)

Table 1. Summary of potential beneficial effects of prebiotics

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Chapter 5

Probiotics

The term 'probiotic' was derived from a Greek word meaning "for life".[1] Probiotics facilitate the fermentation process in the colon, and play an important role in digestive, immunological, and respiratory health.[2]

Definition

The definition of probiotics is based on a 2001 FAO/WHO expert group consensus statement:

> *Probiotics are 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host'*[2,3]
>
> FAO/WHO Expert Group (2001); endorsed by ISAPP (2014)

Examples of probiotics

Numerous probiotic organisms have been researched in infants, including preterm infants, at various dosages and for different durations. Probiotic products may contain one or more bacterial strains.[1] The two most frequently studied bacterial probiotic species are from the *Bifidobacteria* and *Lactobacillus* genera.[1,4]

In 2020, the genus *Lactobacillus* comprises of 261 species. Given the considerable diversity, it was suggested to split the *Lactobacillus* genus into functionally relevant groups. Whole genome analysis was applied to analyse each *Lactobacillus* species. This prompted a reclassification of the Lactobacillus genus into 25 genera, including 23 novel genera.

For example, the new name for *Lactobacillus rhamnosus* is *Lacticaseibacillus rhamnosus*. However, the abbreviations of microorganisms remained the same (i.e., *L rhamnosus*). Notably, while genus level names have changed, species level names have not changed.[5]

Ideally, probiotics should be well characterized, and known to be non-pathogenic, genetically stable, robust, and able to survive processing/storage conditions and gut transit.[1] Additionally, the health effects and required dose should be demonstrated in human studies.

Benefits of probiotics

Probiotics appear to have certain beneficial class effects on the host infant/child,[3] although the mechanisms by which probiotics confer these benefits remain largely unclear.[6]

Probiotic effects are highly strain-specific, and cannot be generalized for the most part.[1,3,6] However, potential common effects may include protection against pathogens/infection, immune system benefits, and synthesis of important nutritional elements such as some vitamins.[6] These are described in more detail below.

A FAO/WHO Expert Consensus document states that "*adequate scientific evidence exists to indicate that there is potential for the derivation of health benefits from consuming food containing probiotics*", but that further evidence is needed to confirm a number of these health benefits.[2]

Normalization of a perturbed microbiota

One of the main advantages of probiotics is in their ability to normalize the gut microbiota, conferring important benefits as described below. Probiotics multiply and colonize the intestinal tract of the host, helping ensure a proper balance between pathogens and commensal bacteria, and shifting the balance toward that found in breastfed infants.[7]

Competitive inhibition of pathogens

Probiotics such as *Bifidobacterium lactis* Bb12 and *L. rhamnosus* GG have been shown to effectively inhibit colonization by pathogenic bacteria (**Figure 12**),[4,8] including various species/strains of *Clostridium*, *Campylobacter*, *Salmonella*, *Escherichia coli*, *Shigella, Staphylococcus,* and *Yersinia.*[9,10] Lactic acid bacteria and *Bifidobacteria* have also been shown to inhibit viral pathogens.[6] It has been reported that certain probiotics (e.g. *Bifidobacterium bifidum, Lactobacillus acidophilus,* and *L. rhamnosus* GG) could play a significant role in preventing or alleviating gastrointestinal conditions such as *Clostridium difficile*-associated diarrhea, and *Helicobacter pylori* infection.[2,11-13] The anti-infective benefits of probiotics may also extend to prevention of respiratory tract infections.[14]

Probiotics directly protect against pathogen colonization and infection in various ways, including:[2-4,6]

- Competing with pathogens for nutritional sources and adhesion sites
- Stimulation of mucus secretion to prevent pathogen adhesion

- Secretion of antimicrobial substances
- Supporting the integrity of the epithelial barrier function
- Modulation and regulation of immune responses

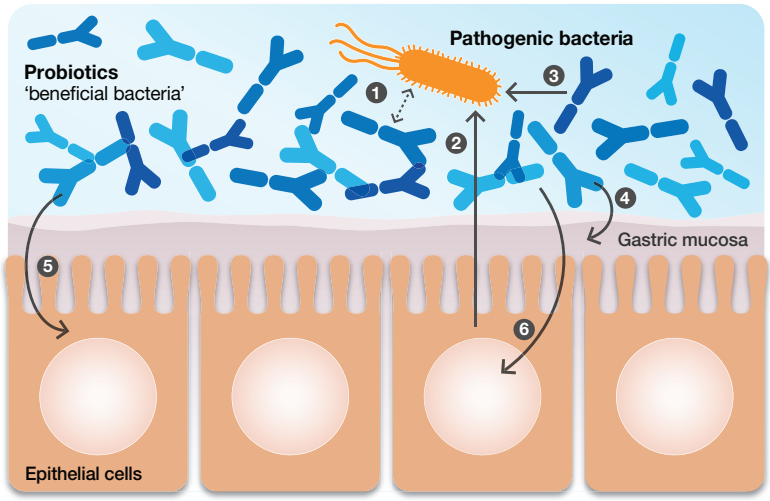

Figure 12. Competitive exclusion of pathogenic bacteria by probiotics. Adapted with permission from S. Karger AG, Basel [*Dig Dis*. Girardin & Siedman 2011;29:574–87]

Regulation of gut motility

As with prebiotics, placebo-controlled trials have shown improvement in gut motility and gastric emptying in infants fed probiotic-containing formula (*Lactobacillus reuteri* DSM 17938), mimicking the gut motility of breastfed infants.[15] This effect may help improve feeding tolerance.

Improvement of colic symptoms

A 2022 meta-analysis, which included data from nine randomized trials involving 587 infants with colic,

demonstrated that probiotics effectively treat and prevent colic, suggesting that probiotics can play an active role in the treatment and prevention of colic, especially within four weeks of probiotic treatment. Randomized trials using *L. reuteri* DSM 17938 showed consistently positive results.[16]

In accordance with the 2022 position paper on probiotics for the management of pediatric gastrointestinal disorders from the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), healthcare professionals may recommend:[17]

- *L. reuteri* DSM 17938 (at least 10^8 CFU/day for at least 21 days) for the management of infant colic in breastfed infants
- *B. lactis* BB-12 (at least 10^8 CFU/day, for 21-28 days) for the management of infant colic in breastfed infants

Due to insufficient evidence, no recommendation can be made:

- For or against the use of *L. reuteri* DSM 17938 in formula-fed infants.
- For or against the use of any of the probiotics studied so far for preventing infant colic due to insufficient evidence.

Immune-modulating effects

Some probiotics, including specific *Lactobacillus, Bifidobacterium,* and *Streptococcus* strains, appear to have immunomodulatory properties, with beneficial effects on cell-mediated immunity and inflammation.[1,6,18] Modulation

of host immunity with probiotic therapy thus represents a promising area of research,[2] particularly in infants where the most pronounced immune-modulating effects are observed.[19]

The mechanisms of these immune function effects are complex and not well understood, but appear to involve both the innate and adaptive immune systems,[11,20] particularly inhibition of the production of immunoglobulin (Ig)E.[21] The effects are largely strain- or species-specific.[14,17] Direct effects may include secretion of factors that mediate host immune responses and immune cell signaling,[1,2,11,22,23] as well as regulation of inflammatory pathways.[4,11,23] Indirect effects include enhancement of the intestinal epithelial barrier, stimulation of mucus production, and competitive inhibition of pathogenic bacteria.[23]

The immunomodulatory effects of probiotics have been demonstrated in various preclinical studies. For example, *Bifidobacterium breve* M-16V demonstrated significant suppressive effects on allergic responses and reduced skin reactivity in murine models, with corresponding reductions in serum markers of sensitization (IgE and IgG) and suppressive effects on T-helper type 2 immune responses.[24,25]

Prevention and/or reduction of infectious or antibiotic associated diarrhea

Data supporting the use of probiotics such as *L. rhamnosus* GG and *Saccharomyces boulardii* as an intervention in cases of acute viral diarrhea (particularly rotoviral) is available and reasonably well documented.[26-31]

Based on the available evidence, the ESPGHAN Working Group on Probiotics recommends the use of probiotics such as *L. rhamnosus* GG and *S. boulardii* in the management of acute gastroenteritis and for prevention of antibiotic-associated diarrhea in infants and children.[26,28,32,33] The use of *L. rhamnosus* GG may be recommended for the prevention of nosocomial diarrhea.

In accordance with the 2022 position paper on probiotics for the management of pediatric gastrointestinal disorders from the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), healthcare professionals may recommend:[17]

- High doses (≥5 billion CFU per day) of *S boulardii** or *L. rhamnosus* GG started simultaneously with antibiotic treatment to prevent antibiotic-associated diarrhea in outpatients and hospitalized children, if the use of probiotics is considered due to the presence of risk factors including class of antibiotic(s), duration of antibiotic treatment, age, need for hospitalization, comorbidities, or previous episodes of antibiotic-associated diarrhea.

*In many of the trials, the strain designation of *S. boulardii* was not available. However, if available, or assessed retrospectively, most used was that recently designated as *S. boulardii* CNCM I-745.

Prevention of allergic manifestations

The immunomodulatory effects of probiotics also appear to translate to a reduction in the risk and severity of allergic

diseases,[9,20,34] although the evidence is somewhat inconsistent and requires further confirmation.[20,34] Several small trials have demonstrated allergic symptom improvement with selected *Bifidobacterium* and *Lactobacilli* strains administered to infants/children with AD.[35-37] In addition, it has been shown that the immune-related negative effects of not breastfeeding may be mitigated by inclusion of *B. lactis* Bb12 in infant formula.[38]

Pre-clinical experimental studies and clinical data indicated that *B. breve M-16V* may have a protective effect against allergy development by impacting gut microbiota composition, gut barrier function and the overall immune function.[39-41]

Infant formulas containing *B. breve M-16V* in combination with prebiotics (i.e. synbiotics) have been shown to reduce the number of overall allergic symptoms in infants with cow's milk allergy (CMA). Indeed, a meta-analysis in 2021 concluded that infants receiving formula containing *B. breve M-16V* in combination with prebiotics had lower rates of all-cause clinical symptoms and fewer GI, skin and/or respiratory symptoms.[42] A further retrospective cohort study found that infants receiving formula containing *B. breve M-16V* in combination with prebiotics had a higher probability of achieving asymptomatic management without hypoallergic formula with a shorter clinical course of symptoms.[43] Furthermore, studies have shown that formulas with synbiotics (*B. breve M-16V* in combination with prebiotics) in infants with CMA support the gut microbiota, prompting favorable shifts in gut microbial composition that are more reflective of the gut microbiota of healthy breastfed infants. For

example, higher levels of *bifidobacteria* and lower levels of *Eubacterium rectale/Clostridium coccoides* group.[44-49]

Despite low levels of evidence, World Allergy Organization (WAO) guidelines suggest there may be a likely net benefit from administering probiotics to infants at high risk of developing allergy, primarily in terms of eczema prevention.[50] However, it requires specific studies to support the individual capacities and beneficial of the specific strains.

WAO guidelines also suggest a possible benefit from using probiotics prenatally, in pregnant women at high risk of giving birth to an allergic child.[50] However, currently, the evidence is not strong,[50] and it remains unclear which strain to use.

Note that the data supporting the benefits of probiotics for allergy prevention are frequently reported with probiotics administered as part of a synbiotic group (see **Chapter 3**).

In accordance with the 2022 position paper on preventing the development of food allergy in infants and young children (EAACI), no recommendation can be made: [51]

- For or against the use of vitamin supplements, fish oil, prebiotics, probiotics or synbiotics in pregnancy, when breastfeeding or in infancy.

In practice, healthcare professionals should apply clinical judgment and offer tailored advice based on an individual patient's personal circumstances.

Prevention of necrotizing enterocolitis in preterm infants

Evidence suggests that abnormal development of the gut microbiota, resulting in dysbiosis, may contribute to the pathogenesis of NEC in preterm and other high-risk infants.[52] NEC is a leading cause of neonatal morbidity and mortality.

While the evidence for *pre*biotics for the prevention of NEC is not compelling,[53] the data supporting the use of specific *pro*biotics is robust and dates back at least three decades.[54,55] Clinical trials and well conducted meta-analyses suggest a highly important role for probiotics – particularly some *Lactobacillus* and *Bifidobacterium* species – in the prevention of NEC;[4,56] however, further strain-specific research is needed.[56]

In accordance with the 2022 position paper on probiotics for the management of pediatric gastrointestinal disorders from the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), healthcare professionals may recommend:[17]

- *L. rhamnosus* GG (at a dose ranging from $1x10^9$ CFU to $6x10^9$ CFU) or the combination of *B. infantis* BB-02, *B lactis* BB-12, and *S. thermophilus* TH-4 at 3.0 to 3.5×10^8 CFU (of each strain) for reducing the risk of NEC in preterm infants, provided all safety issues are met.

Due to insufficient evidence, no recommendation can be made:

- For or against *L. reuteri* DSM 17938 or the combination of *B. bifidum* NCDO 1453 and *L. acidophilus* NCDO 1748.

Due to lack of efficacy, healthcare professionals may not recommend:

- *B. breve* BBG-001 or *S. boulardii*

Nutritional benefits

Non-clinical studies suggest some nutritional benefits of probiotics. Probiotic organisms, such as *L. reuteri, Lactobacillus plantarum, Bifidobacterium adolescentis* and *Bifidobacterium pseudocatenulatum*, are active producers of B group vitamins (B1, B2, B3, B6, B8, B9, and B12).[9] Some *Lactobacillus* and *Bifidobacterium* species have also been shown to enhance the absorption of vitamins and minerals from the gut, and stimulate the generation of amino acids and short chain fatty acids, and produce important digestive enzymes (e.g. lipase, esterase).[9,57,58]

Other effects

Other possible (strain-specific) effects of probiotics may include some neurological and endocrinological effects;[3] but these have not been described specifically in infants and children.

Safety of probiotics

Probiotics are generally well tolerated in infants.[1]

With Qualified Presumption of Safety (QPS)-approved probiotics, there is very little risk with regard to inducing, or being associated with the etiology of, disease.[2,59] However, a Joint FAO/WHO Expert Consultation document suggests that there is a need to establish clear guidelines based on practical criteria, to ensure safety with probiotics.[2] This document recommends that probiotic bacteria containing transmissible drug resistance genes are not used in foods.[2] There have been no pathogenic or virulence properties found for *Bifidobacteria, Lactobacilli*, or *Lactococci*.[2]

Despite the positive clinical data supporting the benefits and safety of live probiotics in preterm infants, concerns around safety and dosing in infants with an immature gut epithelial barrier or impaired immune defenses have limited their use.[1,4,60] Interest is therefore increasing with regard to the use of pre- and postbiotics in infants instead – particularly in those born prematurely.[4,60]

Quality control

Quality control is important to ensure the safety of probiotic-containing products. The ESPGHAN Working Group on Probiotics recently evaluated the available data and suggested a more stringent quality control process to ensure that the probiotic content shown on the label meets the actual content throughout the shelf life of the product, with no contamination present.[61]

Summary

Preclinical effects

- Modulation of intestinal barrier function (e.g. E. coli Nissle 1917, L. rhamnosus GG, L. casei DN-114001)[23]

Synthesis of vitamins[9] and other nutritional elements[9] (e.g. L. *reuteri, L. plantarum, B. adolescentis and B. pseudocatenulatum*)

Clinical effects

- Normalization of perturbed microbiota[9] (e.g. *B. breve* M-16V)[44-49]
- Protection against pathogenic bacteria[3,6] (e.g. *B. lactis Bb12, L. rhamnosus* GG)
- Stimulation of the immune system[1,6] (e.g. *B. breve* M-16V)[24,25]
- Reduction in allergy risk[9,19,20,42,62] (e.g. *B. breve* M-16V)
- Management of acute gastroenteritis[63] (e.g. *S. boulardii, L. rhamnosus* GG)
- Prevention of nosocomial diarrhea and antibiotic-associated diarrhoea[14,26,29,63] (e.g. *S boulardii, L rhamnosus* GG)
- Prevention of NEC[4,52,54,55,63-65] (e.g. *L. rhamnosus* GG, *B. infantis* BB-02, *B lactis* BB-12, *S. thermophilus* TH-4)
- Management of H pylori infection[13] (e.g. *Lactobacilli, S. boulardii*)
- Management of functional abdominal pain disorders[66] (e.g. L. *rhamnosus* GG, *L. reuteri* DSM 17938)
- Management of colic[63] (e.g. L. reuteri DSM 17938, B. lactis BB-12)

NEC, necrotizing enterocolitis

Table 2. Summary of potential benefits of probiotics

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Chapter 6

Synbiotics

The term synbiotic derives from the Greek prefix "syn", which means "together" and the suffix "biotic", which means "pertaining to life".

Definition

The definition of synbiotics was updated in 2019 by the International Association for Probiotics and Prebiotics (ISAPP).[1]

> *Synbiotics are a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host*
>
> ISAPP consensus statement[1]

Complementary and synergistic synbiotics

Synbiotic are further classified in complementary and synergistic synbiotics (**Figure 13**).

With a 'synergistic synbiotic' the substrate (such as a proven prebiotic) is designed to be selectively utilized by the co-administered microorganism(s). On the other hand, a 'complementary synbiotic' is a synbiotic composed of a probiotic combined with a prebiotic, which is designed to target autochthonous microorganisms. In order to classify a complementary synbiotic, minimum criteria for the existing probiotic and prebiotic must be met for both components.[1]

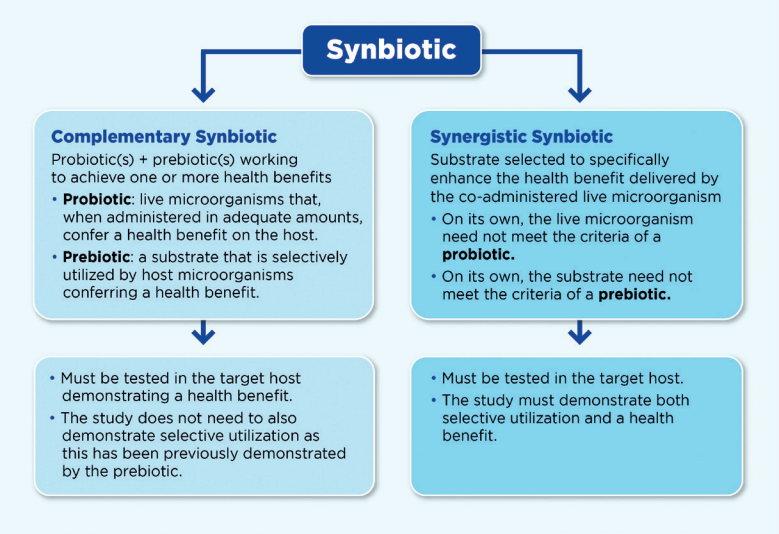


Figure 13. Synbiotic categories.[1]

Benefits and uses of synbiotics

The benefits of pre- and probiotics have been previously described. Administration in combination, as a synbiotic mixture, may enhance some of these benefits.

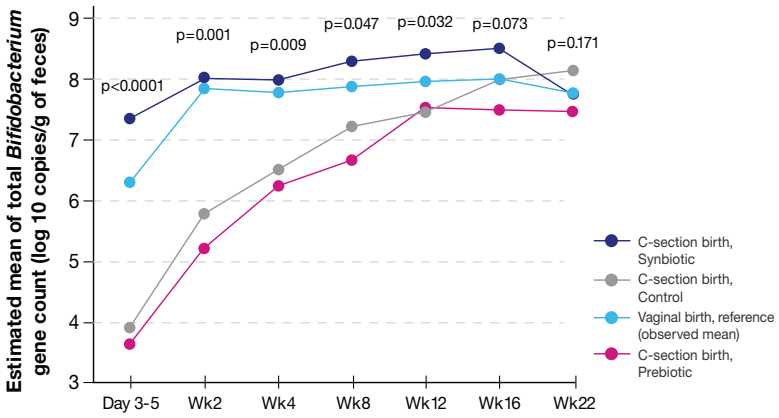
According to the 2022 societal paper on synbiotics in the management of pediatric gastrointestinal disorders from the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN),[2] no recommendation can be made *for* or *against* the use of evaluated synbiotic preparations in the treatment of acute gastroenteritis, prevention of necrotizing enterocolitis, management of Helicobacter pylori infection, inflammatory bowel disease, functional gastrointestinal disorders, and allergy in infants and children.

Effect on the gut microbiota

Clinical data indicate that synbiotic-supplemented infant formula (*Bifidobacteria/Lactobacilli* + oligosaccharides) significantly increases early-life colonizers including *Bifidobacteria* as the most dominant species compared with non-supplemented formula, supporting the development of a robust host–microbiota mutualism.[3]

Another clinical study with 290 healthy infants, investigated the bifidogenic effect of a synbiotic (scGOS/lcFOS 9:1 + *B. breve* M-16V) enriched infant formula. In this study, the 6-weeks long intervention significantly increased bifidobacterial levels bringing them closer to those that were breastfed.[4]

In a multinational, double-blind trial, 183 healthy, full-term, cesarean-delivered infants were randomized to prebiotic (scGOS/lcFOS 9:1), synbiotic (scGOS/lcFOS 9:1 + *B. breve* M-16V), or control formula groups; a vaginally-delivered group was used as a reference cohort. Synbiotic supplementation (but not prebiotic supplementation alone) showed a bifidogenic effect on the gut microbiota, restoring the *Bifidobacterium* colonization delay and dysbiosis characteristic of caesarean-delivered infants[5] (**Figure 14**). Another trial using this synbiotic mixture confirmed these results, also showing restored levels of *Bacteroides,* a bacteria species largely depleted in caesarean-born babies and associated with immune benefits.[6,7]



Adapted from: Chua et al. JPGN 2017;65:102-106

Figure 14. Early synbiotic intervention increases the *Bifidobacterium* count in cesarean-born infants, mimicking that of vaginally delivered infants[5]

As described in an earlier chapter, infants at risk of developing allergy (due to exposure to environmental factors such as cesarean section delivery, or antibiotic use), and those with established CMA, can present gut microbiota dysbiosis in early life. Given that a bifidogenic environment in the gut microbiota is important for immune development in infants, restoring a challenged gut microbiota through the use of synbiotics, may help maintain appropriate immune function.

Immune function and allergy management

Due to the known immunomodulatory effects of pre- and probiotics, synbiotic mixtures such as scGOS/lcFOS plus *B. breve* M-16V are an attractive therapeutic proposition for further enhancing immune function.[8] It has been suggested that the synbiotic concept may be involved in suppression of IgE-mediated immune responses.[9]

In murine models, synbiotic mixtures have demonstrated enhanced immune regulatory responses and reduced Th2 effector responses,[10-12] which may have important implications for optimal maturation of the immune system in humans/infants.

Knowing that a bifidogenic environment in the gut microbiome is important in the immune development of an infant, restoring this dysbiosis is a key element for infants born via C-section, exposed to antibiotics, or for infants with an established allergy. Synbiotics have been shown to restore the delayed colonization of *Bifidobacteria* in cesarean section delivered infants, bringing the levels closer to that of vaginally born and breastfed infants.[5] In one study also an indication for reduced (allergic) skin symptoms was observed in C-section infants consuming synbiotics.[5] However, the evidence is not consistent reinforcing that specific combination of prebiotics and probiotics have specific individual capacities.[13]

In another example in infants with atopic dermatitis, a 12-week intervention with scGOS/lcFOS and *B. breve* M-16V restored the gut microbiota closer to the healthy breastfed profile,[14] and resulted in a lower prevalence of asthma-like symptoms and asthma medication use after one year of follow-up, suggesting long-term effects of nutritional interventions in early life.[15] In addition, this study has shown a significantly lower incidence of diaper dermatitis in infants receiving synbiotics, compared with those receiving standard formula.[14]

The use of synbiotics for the treatment of allergic disease has also received attention. A meta-analysis in 369 infants and children showed evidence supporting the use of synbiotics – particularly containing mixed strains of bacteria – for the treatment of AD. The results were most pronounced in children over 12 months of age.[13]

Summary

Clinical effects
Improved stool characteristics[16] (e.g. *B. longum* BL999 and *L. rhamnosus* LPR + GOS/scFOS)
Preclinical effects
Enhanced bifidogenic effects[3,5] (e.g. *B. breve* M-16V + scGOS/lcFOS)
Immunomodulatory effects[5,8,15] (e.g. *B. breve* M-16V + scGOS/lcFOS)
Enhanced SCFA* production[12] (e.g. *B. breve* M-16V + scGOS/lcFOS)
Improved viability of probiotics[8,17]

*SCFA, short chain fatty acid

Table 3. Summary of potential effects of synbiotics

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Chapter 7

Postbiotics

Unlike the probiotic concept, in which live bacteria must be ingested and produce beneficial health effects in the host, the postbiotic concept comprises the nonviable microorganisms that may also include the associated metabolites arising in the food product as a result of fermentation facilitated by bacterial metabolic activity. The bacteria can act as a "microbial factory" to enrich the food matrix, thus conferring health benefits that do not require bacterial viability. It is well established that some products of bacterial fermentation and/or remaining non-viable bacterial materials possess bioactive properties. A preparation containing all these components is denoted as a postbiotic.[1]

Since postbiotics do not require bacterial viability or colonization in the host,[1,2] they may have several advantages as nutritional components. Postbiotics do not contain potentially harmful bacterial components; in addition, they show relative stability during storage, and are unaffected by emerging antibiotic resistance.[2,3]

In contrast to the abundant data on pre- and probiotics, the field of postbiotics in food and infant formula is rapidly emerging and ongoing research is being performed in this area.

Definition

Although there is no universally accepted definition of postbiotics yet,[4] some definitions have been proposed in the literature. Aguilar-Toalá and colleagues proposed that "*Postbiotics refers to soluble factors (products or metabolic byproducts), secreted by live bacteria, or released after bacterial lysis, such as enzymes, peptides,*

teichoic acids, peptidoglycan-derived muropeptides, polysaccharides, cell surface proteins, and organic acids".[2] This proposal has been further fine-tuned and made more specific in the context of food for human consumption.

The definition of postbiotics was published in 2019 by the International Association for Probiotics and Prebiotics (ISAPP).[5]

> *Postbiotics are a 'preparation of inanimate microorganisms and/or their components that confers a health benefit on the host'*
>
> ISAPP consensus statement[5]

Fermentation by naturally occurring microorganisms can be regarded as a bioactive enrichment of food. Indeed, bacteria used during this process can be used to naturally enrich the food matrix with a broad range of bioactive components that may confer different health benefits.

Thus, postbiotics are usually present in the food matrix that was fermented, but could also be derived from the fermentation media (**Figure 15**). It should be noted, however, that purified components synthesized by microorganisms such as antibiotics are not considered postbiotics.

The main bioactive components produced during fermentation are organic acids, microbial cell wall components, proteins, lipids, carbohydrates, vitamins, or other complex molecules[1,4,6] (**Figure 16**). Other processes used in the production of postbiotics include thermal inactivation (e.g., autoclaving),

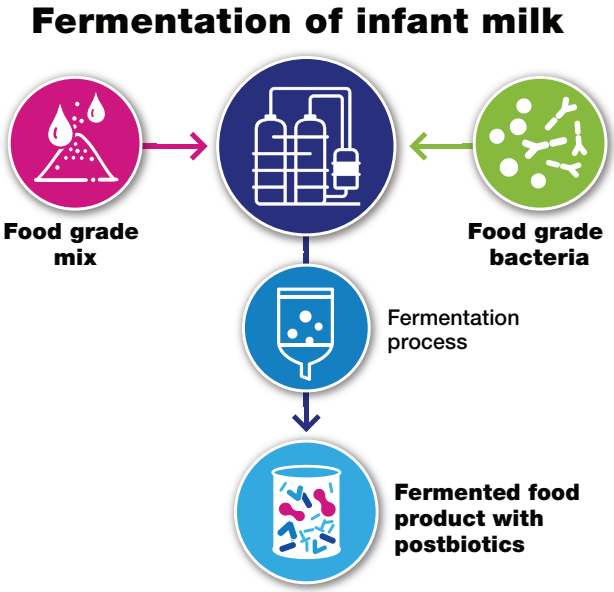

Figure 15. Fermentation of infant milk to produce postbiotics

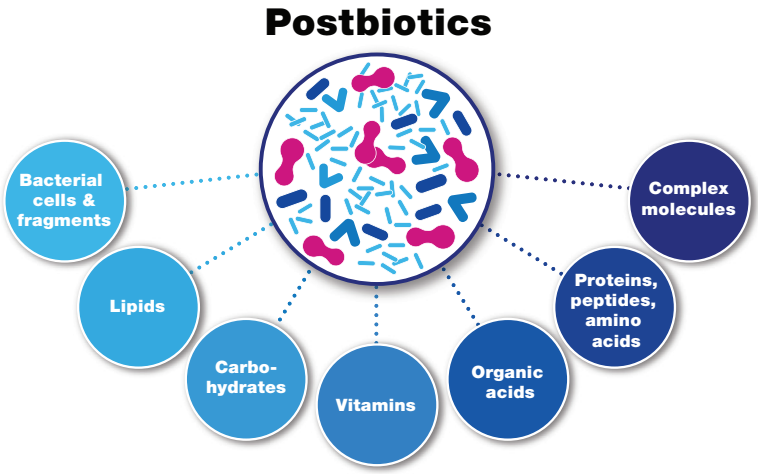

Figure 16. Examples of postbiotics[2,5,9-11]

ultraviolet inactivation, gamma radiation, chemical treatment (e.g., with formalin, enzymes) and sonication.[7] Notably, selection of the right bacterial strains is crucial, since the ability of microbial cultures to produce bioactive metabolites is commonly a strain-dependent trait. Consideration should also be given to the optimum conditions required for fermentation to produce bioactive components. Postbiotics thus arise as a result of a specific food matrix, a particular bacterial strain, a unique fermentation process, and optimal conditions; hence, not all postbiotics are considered to be the same.[8]

The range of mechanisms by which various postbiotics confer their unique benefits have not yet been fully elucidated. However, scientific data indicate that postbiotics have potential physiological functions at both the local and systemic level in the host.[4] These functional properties can positively affect the microbiota homeostasis, host metabolic and immunological responses, and host resilience to detrimental changes[1,5] (**Figure 17**).

Benefits of postbiotics

Along with pre- and probiotics, the postbiotic concept is emerging as a further source of support for host health through improvement of distinct physiological functions. Several studies have reported beneficial effects of specific bioactive metabolites produced by a number of microorganisms. Here we present potential benefits of postbiotics; although, these components would not all fall under the postbiotics definition proposed above. Not all are produced through food-grade fermentation processes and/or in their fermentation matrix.

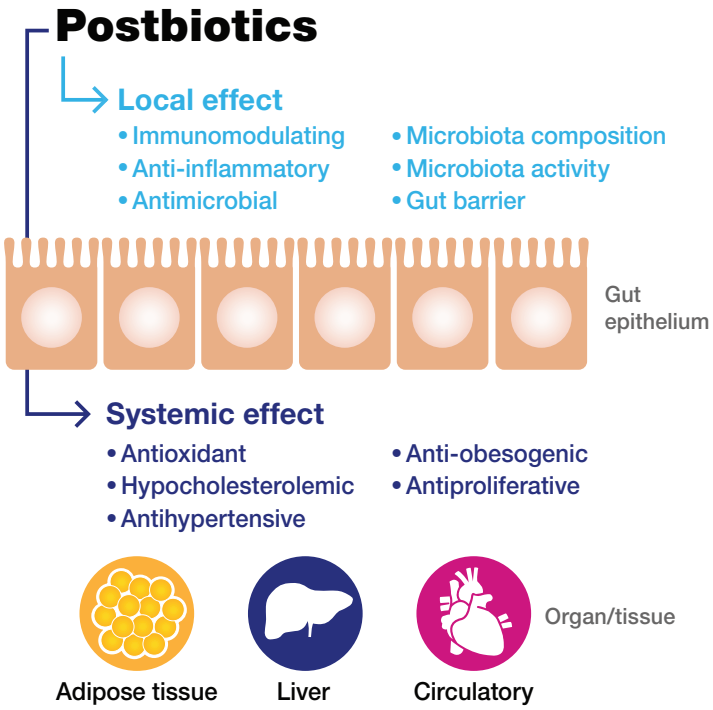

Figure 17. Local and systemic effects of postbiotics. Adapted from: Aguilar-Toalá et al. *Trends Food Sci Technol.* 2018[2]

In most reports, bioactive components produced by *Bifidobacterium* and *Lactobacillus* species have been studied.[5] Evidence suggests the potential of postbiotics to be systemically available and thereby act on different organs and functions beyond the gut. Some have been shown to support intestinal barrier function,[11] modulate inflammatory signaling pathways,[10,12] and confer antimicrobial and immunomodulatory effects in the gut (**Figure 17** and **Table 4**). These actions may positively impact gut microbiota homeostasis and host metabolic and

signaling pathways, thus representing a highly promising opportunity in the field of functional foods.[1,5]

Local effects on the gut epithelium (pre-clinical)
Immunomodulating[13]
Anti-inflammatory[14]
Antimicrobial[15]
Enhancement of intestinal barrier function[16]

Systemic effects on organs/tissues (pre-clinical)
Antioxidative
Hypocholesterolemic
Antihypertensive
Anti-obesogenic
Antiproliferative
Anxiolytic
Antidepressant

Table 4. Summary of key potential effects of postbiotics

Examples of pediatric trials with postbiotics
evaluating clinical outcomes

- **Fermented infant formula**
- **Management of acute gastroenteritis**
- **Prevention of common infectious diseases**
- **Atopic eczema & CMA**
- **Allergic rhinitis**
- **Lactose malabsorption**

Limited data I but all RCTs

COUNTRY/ REGION	PARTICIPANT CHARACTERISTICS (N)	INTERVENTION AND CONTROL GROUP	DURATION OF INTERVENTION	MAIN CONCLUSION	REF.
Fermented formula (healthy infants)					
Italy	Age 0–4 months (n = 90)	Fermented formula with BB C50 and ST 065 vs breastfeeding or standard infant formula	0–4 months	A 2015 systematic review showed that fermented formula, compared with the use of standard infant formula, does not offer clear additional benefits, although some benefit on gastrointestinal symptoms cannot be excluded; no negative health effects have been documented [59]	172
France	Age 0–12 months (n = 129)	Fermented formula with BB C50 and ST 065 vs standard infant formula	0–12 months		184
France	Age 0–4 months (n = 30)	Fermented formula with BB C50 and ST 065 vs standard infant formula	0–4 months		173
France	Age 4–6 months (n = 968)	Fermented formula with BB C50 and ST 065 vs standard infant formula	5 months		166
France	Age 0–3 months (n = 109)	Fermented formula with BB C50 and ST 065 vs standard infant formula	15 days		185

COUNTRY/ REGION	PARTICIPANT CHARACTERISTICS (N)	INTERVENTION AND CONTROL GROUP	DURATION OF INTERVENTION	MAIN CONCLUSION	REF.
Fermented formula in preterm infants					
Italy	Preterm infants 30–35 weeks of gestational age, Age 0–3 days (n = 58)	Preterm infant formula, heat-inactivated fermented formula with BB C50 and ST 065 vs preterm infant formula	During hospital stay; 2–5 weeks	Reduced incidence of abdominal distension in infants fed preterm fermented formula	161
Management of acute gastroenteritis					
France	Age 1–48 months (n = 71), acute diarrhoea	Heat-killed *L. acidophilus* LB vs placebo	4 days	A 2014 meta-analysisa showed that *L. acidophilus* LB reduced duration of diarrhoea in hospitalized, but not outpatient, children compared with a placebo; the chance of a cure on day 3 was similar in both groups, but *L. acidophilus* LB increased the chance of cure on day 4 (ref.162)	186
Ecuador	Age 10 months (n = 80), acute diarrhoea	Heat-killed *L. acidophilus* LB vs placebo	4 days		187
Peru	Age 3 months to 4 years (n = 80, acute diarrhoea (less than 3 days)	Heat-killed *Lactobacillus* LB vs placebo	4.5 days		188
Thailand	Age 3–24 months (n = 73), acute diarrhoea without severe dehydration	Lyophilized heat-killed *L. acidophilus* LB vs placebo	2 days		189
Finland	Age <4 years (n = 41), acute rotavirus diarrhoea	Heat-inactivated *L. casei* vs viable *L. casei* 1010 CFU	5 days	Equal clinical recovery from rotavirus diarrhoea	163
Prevention of common infectious diseases					
Italy	Age 12–48 months (n = 377), healthy children attending day-care or preschool at least 5 days a week	Cow's milk + postbiotics or rice with fermented milk with heat-inactivated *L. paracasei* CBA L74 vs placebo	3 months	Reduced risk of some common infectious diseases such as gastroenteritis and respiratory tract infections (*incl. pharyn gitis*, laryngitis, tracheitis) observed during the study period	165
Italy	Age 12–48 months n = 146), healthy children, attending day-care or preschool for at least 5 days a week	Lyophilized heat-killed *L. paracasei* CBA L74 vs placebo	3 months	Reduction in some common infectious diseases, such as otitis media and pharyngitis	168
Pakistan	Age 6–12 months (n = 75), healthy infants with high risk of diarrhoea-related mortality	Micronutrient sachets with heat-inactivated *L. acidophilus* vs micronutrient sachets or place- bo sachets	2 months	No statistically significant difference in diarrhoea prevalence between the micronutrient with *L. acidophilus* and placebo groups	167

Continued

COUNTRY/ REGION	PARTICIPANT CHARACTERISTICS (N)	INTERVENTION AND CONTROL GROUP	DURATION OF INTERVENTION	MAIN CONCLUSION	REF.
Atopic eczema and cow's milk allergy					
Finland	Mean age 5.5 months (n = 35), infants with atopic eczema and cow's milk allergy	EHWF + live or heat-inactivated *L. rhamnosus* GG vs placebo	Mean 7.5 weeks	Supplementation of EHWF with viable but not heat-inactivated *L. rhamnosus* GG is a potential approach for the management of atopic eczema and cow's milk allergy	169
Allergic rhinitis					
Taiwan	Age >5 years (n = 90), perennial allergic rhinitis for more than 1 year	Live or heat-killed *L. paracasei* 33 or placebo	30 days	In both intervention groups, the overall quality of life improved; heat-killed *L. paracasei* 33 was not inferior to live *L. paracasei* 33; no obvious adverse effects	190
Lactose malabsorption					
Indonesia	Age 10–12 years (n = 86), lactose malabsorption	Killed and live *Lactobacillus helveticus* R-52 and *L. rhamnosus* R-11	2 weeks	Decrease in breath hydrogen test in both groups	191

BB C50, *Bifidobacterium breve* C50; EHWF, extensively hydrolysed whey formula. *L. acidophilus, Lactobacillus acidophilus; L. casei, Lactobacillus casei; L. paracasei, Lactobacillus paracasei*; *L. rhamnosus* GG, *Lacticaseibacillus rhamnosus*; ST 065, *Streptococcus thermophilus* 065 [a]Based on material presented in referenced systematic reviews.

Continued

Bioactive metabolites from *L. plantarum*, when combined with the prebiotic inulin, have been shown to inhibit proliferation of pathogenic bacteria. These antimicrobial properties may be attributable to the presence of specific components with antimicrobial activity.[15] In addition, effector molecules from *Lactobacillus* species were shown to be able to protect against the inflammatory properties of invasive *Salmonella* on healthy tissue and downregulate ongoing inflammatory processes in inflammatory bowel disease (IBD) tissue.[17] Metabolites from *Lactobacillus casei* DG were shown to mitigate the inflammatory response in an *ex vivo* organ culture model of post-infectious IBS patients.[18]

Specific postbiotics have been shown to help stimulate the growth and activity of specific components of the gut microbiota.[19-21] Some of these have been shown to directly inhibit pathogens such as *Listeria*, *Salmonella*, *Escherichia coli*, and *Enterococcus* strains.[2,5,22] For example, postbiotics from *B. breve* C50 showed a reduction in pathogens including *Clostridium perfringens* and clostridial spores, a reduction in fecal pH, and an increase in the number of bifidobacterial species after seven days of consumption.[23]

Postbiotics in infant formula

In infant formulas, the concept of postbiotics is not widely used; although, specific fermented infant formulas with postbiotics have been commercially available in Europe for decades. The postbiotics in fermented formulas are generally derived from fermentation of a milk matrix by food-grade bacteria such as *Bifidobacterium, Streptococcus,* and/or *Lactobacillus* strains.[2,22,24] Inactivation of the bacteria during post-fermentation production processes such as homogenization, pasteurization, sterilization, and/or spray-drying, ensures that few or no viable bacteria remain in the final product.[25]

Fermented formulas have the potential to improve some digestive symptoms, including lower gastrointestinal symptoms.[25,26] For example, a 2015 systematic review of the available literature concluded that "*infants that could potentially benefit from fermented formulas are those with digestive discomfort (colics, bloating) and infants with diarrhea*".[25] A more recent 2022 systematic review of the available literature

concluded that *"infant formula with postbiotics are safe and well tolerated by infants who cannot be breastfed"*.[27] In addition, there is some rationale for harnessing the immunomodulatory activity of postbiotics to provide other benefits, such as improving atopic dermatitis symptoms.[2] Also, postbiotics have recently been suggested as a potential preventive strategy against Necrotising Enterocolitis (NEC) in preterm infants.[22]

Potential benefits of specific postbiotics in infant formula

A recent systematic review concluded that infant feeding with postbiotics are safe and well tolerated.[27] Another review indicated that postbiotics have direct immunomodulatory effects, such as influencing cytokine expression and release.[13] Although no firm conclusions can be made on clinical efficacy of one formula over another, emerging data suggests promising clinical benefit (e.g., in infant colic).

Postbiotics derived from *Lactobacillus paracasei* CBA L74

Lactobacillus paracasei CBA L74 is used to prepare commercial fermented infant, follow on, and young child formula, by fermenting cow's skim milk. In the final product, non-viable bacteria and fermentation products are present, corresponding to 5.9 x 10^{11} CFU per 100g.[28]

Pre-clinical data

In pre-clinical research, postbiotics from *L. paracasei* CBA L74 have been reported to have anti-inflammatory effects on dendritic cells in response to the pathogen *Salmonella typhimurium*. The

postbiotics inhibited pro-inflammatory cytokines, while not affecting IL-10. It was shown that this effect was not induced by the non-viable *Lactobacillus* cells and fragments, but rather by the metabolites produced. In the same study, the fermented milk displayed a protective effect against colitis and against an enteric pathogen infection (*S. typhimurium*) in a mouse model.[29]

Clinical data

In a clinical study in 377 healthy children aged 12-18 months who were attending daycare, dietary supplementation with the *L. paracasei* CBA L74 fermented young child formula prevented common infectious diseases, including upper respiratory tract infections and acute gastroenteritis. This preventive effect was accompanied by a reduction in medication use (e.g. antibiotics, antipyretics, or steroids). In addition, an increase in fecal biomarkers of innate and acquired immunity were observed, and a negative association between these biomarkers and the occurrence of common infectious diseases was observed.[28]

Postbiotics derived from *B. breve* C050 and/or *S. thermophilus* 065

B. breve C50 and *S. thermophilus* 065 are both used in the preparation of commercial infant and follow on formula, in which a milk matrix is fermented.

Pre-clinical data

In a preclinical model, postbiotics derived from *B. breve* C50 induced prolonged dendritic cell survival and maturation, and induced high IL-10 production through TLR-2, suggesting

immune regulatory functions.[30] Moreover, postbiotics from this strain when combined with postbiotics from *S. thermophilus* C65 have been reported to reinforce the intestinal barrier capacity and stimulate Th1 response in a mice model.[31]

Clinical data

The effects of an infant formula with postbiotics derived from *S. thermophilus* 065 and *B. breve* C50 was tested in newborn infants. Eleven infants received the test formula, while nine controls received a standard infant formula without postbiotics. The microbiota of the active group showed a higher number of bifidobacteria and a decrease in the number of adult-like species, and the antipoliovirus IgA titers increased significantly after a challenge ($p<0.02$) in the active group versus the control group without postbiotics.[21]

In a third clinical study involving 90 healthy term newborn infants, the fecal pH of those who used an infant formula with postbiotics was significantly lower compared to infants fed a standard formula ($p<0.05$), being similar to the faecal pH of human milk fed infants.[32] In the same study, it was shown that the infant formula with postbiotics from *B. breve* C50 and *S. thermophilus* 065 induced a significantly higher thymus size, which was closer to the thymus size of human milk fed infants.[32]

Another randomized, controlled trial investigated the incidence of acute diarrhea and its severity in healthy infants fed a formula containing postbiotics from *B. breve* C50 and *S. thermophilus* 065, compared to infants using a standard infant

formula without postbiotics. Diarrhea incidence, duration of diarrheal episodes, and number of hospital admissions did not differ significantly between the groups. However, episodes of diarrhea were less severe in infants using an infant formula with postbiotics, indicated by fewer cases of dehydration, medical consultations, oral rehydration solution prescriptions, and changes to other formulas.[33]

Finally, a randomized trial has also demonstrated that consumption of an infant formula with postbiotics from *B. breve* C50 and *S. thermophilus* 065 decreased the incidence of potentially allergic adverse events, suggesting an improvement of oral tolerance to cow's milk in infants with high risk of atopy.[34]

Postbiotics derived from *B. breve* C050 and *S. thermophilus* 065 combined with prebiotics scGOS/lcFOS

Clinical data

A randomized, controlled, double blind trial investigated the safety and efficacy of an infant formula with prebiotics scGOS/lcFOS 9:1 at a level of 0.8 g/100 mL and postbiotics derived from *S. thermophilus* 065 and *B. breve* C50, using the Lactofidus™ fermentation process. The study included 432 healthy infants divided into four different groups, receiving formula with prebiotics and postbiotics (with two different levels of postbiotics), formula with prebiotics only, and formula with postbiotics only. The study showed that the combination of postbiotics and prebiotics was safe and well tolerated, and supported normal growth.[35] Also, the study demonstrated that the formula with prebiotics and postbiotics led to less crying

and lower reported incidence of infantile colic.[36] Since infantile colic is correlated with low-grade systemic inflammation,[37] these findings may indicate an effect of the new nutritional concept on inflammatory immune regulation.

A second randomized, controlled, double-blind clinical study investigated infant formula with postbiotics from *B. breve* C50 and *S. thermophilus* 065 and prebiotics scGOS/lcFOS (0.8 g/100 mL) in a 9:1 ratio, using the Lactofidus™ fermentation process (n=200). The control formula did not contain prebiotics and postbiotics. Breastfed infants were included as reference group. The combination of specific prebiotics and postbiotics in infant formula was shown to be safe and well tolerated.[38] Compared to the control group, the composition and metabolic activity of the fecal microbiota among infants fed prebiotics and postbiotics was more aligned with that of breastfed infants. A lower pH, higher levels of acetic acid and sIgA, increased number of *bifidobacteria*, and decreased *C. difficile* occurrence were observed in the gut microbiota of infants using prebiotics and postbiotics.[39,40] Moreover, infants using prebiotics and postbiotics had significantly softer stools versus the control group.[41]

A third randomized, controlled, double-blind clinical study investigated the effect of a fermented infant formula with postbiotics (e.g. 3′-GL) derived from *B. breve* C50 and *S. thermophilus* 065 and prebiotics (scGOS/lcFOS 9:1) in infants (n=280). The control formula did not contain prebiotics and postbiotics. Compared to the control group, the median secretory IgA (sIgA) concentration in the experimental formula was

significantly higher and was more similar to the concentrations found in the breastfed-reference group. The experimental formula resulted in a microbiota composition and metabolic activity closer to the breastfed infants' microbiome.[42]

A fourth randomized, controlled, double-blind clinical study investigated the effect of a partly fermented infant formula with prebiotics (scGOS/lcFOS 9:1), postbiotics (e.g. 3′-GL) derived from *B. breve* C50 and *S. thermophilus* O65, 2′-FL, and milk fat in formula fed infants (n=215). The control formula contained no postbiotics. Equivalence in daily weight, length, and head circumference gain up to 17 weeks of age between the postbiotic group and control group was observed. A partly fermented infant formula with postbiotics was safe and well tolerated in healthy term infants.[43]

Postbiotics with additional components

Clinical data

A randomized, controlled, double-blind clinical study investigated the effect of a partly fermented formula with postbiotics, prebiotics (scGOS/lcFOS 9:1) and locust bean gum (n=182). The control formula contained postbiotics and locust bean gum only. The experimental formula was well tolerated, safe and supported adequate growth. In addition, there was greater improvement of GI symptom burden in infants with more severe symptoms.[44]

One challenge with infant formulas containing postbiotics (as part of the active arm or control arm) is the lack of standardization and the varying levels of postbiotics present.

An open, prospective, observational study investigated the effect of a locust bean gum-thickened formula containing postbiotics derived from *B. breve* C50 and *S. thermophilus* O65 in infants (n=2604). The formula decreased infant regurgitation, was well tolerated, and improved parental quality of life. Stool composition and frequency of the infants remained within the normal range.[45]

Gut microbiome data

A study involving collection of fecal samples from a randomized, controlled, double-blind clinical study that investigated the effect of infant formula with prebiotics (scGOS/lcFOS 9:1) and postbiotics (e.g. 3′-GL) derived from *B. breve* C50 and *S. thermophilus* O65 compared with a standard infant formula (n=200). Results showed that the experimental formula may trigger responses in the intestinal microbiota composition that brings the resulting fecal metabolite profile of formula-fed infants closer toward those observed in breast-fed infants.[46]

Safety of postbiotics in infant formula

No negative health effects of infant formulas with specific postbiotics have been documented,[25] and infant formulas with postbiotics are reported to support a normal growth trajectory. This was confirmed in a recently published systematic review by Szajewska et al,[25] in which data were analyzed from five randomized trials involving 1326 infants who received either formula fermented with *B. breve* C50 and *S. thermophilus,* or unfermented infant formula. Compared with infants receiving standard formula, those receiving formula with postbiotics showed similar weight and length gains.[25]

Postbiotics have been suggested as a potential preventive strategy against NEC in preterm infants, to avoid the risk of administering live microorganisms that could translocate and cause infection. Well-designed trials investigating the efficacy and safety of postbiotics for the prevention or treatment of NEC should confirm this.[22]

As postbiotic signatures are dependent on bacterial strains and processes, the safety and suitability of specific postbiotics in infant formula remains to be confirmed.

Summary

Preclinical effects

- Anti-inflammatory properties[29] (e.g. derived from *L. paracasei* CBA L74)
- Protection against colitis and enteric pathogen infection[29] (e.g. *S. typhimurium*) (e.g. derived from *L. paracasei* CBA L74)
- Prolonged dendritic cell survival and maturation[29] (e.g. derived from *L. paracasei* CBA L74)
- High IL-10 production through TLR-2[30] (e.g. derived from *B. breve* C50)
- Reinforcement of intestinal barrier capacity[31] (e.g. derived from *S. thermophilus* C65)
- Stimulation of Th1 response[31] (e.g. derived from *S. thermophilus* C65)

Non-clinical and Clinical effects

- Prevention of common infectious diseases, including upper respiratory tract infections and acute gastroenteritis[28] (e.g. derived from *L. paracasei* CBA L74)
- Increased fecal biomarkers of innate and acquired immunity[28] (e.g. derived from L. *paracasei* CBA L74)
- Increased poliovirus-specific intestinal antibody response[21] (e.g. derived from *S. thermophilus* 065 and *B. breve* C50)
- Less severe diarrhea[33] (e.g. derived from *S. thermophilus* 065 and *B. breve* C50)
- Similar thymus indexes as human milk-fed infants[32] (e.g. derived from *S. thermophilus* 065 and *B. breve* C50)
- Modulation of the gut microbiota with higher proportion of bifidobacteria and with fewer adult-like species[21] (e.g. derived from *S. thermophilus* 065 and *B. breve* C50)
- Up-regulation of fecal secretory IgA in preterm infants[47] (e.g. derived from *S. thermophilus* 065 and *B. breve* C50)
- Lower incidence of infantile colic[36] (e.g. derived from *S. thermophilus* 065 and *B. breve* C50)
- Modulation of the fecal microbiota and activity towards human-milk fed infants[9,40,46] (e.g. derived from *S. thermophilus* 065 and *B. breve* C50)
- Supports adequate growth[43] (e.g. derived from *S. thermophilus* 065 and *B. breve* C50)

Table 5. Potential benefits of some specific postbiotics in infant formula

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Chapter 8

The future of biotics in infant health

Research is continuing into defining the concept of the healthy microbiota. The National Institutes of Health-supported 'Human Microbiome Project' (https://hmpdacc.org/) was established in 2008, with the purpose of characterizing microbial communities from hundreds of healthy individuals.[1] This project is expected to revolutionize future research into biotics and their applications, including infant health and development.[2]

Future biotics research

As discussed above, the efficacy of different biotics in preventing and treating some disorders such as allergies and gastrointestinal problems and infections is becoming well established. As more probiotic organisms – and the specific prebiotics that fuel them – are discovered, it is likely that strain-specific applications will continue to strengthen and expand.[3] Importantly, future research into prebiotics is expected to yield more components with structures identical to functional HMOs in human milk, improving the functionality of prebiotic-supplemented formulas.

Postbiotics research is still in its infancy, but is a highly promising area of discovery in both preventative and treatment scenarios (e.g. H.pylori eradication, management of IBS, chronic diarrhoea).[4,5] The 2021 ISAPP definition aimed to offer clarity on an emerging term that is likely to evolve as the research advances. There is a need for well-designed trials evaluating specific fermentation processes and postbiotics, and their utility in infant formulas, including in

high-risk preterm infants.[4] As new fermentation processes and formulations become available, including formulations also containing added prebiotics, more studies evaluating the benefits of these modifications are planned.[6]

Other areas of research

On a practical note, different methods of biotics administration through functional foods and supplements are being investigated.[3] There has been some concern around the shelf life of live probiotic bacteria in foods, and poor survival during transit through the gastrointestinal system. Recent research efforts are continuing to focus on improving bacterial survival through technologies such as microencapsulation.[7]

Other research is focusing on the potential role for prebiotics and probiotics in the approach to overcoming global antibiotic resistance, with applications both in humans and in the food production industry.[2,8] In addition, while the evidence is not yet strong, there is also increasing rationale for the use of probiotics alongside antibiotics as standard practice, to help maintain a healthy gut microbiota composition.[9] At the same time, there is increasing interest in postbiotics for gut microbiota modulation and health promotion. Personalised nutrition and precision medicine influence the future application of probiotics and prebiotics, with enhanced knowledge on modulation of microbial signatures of health and disease.

Concluding remarks

While the study of biotics in helping modulate the gut microbiota is warranted, it is important that such interventions are considered alongside other strategies that help address the cause of the dysbiosis in the first place, such as birth/delivery method, type of feeding, and environmental factors.[10]

Human milk will always remain the gold standard in infant nutrition. However, for infants who cannot be exclusively breastfed, pre-, pro-, and postbiotics, and combinations thereof, are promising bioactive components to mimic human milk functionality and support immunity through the gut in infancy. Further research is anticipated to strengthen the data around the use of these components, and it is expected that biotics will eventually become prerequisite ingredients in infant formulas.

Indeed, momentum continues to grow as the scope of preventative and therapeutic uses for biotics further expands.

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THE BIOTICS FAMILY IN EARLY LIFE is the fourth book in an educational series on the first 1000 days of life. This book examines the influence of the gut microbiota on immunity, and discusses the building evidence supporting the use of nutritional biotics in early life, to ensure the development of a balanced microbiota and normal immune function development.

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