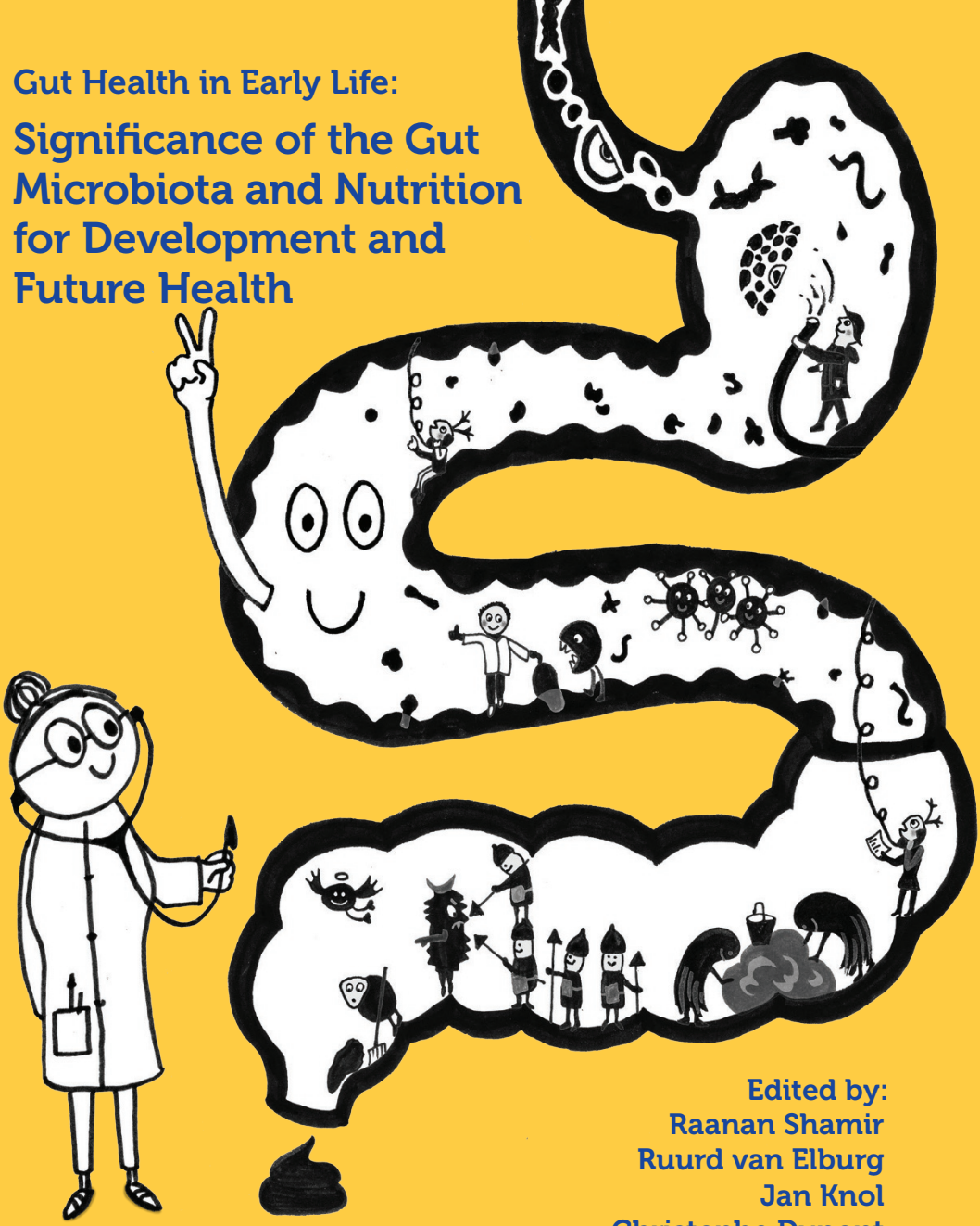
Gut Health in Early Life:
Significance of the Gut Microbiota and Nutrition for Development and Future Health



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Essential Knowledge Briefings

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Glossary

ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FOS	fructo-oligosaccharides
GOS	galacto-oligosaccharides
GI	gastrointestinal
HMOS	human milk oligosaccharides
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
IgA	immunoglobulin A
IgE	immunoglobulin E
lcFOS	long chain fructo-oligosaccharides
NEC	necrotizing enterocolitis
OS	oligosaccharides
SCFAs	short chain fatty acids
scGOS	short chain galacto-oligosaccharides
WHO	World Health Organization

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Chapter 1

The infant digestive system and its dynamic functions

Introduction

The period from conception through early life is a unique and fascinating period of growth and development that lays the foundation for future health. The first 1,000 days in particular, from the point of conception until around the child's second birthday, is often cited as a critical window of opportunity. Worldwide epidemiological, clinical, and non-clinical studies have related the influence of certain environmental factors in early life to differences in the expression of genetic and biological characteristics, which in turn influences patterns of health and disease in later life.[1]

Significant changes in nutrition during early life, from ***in utero*** sources to ingestion of milk, followed by the introduction of solid foods, are some of the most important programming mechanisms influencing the development of the body's biological systems during this period.[1] In particular, the importance of human milk during early life is well established.[1]

Healthy development of the gut is of major importance for a variety of reasons. The gut contributes to overall health by ensuring digestion and absorption of nutrients and fluids to prevent undernutrition and dehydration; it also provides a barrier against infectious agents, induces mucosal and systemic tolerance to prevent allergy, and provides signals to the brain to maintain homeostasis.[2]

This Essential Knowledge Briefing is the first in a series that examines gut health and development in early life. It is intended to serve as a practical guide for healthcare professionals who have a special interest in infant health. This first Essential Knowledge

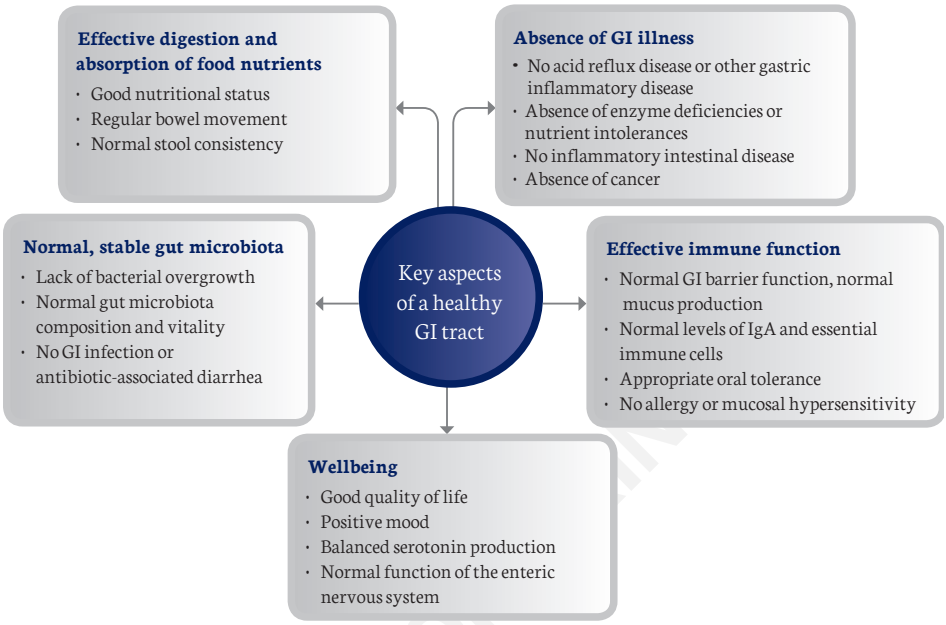
Briefing discusses the role of the developing gut microbiota in human health and disease, both in the short and long term, and contains up-to-date information on the types of microbes commonly present in the gut, the range of functions they perform, and the factors that affect colonization and shape the development of the gut microbiota during early life. It also investigates the potential for improving gut health by deliberately modifying the composition of the gut microbiota in infants. The second Essential Knowledge Briefing discusses the diagnosis and treatment of common digestive problems in pregnant women and infants.

The adult gut in perspective

- **70-80% of the body's immune cells** are concentrated in the gut, creating a gut-specific immune system[3]
- There are **100 million neurons** located along the gut which produce various neurotransmitters that regulate mood and satiety[4]
- **95% of the body's total serotonin** is located in the gut[5]
- Roughly **100 trillion bacteria** reside in the gut[6]

Optimal gut function

The term "gut health" covers multiple aspects of the gut, including the effective digestion and absorption of nutrients, optimal gut barrier function, a normal and stable gut microbiota composition, effective immune status, and a state of general wellbeing[2] (**Figure 1**). From a medical perspective, it is difficult to exactly define and measure gut health. Gut health is defined as a "state of physical and mental well-being in the absence of gastrointestinal (GI) complaints that require the consultation of a doctor, in the absence of indications or



GI, gastrointestinal; IgA, immunoglobulin A.

Figure 1. Potential indicators for a healthy gut*[2]

* These are general indicators that are not specific to infancy

risks of bowel disease, and in the absence of confirmed bowel disease".[2]

Dynamic functions of the digestive system

A normal-functioning GI system can effectively digest food and absorb nutrients, providing all the energy and nutrients the body needs, while regularly disposing of waste material. After initial digestion in the stomach, absorption takes place in the small and large intestines, enhanced by projections from the GI lining known as villi (**Figure 2**), which increase the intestine's effective surface area for absorption. The small intestine absorbs nutrients released

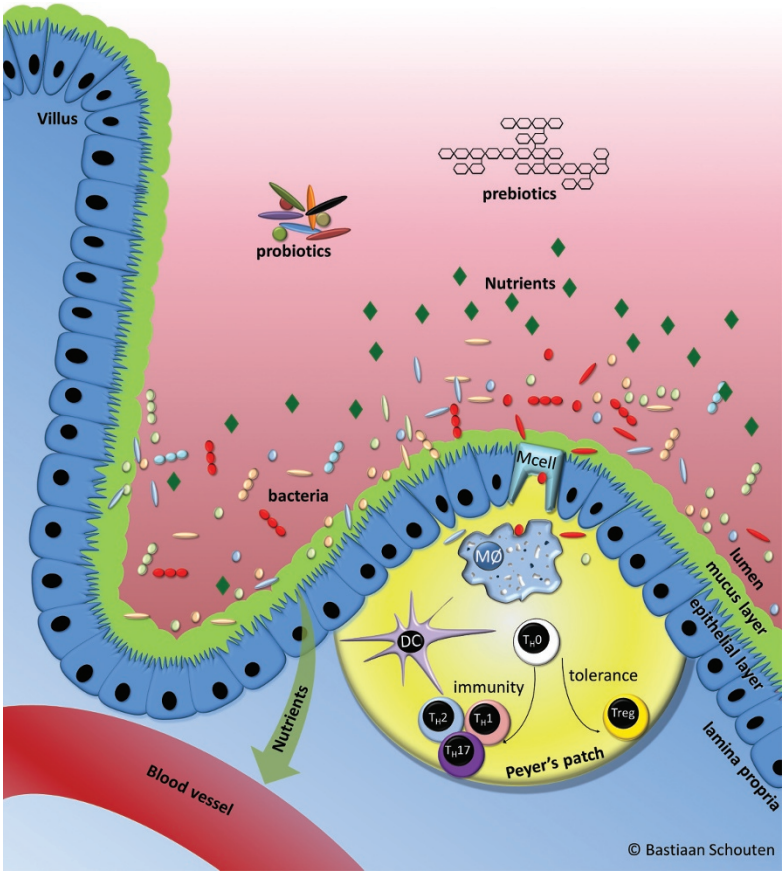


Figure 2. Schematic representation of a part of the small intestine, including a villus.

Nutrients are digested and absorbed by the GI tract into the blood stream. There are interactions with prebiotics and probiotcs in the lumen of the small intestine. During this process there is a monitoring of the immune system, including dendritic cells (DC), macrophages (M0) and multiple T helper cells (TH0, TH1, TH2, TH17 and Treg) in the Peyer's patch.

Figure courtesy of Baastian Schouten, Danone Nutricia Research, The Netherlands

from food material; food that cannot be digested by these enzymes then makes its way into the large intestine, where much of it is broken down by enzymes released by microorganisms in the gut (the gut microbiota– see **Chapter 2** and **Chapter 3**).

GI motility is an important aspect of gut function, and is controlled by the presence of food, by autonomic nerve function, and by input of gut hormones. Feeding initiates stomach wall contractions, followed by gastric emptying, peristalsis, and other patterns of motility.[7] GI motility also appears to be influenced by the composition of the gut microbiota.[8]

The gut has a number of important functions aside from digestion and absorption. The epithelial lining of the gut, along with a protective layer of mucus lining the intestinal lumen, is collectively referred to as the "GI barrier". The GI barrier is more than simply a mechanical barrier; it is a complex functional entity that provides defense via a dynamic immune system, executes metabolic functions, and enables communication between the gut microbiota and the brain through immunological, endocrine, and enteric nervous system pathways – referred to as the "gut-brain axis".[2,9] Thus, the enteric nervous system is sometimes called the "second brain"[10] (**Figure 3**). The gut-brain axis is also mediated by luminal epithelial chemosensors, which can respond to and transmit signals regarding bacterial metabolites present in the luminal space.[11]

The complex interplay of all of these factors is essential for the proper development and functioning of the immune system, and for the development of the brain itself from the time of birth.[11]

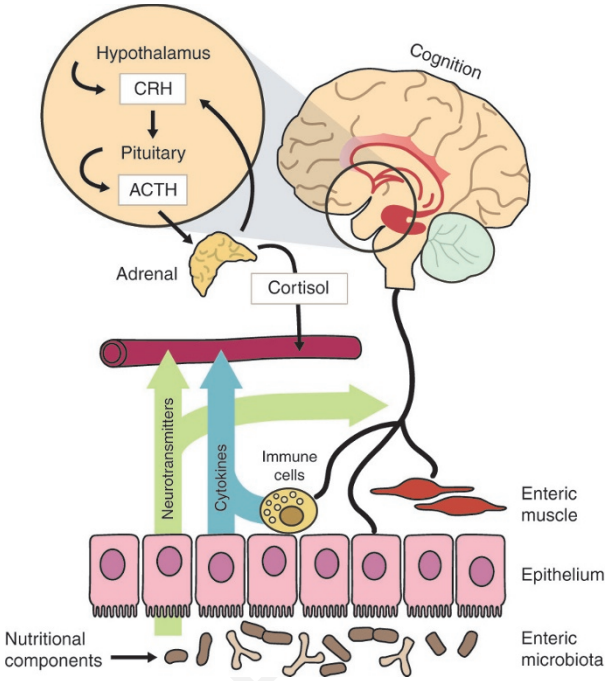


Figure 3. The reciprocal interaction between the gut microbiota and the brain

The reciprocal interaction between gut microbiota and the brain. Gut microbiota may modulate brain function and development through immune signaling (e.g., pro- and anti-inflammatory cytokines, chemokines and immune cells), endocrine and neural pathways. Conversely, the brain may influence the gut through neurotransmitters that impact on immune function and through alterations in cortisol levels, intestinal motility and permeability. Nutritional components may exert effects on each of these communication pathways. ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone.

A greater understanding of gut development during infancy is vital for both immediate and long-term interventions aimed at maintaining wellbeing. Thus, clinical research, particularly with respect to the dynamic development, establishment, and functions of the intestinal microbiota in the first months and years following birth, is a rapidly expanding field with potential to influence health throughout life.

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Chapter 2

The power of the gut microbiota

The gut microbiota and its distribution

Microbes, particularly bacteria, colonize every surface in the body that is exposed to the external environment, including the skin, oral/nasal cavities, and the urogenital and GI tracts.[1] In addition, several organs of the body that are considered sterile, including the lungs,[2] mammary glands,[3] and the placenta,[4] have been found to house unique and dynamic microbial communities.

Of all sites, the gut, particularly the colon, is the most heavily populated,[1,5] with approximately 1,000 different species of known prevalent bacteria.[6,7] Within the gut of each individual, a group of approximately 160 of these species can be found.[8] Gut bacteria include both "commensal" (resident) bacteria and transiently introduced bacteria that co-exist in a complex state of symbiosis and equilibrium.[9] The human colon harbors approximately 10^{14} bacterial cells – ten times the number of cells that constitute the entire human body[1,3,10] – and houses a diverse, dynamic microbial ecosystem which is essential to gut function.[3] This complex array of commensal microbes in the gut is commonly known as "the gut microbiota".

Gut microbes predominantly belong to four major phyla: *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria*. The composition of the gut microbiota is influenced by a complex variety of physiological, cultural, and environmental factors, including:[5,9,11-13]

- Mode of delivery
- Gestational age at birth
- Familial environment
- Diet

- Disease
- Stress
- Lifestyle
- Hygiene
- Antibiotic use

The distribution of gut microbiota varies by GI location[1] (**Figure 4**).[15] Conditions that influence this distribution include intestinal motility, pH, nutrient supply and composition, and GI secretions such as acid, enzymes, and mucus.[1] The population of microbes increases in density from the stomach to the small intestine, and from the small intestine to the large intestine, reflecting the progressively increasing pH and different digestive functions of these successive organs. For example, a dense and diverse microbial ecosystem is found in the colon, where microbes ferment undigested food.[1,14]

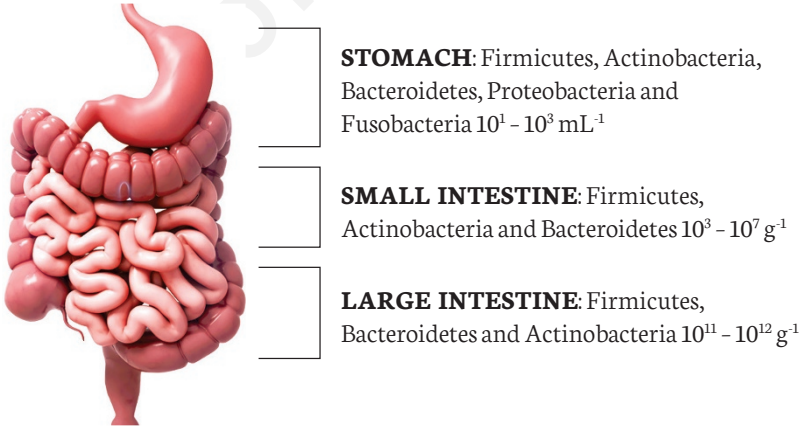

Figure 4. Distribution of key bacterial phyla in the human gastrointestinal system[15]

A personal microbiota "signature"

While several common bacterial phyla and genera comprise the gut microbiota, the composition at species level varies widely between individuals,[5,11] and is unique to each individual.[6,16] Interestingly, while the host genotype plays an important role in determining the bacterial composition in the gut,[17] identical twins only share 50%-80% of the species in their gut microbiota.[5,17] The composition of the microbiota also varies within the same individual over time,[5,9] largely due to incidental environmental factors.[18] However, the composition usually reverts back to its original composition following any short-term disruptions caused by, for example, disease or antibiotics.[19] Thus, it is virtually impossible to define a universal standard in gut microbiota composition.[11]

However, despite these large inter-individual differences in microbial community composition, the functionality of the gene content of the gut "microbiome" (the collective genome of the microorganisms) is broadly comparable across the human population and constitutes a core microbiome at the functional level.[5] Rather than a core group of species, current thinking focuses on defining the core functions performed by microbes in a healthy gut.

Beneficial functions of the gut microbiota

The gut microbiota has multiple functions that include nutritional, physiological, metabolic, and immunological functions (**Figure 5**).[1]

1. Digestion of nutrients

The gut microbiota is collectively involved in the efficient processing of nutrients, including several nutrients that the gut

lacks the necessary enzymes to digest on its own, such as starch and dietary fiber.[1] The host-microbe relationship is a symbiotic one; microbes in the gut, particularly the colon, can utilize these indigestible nutrients as a readily-fermentable food source for their own growth, while enhancing nutrient bioavailability and absorption by generating by-products which are useful for the human host.[3,20,21]

By-products include compounds such as short chain fatty acids (SCFAs), including acetic acid, lactic acid, and butyric acid, from degradation of unabsorbed poly- and oligosaccharides (OS), which are absorbed in the colon and used as a source of energy by the host.[1,14,20-22] It is estimated that SCFAs contribute approximately 10% of the human energy requirement.[20]

In addition, gut microbes synthesize a variety of essential micronutrients such as vitamin B_{12}, vitamin K, and folate that humans are unable to synthesize themselves.[1,20,23] Certain gut microbes are also capable of metabolizing bile acids, which is a critical step in bile acid recycling and homeostasis.[24]

2. Defence against pathogens

The gut microbiota participates in the body's defense against pathogens by actively limiting pathogen colonization in the gut. This is accomplished in several ways, including:

- Competing for nutrients (and adhesion sites) to competitively inhibit the growth of other microorganisms[6]
- Producing antimicrobial peptides (bacteriocins)[1,3,6]

- Facilitating growth and changes in the epithelial surface,[20] thus influencing the development, structure, and function of the epithelial barrier[3,25]
- Stimulating the immune system (for example, production of immunoglobulin A [IgA]) to manage the composition of gut microbes[3]
- Impacting GI motility[24]

In addition to microbial defence against pathogens, the mechanical properties of the epithelial barrier are important. The epithelial gut lining is covered by a protective layer of mucus that entraps pathogens and minimizes direct microbial contact with the epithelium,[25] enhances clearance of pathogens from the gut,[22] and provides a medium where gut bacteria can grow, colonize, and interact with immune system cells.[20,26,27] The epithelial barrier is not fully developed in newborn infants, and undergoes a critical period of development during infancy.[3]

3. Development of immune system

Immunological homeostasis depends on a balanced indigenous gut microbiota and appropriate timing and dosing with respect to the introduction of dietary antigens. The intestinal microbiota plays a key role in promoting and guiding the development of the mucosal and innate immune system in infancy,[3,6,9,28] which includes establishing and regulating the intestinal surface barrier.[3]

The gut microbiota also plays a key role in the development of the adaptive immune system, specifically:[3]

- signaling development of key intestinal lymphocyte subsets such as B cells, T helper (Th) effector cells and regulatory T (Treg) cells
- establishing the ratio of Th1 to Th2 effector cells which determines systemic immune responses

Animal models have linked the appearance and migration of mucin-containing goblet cells with the activation of the immune system by colonizing microbes; a healthy gut has a mucosal barrier that is twice the thickness of that in a microbe-free gut.[29] Furthermore, the gut microbiota affects gut development, through its role in the development of a robust villous capillary network and, by extension, a healthy intestinal blood vessel network.[14]

The infant immune system is immature and skewed towards a Th2-dominated response in order to keep the pregnancy intact during gestation. The first few months after birth thus represent a period of increased susceptibility to infection, before an age-dependent maturation of the immune system occurs.[3] Exposure to various environmental microbial components is thought to play an important role in this maturation process, and the literature suggests that specific early exposure of the gut to a variety of

microorganisms reduces the risk of developing inflammatory, autoimmune, and atopic diseases such as eczema and asthma in early childhood.[3]

4. Other effects

The intestinal microbiota is involved in the development and maintenance of intestinal homeostasis,[10] energy homeostasis,[26] and GI sensory and motor function.[1]

There is also increasing evidence to suggest an association between the gut microbiota and psychological wellbeing and behavior, including mood and stress response, via the gut-brain axis.[26,30] Some studies have suggested a link between gut-related pathologies and psychological disorders such as depression.[30]

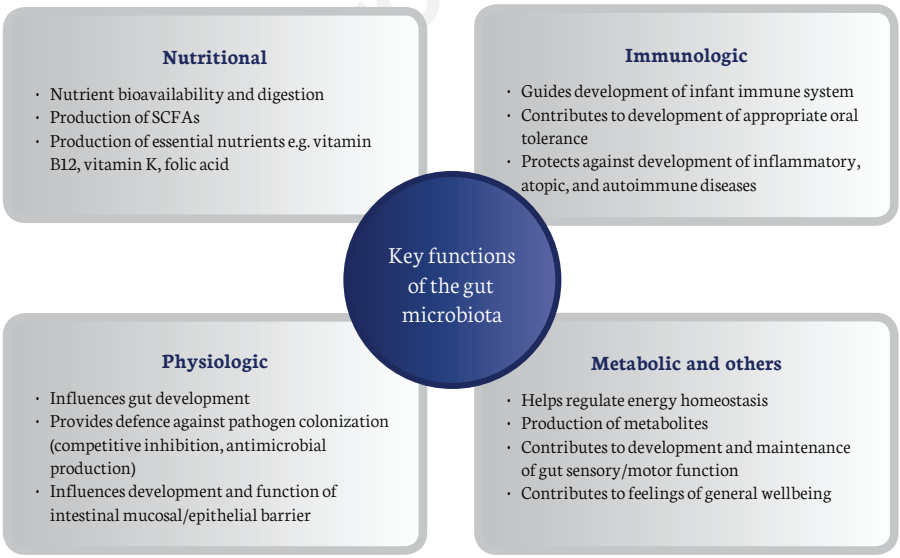

Figure 5. Beneficial functions of the gut microbiota[1,3,6,9,20,26,30]

The role of the gut microbiota in health and wellbeing

The association between the gut microbiota and health and disease is apparent from the earliest stages of life, and continues as the infant grows and develops.[3,20]

A healthy gut is associated with a diverse and balanced, stable, well-functioning microbial ecosystem within (**Figure 6**), and it is becoming well established that disturbance of the complex equilibrium of the gut microbiota is associated with

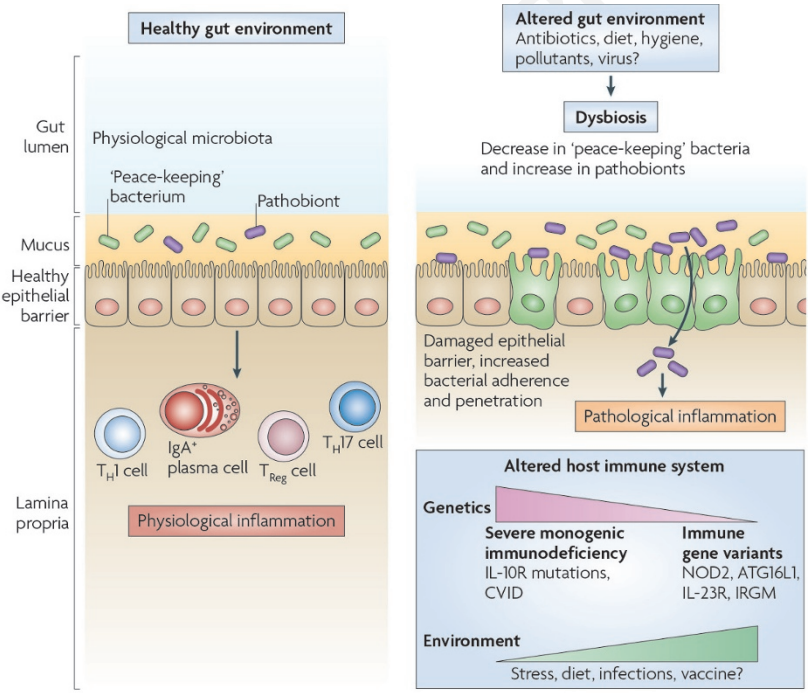

Figure 6: Comparison of a healthy versus altered gut microenvironment

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the development of various disorders, including metabolic, immunologic, and even psychological/behavioral disorders.[20] Disturbances or imbalances in gut microorganism communities are frequently referred to as "dysbiosis" – an old term that is gaining new interest with the advent of escalating research into the influence of the gut microbiota on health and disease.

Immediate health susceptibilities from dysbiosis in the growing infant may include infections, colic, and general digestive discomfort; however, dysbiosis may also increase the risk of developing a range of other diseases and medical conditions, including allergy, autoimmune diseases, food intolerance, digestive disorders such as irritable bowel syndrome (IBS), autism, and, in the longer term, conditions such as obesity, diabetes, and psychological disorders including anxiety and depression.[1,7,12,20,22,24,31-33] These wide-reaching effects reflect the wide spectrum of functions of the gut microbiota.

In most cases, the precise nature of the association between dysbiosis and the occurrence of pathological conditions, and whether dysbiosis is a cause or effect, are yet to be fully elucidated.[3,24] However, an increasing body of literature supports a direct association, emphasizing the importance of developing and maintaining a healthy gut during infancy to help ensure general health and wellbeing.

1. Allergy

The prevalence of allergy in infants with no family history of allergy is approximately 10%, rising to 20%-30% among infants

with a first-degree relative with allergy.[34] Neonates, with their immature innate and adaptive immune systems, may be unable to always initiate appropriate immune responses. In the early months and years after birth, the mucosal immune system gradually matures alongside the development of the infant's gut microbiota,[20] which appears to modulate immunologic and inflammatory systemic responses,[34] providing increasing protection from antigens in the environment.[20]

A hypersensitive immune system gives rise to allergic reactions, whereby normally harmless substances in the environment, termed as allergens, trigger the immune system. These reactions are acquired, and they lead to excessive activation of mast cells and basophils by immunoglobulin E (IgE).[3]

Studies show that infants and young children with allergies harbor a different gut microbiota profile from those without allergies, particularly different levels of *Bifidobacterium* species.[6,28] In Western countries, where increased hygiene appears to have changed the gut microbiota of infants, the prevalence of allergic conditions has increased dramatically in recent years,[35] further supporting the theory that the gut microbiota is involved in immune system development.

2. Development of metabolic disorders

As discussed above, the intestinal microbiota plays a crucial role in the digestion of food and the processing of nutrients. When the microbiota is disrupted, metabolic pathways, including those involved in nutrient harvest, also show disruption, and

such disturbances have been shown to be associated with obesity and insulin resistance.[1,36,37] Some studies suggest that an altered microbial composition in the gut may increase the efficiency of food conversion, providing the host with increased amounts of usable energy in the form of SCFAs and sugars, which is efficiently stored as fat.[1] One study of gut microbiota transplant from lean individuals to recipient individuals with metabolic syndrome showed a significant improvement in insulin sensitivity 6 weeks after infusion.[37] However, whether an altered microbial composition is a direct cause of obesity and insulin resistance, or results from unhealthy dietary changes, remains unclear.[1]

An association between a lack of diversity in the intestinal microbiota and the development of metabolic disorders such as obesity and type II diabetes has also been demonstrated, and altered microbial ratios have been correlated with insulin resistance.[36] Furthermore, it has been recently shown that certain drugs used in patients with type II diabetes work via their effects on the gut microbiota.[38] Dysbiosis has also been associated with non-alcoholic fatty liver disease and the metabolic syndrome; animal studies and pilot studies in humans using probiotics to modulate the gut microbiota have shown this approach to be a promising add-on therapeutic tool.[39]

3. Brain development, behavior, and mood

Microbial colonization in the infant has been shown to coincide with key neurodevelopmental periods, and some evidence suggests an association between disruption of this colonization process and central nervous system dysfunction, with the potential to lead to adverse psychological health outcomes later in life.[32]

In addition, an increasing body of evidence indicates that gut microorganisms may directly interact with elements of the host's neurophysiological system to influence host behavior, mood, stress response, and psychological health, including the development of anxiety and depression, via the gut-brain axis. This appears to involve a complex interplay of both immune and non-immune effects.[30]

It has been suggested that gut microbiota may influence the likelihood of children developing autism. While this link is somewhat speculative, GI complaints are common among children with symptoms of autism, and autistic children show a significantly altered gut bacterial composition compared with non-autistic children.[33] A history of multiple courses of antibiotics, which disrupt the balance of the commensal gut bacteria, is common among children with autistic spectrum disorders.[33]

Therapeutic approaches

An increasing understanding of the role of the gut microbiota in health and disease offers a rational therapeutic target for intervention.[20] Evidence suggests that the focus of medical research should not be solely on the treatment of gut disorders, but should shift towards maintaining gut health, either through primary or secondary preventative steps.[1] Thus, one increasingly common approach in the management of the above conditions involves deliberately modulating the composition of the gut microbiota using probiotics, prebiotics, antimicrobials, or fecal transplant procedures to encourage a healthier microbiota composition[5,12,40] (see **Chapter 4**).

Medical conditions that may be associated with a disrupted gut microbiota[1,7,12,20,22,24,31-33]

Early life:

- Necrotizing enterocolitis (NEC)
- Colic
- GI infections
- Constipation/diarrhea
- Celiac disease
- Antibiotic-associated diarrhea
- Allergy

Beyond infancy and into adulthood:

- Atopy (allergy) and asthma
- Celiac disease
- Colon cancer
- Diabetes (type I and type II)
- GI infections
- Non-alcoholic fatty liver disease
- Obesity
- Psychological disorders
- Rheumatoid arthritis
- Inflammatory bowel disease (IBD)
- Irritable bowel syndrome (IBS)

Chapter highlights

1. Microbes colonize virtually every body surface. The gut is the most densely populated.
2. One of the main functions of the gut microbiota is to enhance digestion of food, assisted by the production of important nutrients such as SCFAs and a variety of vitamins and amino acids.
3. The gut microbiota performs nutritional, metabolic, physiological, immunological, and other functions, and is involved in the development and maintenance of the gut barrier.
4. Appropriate gut microbiota diversity and composition is essential for the maintenance of health and wellbeing.
5. The gut microbiota plays a crucial role in the early development of the intestinal immune system, by training it to distinguish between commensal microbes and pathogenic microbes.
6. An abnormal gut microbiota affects early immune response development and increases the risk of allergic disorders.
7. Dysbiosis may also be associated with infant disorders such as colic, GI infections, constipation, diarrhea, and NEC.
8. Later-life consequences of infant dysbiosis may include atopic disorders, celiac disease, obesity, diabetes, and autoimmune disorders.

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Chapter 3

Early colonization of the gut

The significance of early gut colonization in the infant

The influence of early colonization patterns on the composition of the subsequent adult microbiota is not yet fully understood. However, increasing evidence suggests that the process of microbial colonization and establishment of optimal host-microbe symbiosis during early infancy appears to profoundly impact both early and lifelong health, by positively influencing gut maturation, immune development, physiological function, and metabolism.[1]

Conversely, as described in **Chapter 2**, growing evidence suggests that an imbalance or disturbance in the abundance and diversity of an infant's gut microbiota for any reason may be associated with a wide range of diseases and disorders in the short and long term, including immune and metabolic disorders and atopic diseases.[2] Thus, a greater understanding of the process of gut colonization and microbiota assembly is not merely an academic exercise but is potentially of great practical importance,[3] and highlights the necessity of establishing and maintaining a healthy gut microbiota in infancy.

Establishment of the gut microbiota in early life

1. Pregnancy

The gut undergoes an intense period of development *in utero*, influenced by genetic factors, as well as maternal factors including health and nutritional status.[1,4]

Until recently, the GI system of the developing fetus was considered sterile; however, in the last decade, several species of commensal bacteria have been detected at low levels in umbilical cord blood, amniotic fluid, the placenta, and infant meconium,[5,6] suggesting a small measure of microbial exposure *in utero*.[5,7] However, several studies have particularly shown greater microbial colonization in amniotic fluid of women in preterm labor, suggesting that there is a relationship between amniotic bacterial abundance and gestational age at delivery.[8]

Prenatal maternal factors that may influence the postnatal development of the infant gut microbiota and immune system include stress, diet (including dietary supplementation) during late pregnancy, maternal body mass index, smoking status, and socioeconomic status.[2,7]

2. Birth

During and immediately after birth, pioneering bacteria are introduced to the infant's body and a new microbial ecosystem begins to be established within the gut[4] (**Figure 7** and **Figure 8**). It appears that initial colonization of the infant gut is largely a result of the exposure to microbes in the environment including the maternal vaginal, fecal, and skin microbiota.[1,7,9,10]

The mode of birth affects the composition of the infant gut microbiota; among infants delivered vaginally, a microbial composition similar to that found in the birth canal and gut tends to be observed, while in those born by cesarean section, the microbial composition tends to more closely resemble that of

mother's skin and the hospital environment, reflecting contact with staff and other neonates.[2,9-12] Cesarean-born infants have a less diverse, lower total bacterial count than vaginally delivered infants, with higher levels of *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* species and low or absent *Bifidobacterium* counts.[11]

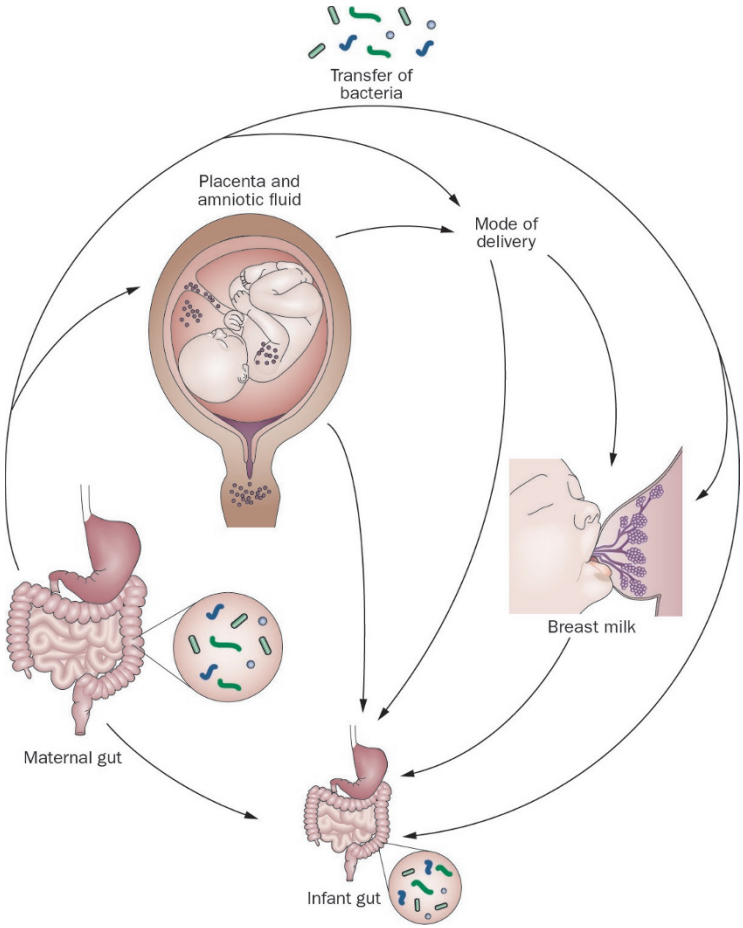

Figure 7: The maternal microbial legacy is transmitted during pregnancy, at birth and during breastfeeding

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Prophylactic antibiotics – standard of care in many countries around the world and in many guidelines for cesarean delivery – as well as a lower probability of being breastfed, may also play a role in the altered microbial composition of infants delivered by cesarean section,[6] contributing to the lower levels of *Bifidobacteria*. Delayed breastfeeding may also contribute to aberrant colonization patterns.[6]

The gut microbiota of infants delivered by cesarean section has been shown to eventually "catch up" with that of their vaginally delivered counterparts in terms of both stability and diversity.[11] However, these aberrant patterns of colonization occur during a critical period of immune and metabolic development. Hence, there may be long-term consequences for infants delivered by cesarean section. Several studies have highlighted that the microbial alterations observed in cesarean-born infants are associated with a subsequent increased risk of developing various diseases, including asthma, eczema, allergy, obesity, chronic immune-related inflammatory diseases, and type I diabetes.[2,13]

3. Infancy: 0–12 months

Immediately after birth, the infant is exposed to the mother's skin and oral microbiota during early bonding.[2] Environmental pathogens in the hospital birth environment have also been shown to influence gut colonization,[14] and even inhaled microbes, which are swept into the gut to the nasopharyngeal cavity and upper airways, contribute to the composition of the gut microbiota.[15]

Early dietary exposure via human milk or infant formula is a central driver influencing the gut microbiota composition[1,6,9] (see **Chapter 4**). Human milk contains "prebiotic" OS – soluble but

non-digestible carbohydrates that reach the colon intact and are known to selectively stimulate the growth of gut bacteria that may positively impact infant health.[6]

Bacteria found in human milk also play a significant role, including *Bifidobacterium*, staphylococci, streptococci, and lactic acid bacteria.[6,16] It is thought that microbes reach human milk through endogenous routes and/or through introduction to the nipple by the infant following exposure to the birth canal and fecal microbiota during delivery.[6] Compared with exclusively breast-fed infants, the fecal microbiota of formula-fed infants is characterized by less diverse *Bifidobacterium* populations.[17]

After initial bacterial inoculation and colonization, rapid and significant changes in microbial abundance and diversity begin to take place as the infant acquires a wider range of microbial species from his or her environment, eventually creating a unique and stable microbial ecosystem within the gut[1,18] (see **Chapter 4**).

The next major stage in the development of an infant's gut microbiota is the introduction of solid foods.[11] Typically, after 4 to 6 months of receiving an exclusively milk diet, solid foods including fruits, vegetables, and cereals, all of which contain *insoluble* indigestible carbohydrates, are gradually introduced into the diets of infants of developed countries.[6] The introduction of these more complex foods promotes colonization of the infant gut with an increasing number and diversity of bacteria.[6]

4. Toddlerhood: 1–3 years

The gut microbiota continues to become established during this period, consistent with the establishment of a varied solid food diet.[19] By approximately 3 years of age, the diversity and complexity of the gut microbiota has stabilized and resembles more closely that of an adult.[4,6,9,20,21] After this, the gut microbiota can still undergo temporary disturbance – for example, through diet, disease, or medication.[19]

General factors influencing early colonization

Throughout the developmental stages outlined above, a range of other physiological, environmental, and cultural factors have been implicated in gut colonization and development of the gut microbiota in early life (**Figure 8**).[1,18] These may include genetic disposition, family size (other siblings), culture, geographic location (developed versus developing countries; urban versus rural living), early exposure to animals, standard of sanitation, infections and antibiotic use, and gestational age.[2,6,7,11,19,22]

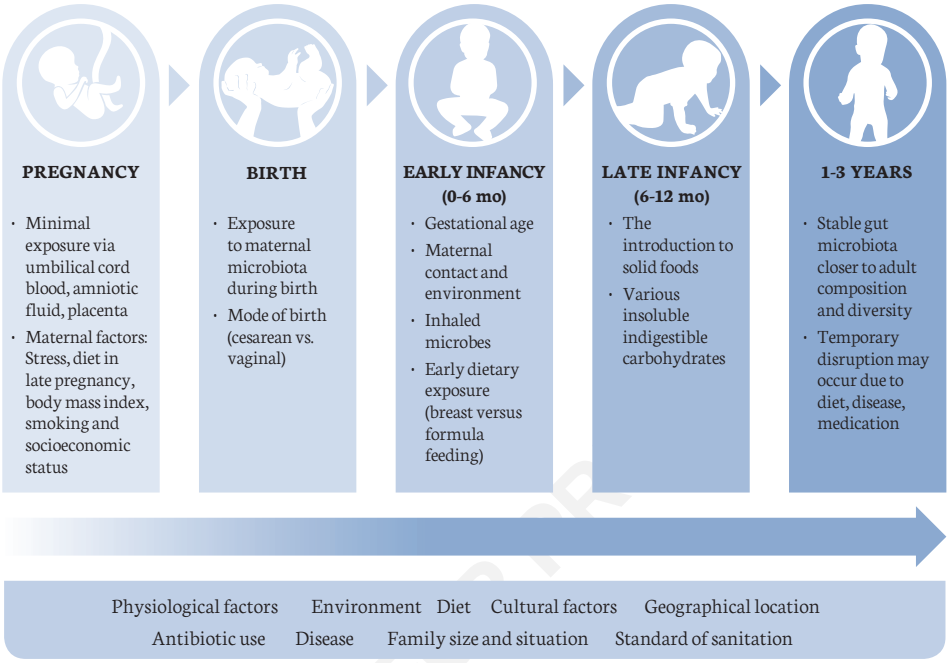

Figure 8: Sources of microbial colonization and factors affecting the development of the intestinal microbiota in early life

Effect of pregnancy on the maternal gut microbiota

During the course of a pregnancy, the maternal body undergoes significant hormonal, immunological, and metabolic changes. An increase in maternal body fat is observed in the first trimester, thought to help prepare the mother for the increased energy demands of pregnancy and lactation. Additionally, reduced insulin sensitivity is observed during the later stages of gestation, which may be associated with changes in immune status.[23] In parallel, the maternal bacterial load in the gut increases between the first and third trimester of pregnancy, with dramatic remodeling of

the microbiota composition resulting in a reduction in microbial diversity in the woman's gut. By the third trimester, there is a wide variation between pregnant women with regard to their gut microbiota compositions.[23]

In non-pregnant individuals, recent evidence suggests that disturbances in the gut microbiota play a key role in the development of metabolic disease, including inducing inflammation, weight gain, and reduced insulin sensitivity. In the context of pregnancy, while some preclinical evidence suggests an association between maternal gut microbiota changes and metabolic/immunological status, the precise relationship and mechanisms remain less clear.[23]

Effects of antibiotics on the infant microbiota

Antibiotic use in infants has been strongly associated with gut microbiota disturbances.[2,6,24] However, differences in antibiotic specificity, dosage, length of treatment course, and administration route make such changes difficult to predict or interpret.[6]

Studies have shown that about one-third of the bacterial species in the microbiota may be disrupted by a course of certain antibiotics, and that these profound changes can persist for weeks or months in infants.[24,25] In general, antibiotic treatment appears to cause delays and disruption in expected patterns of early colonization of ***Bifidobacterium*** and ***Lactobacillus*** species, and allows an overgrowth of other species such as ***Proteobacteria.***[2,6,26] Recent evidence suggests no recovery of the microbiota composition within 4 weeks, and only partial recovery within 8 weeks, the long-term effects of which are unknown.[26]

Delays in gut colonization and a change in the microbiota composition have also been observed among infants whose mothers receive antibiotic treatment perinatally and/or while breastfeeding,[2,6] although, in general, these changes do not appear to persist after the introduction of solid foods.[6]

However, as discussed in **Chapter 2**, while antibiotic-induced microbiota changes tend to be transient, there is nevertheless evidence to suggest that even these transient changes are associated with the development of immune-related and other disorders in the longer term.[6]

Preterm and low-birth weight infants

Shorter gestational length appears to be associated with delayed gut colonization and low microbial diversity after birth – particularly lower proportions of beneficial *Bifidobacteria* – compared with that of full-term infants. This may be either a cause or effect of preterm delivery.[2,6,11,27,28]

In combination with immature gut structure and mucosal immune function,[27] factors that may result in delayed or disrupted bacterial colonization among preterm infants include the frequent use of total parenteral nutrition,[27] delayed enteral feeding,[29] an aseptic neonatal intensive care unit environment,[6,27,30] frequent postnatal antibiotic administration,[6,29,30] and other factors such as prolonged rupture of membranes and ambient pathogen exposure.[29] In addition, antibiotic use during labor and cesarean section is more common among mothers of preterm infants, which may influence colonization at birth.[3,31] It has been demonstrated that the effect of a single intra-partum antibiotic dose administered

to a mother appears to be at least equal to the effect of multiple post-partum doses of antibiotics administered to her infant in terms of microbial colonization disturbance in the infant gut.[3,31]

An abnormal gut microbiota has been implicated in the development of neonatal sepsis, along with a range of GI disorders in the infant, including NEC.[6,32-38] In particular, early empiric use of antibiotics resulting in sustained suppression of microbial diversity and an increased risk of rebound pathogenic overgrowth, coupled with exaggerated and uncontrolled responses of the immature immune system, appear to be key contributors.[27,33,34] Moreover, a longer duration of postnatal antibiotic treatment in preterm infants has been associated with an increased risk of NEC.[39] A high prevalence of certain pathogens has been observed among preterm infants who develop sepsis or NEC.[27,34,37,38]

As well as potentially contributing to morbidity and mortality in preterm infants,[36] a delay in establishment of the gut microbiota may be associated with longer-term effects, such as immune disruption and allergy, and neurodevelopmental delay.[21] In addition, low birthweight infants may be at greater risk for obesity and metabolic disorders in later life, which appear to be related to the infant gut microbiota.[21]

A large meta-analysis of studies in preterm infants showed that support of gut microbiota establishment with probiotics reduced the risk of feeding intolerance, NEC, extended hospitalization, and all-cause mortality.[29] Studies have shown a positive association between gut microbiota diversity and healthy infant weight gain, suggesting that supporting the development of the gut microbiota may assist catch up growth in preterm infants, but this is yet to be definitively demonstrated.[6]

Chapter highlights

1. How the infant gut microbiota develops during early life can have a significant impact on infant health and wellbeing. Dysbiosis in infancy has been associated with a range of short-term disorders, including GI infections, colic, constipation and general digestive discomfort.
2. Evidence suggests that a small amount of bacterial exposure may occur before birth through the amniotic fluid and placenta, but the majority of the colonization process occurs during and after birth through contact with the mother and environment.
3. Breastfeeding plays an important role in the development of the intestinal microbiota.
4. *Bifidobacterium* is a key species in breastfed infants. In most cases, deviations from a normal, stable gut microbiota in infants involve a drop in *Bifidobacterium* levels.
5. The introduction of solid foods at around 4 to 6 months is the second major milestone in the development of the gut microbiota, and results in an increase in the number and diversity of various microbial species.
6. Many factors influence the development of an infant's gut microbiota: prenatal factors, such as the mother's body mass index and the length of gestation; birth factors, such as mode of delivery; and postnatal factors, such as types of feeding, use of antibiotics, and the infant's family environment.

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Chapter 4

Nutrition and gut health during early life

As discussed in **Chapter 3**, the first year of postnatal life is a key period for programming the immune system and establishing the gut microbiota for the remainder of life. The type of feeding and other factors to which the infant is subjected, for example, illness or antibiotics, may have a direct influence on the composition of the gut microbiota and on intestinal epithelial integrity.[1]

The composition of human milk

Human milk provides optimal nutrition for infant growth and healthy development, as it contains a wide range of nutritive and protective compounds specifically tailored to the infant's needs.[1-3] Breastfeeding is one of the factors that have been strongly associated with a lower incidence of infectious diseases and allergy in infancy and childhood, through its contribution to the development of a healthy gut and resident microbiota, and immune system development.[4-8] Breastfeeding is also associated with optimal brain and eye development.[6-8]

In the longer term, breastfeeding also has important implications on public health. Human milk has a beneficial effect on nutrient absorption and metabolism and has been shown to be associated with a lower risk of metabolic disorders such as obesity, hypertension, and hypercholesterolemia in later life.[6-8]

The composition of human milk shows dynamic changes over the lactation period according to the infant's nutritional needs at various stages,[9] and varies according to maternal diet, highlighting the importance of good maternal nutrition.[3] The most abundant compounds in human milk are carbohydrates (predominantly

lactose and OS) and fatty acids, reflecting the primary nutritional role of human milk (**Figure 9**). Other components include human milk oligosaccharides (HMOS), microbes, nucleotides, immunoglobulins, immune cells, cytokines, lysozyme, lactoferrin, and other immune-modulating factors.[1,3]

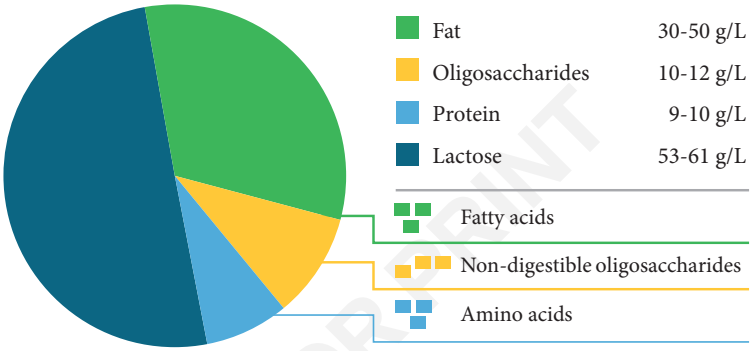


Figure 9: The composition of human milk

Adapted from Newburg DS, Neubauer SH. In: Jensen RG (ed): Human milk composition, Academic Press 1995;273-349.

Lactoferrin is a glycoprotein that binds iron in the milk and within the gut, limiting its availability to pathogens, and can also prevent pathogens binding to the gut barrier.[10] Cytokines, antibodies, and lysozyme are all components of the mature immune system. Like lactoferrin, the antibodies in human milk prevent pathogens from binding to the gut barrier, while lysozyme can directly attack bacterial cell walls and cytokines can reduce inflammation in the gut (**Table 1**). Because the adaptive immune system takes time to develop, newborn infants are initially reliant on the innate immune system of the gut, which is partially contributed to by these bioactive compounds in human milk.[10]

Table 1: Compounds with immunological properties in human milk[11]

Anti-microbial compounds		
Immunnoglobulins: sIgA, sIgG, sIgM	Haptocorrin	Maternal leukocytes and cytokines
Lactoferrin, lactoferricin B and H	Mucins	sCD14
Lysozyme	Lactadherin	Complement and complement receptors
Lactoperoxidase	Free secretory component	ß-Defensin-1
Nucleotide-hydrolyzing antibodies	OS and prebiotics	Toll-like receptors
K-casein and α-lactalbumin	Fatty acids	Bifidus factor
Tolerance/priming compounds		
Cytokines: IL-10 and TGF- β	Anti-idiotypic antibodies	
Immune development compounds		
Macrophages	Growth factors	Nucleotides
Neutrophils	Hormones	Adhesion molecules
Lymphocytes	Milk peptides	
Cytokines	Long-chain polyunsaturated fatty acids	
Anti-inflammatory compounds		
Cytokines: IL-10 and TGF- β	Adhesion molecules	Lactoferrin
IL-10 receptor antagonist	Long-chain polyunsaturated fatty acids	sCD14
TGF-α and IL-6 receptors	Hormones and growth factors	Osteoprotegerin

IL, interleukin; OS, oligosaccharides; sCD14, soluble cluster of differentiation 14; sIg, serum immunoglobulin; TGF, tumor growth factor

Human milk also contains immune system cells such as macrophages.[10] Along with the other immune system components, immune system cells are particularly abundant in the milk produced just before and after birth, known as colostrum.[10] In addition to providing the infant with important commensal bacteria and protection against pathogens, certain components of human milk directly stimulate the development of the infant's own immune system.[1]

Human milk oligosaccharides

Extensive research has been conducted into the beneficial role of HMOS in infant health.[2] HMOS in human milk are one example of naturally occurring prebiotics – non-digestible food ingredients that actively promote the growth of beneficial microorganisms in the intestines. HMOS are a group of over 1,000 structurally diverse carbohydrate molecules that promote the growth of specific bacteria, particularly ***Bifidobacteria.***[5] As mentioned in **Chapter 2**, these bacteria can use HMOS as a source of energy, and fermentation of HMOS in the colon by commensal bacteria produces useful byproducts for the host, including SCFAs.[12-15] This prebiotic effect is considered to be of major benefit in infants because it helps shape a healthy gut microbiota to stimulate the developing immune and metabolic systems.[16] In addition, HMOS bind pathogens, preventing their adhesion to the mucosal surface.[1,4,12]

HMOS patterns show individual differences between mothers, linked to specific enzymes coded by a small number of known genes.[17] There are four known HMOS groups that correlate

with the genetic basis of the Lewis blood group system.[17] HMOS patterns also vary over the course of the lactation period in an individual mother.[17,18] Thus, the level of HMOS-associated protection against pathogens is influenced by a complex interplay between factors such as maternal genotype, infant genotype, and infant exposure to a given set of pathogens.[19]

Human milk contains 20–23 g /L (colostrum) and 12–13 g/L (mature milk) free HMOS.[20] This is 10- to 100-fold the concentration of OS found in cow's milk. Furthermore, the structural diversity within the OS fraction in human milk exceeds that found in cow's milk. With multiple core structures and multiple linkage sites of each core, HMOS exist in various isomeric forms. These combinatorial possibilities could theoretically produce 1,000 different HMOS.[19]

Benefits of short-chain fatty acids

SCFAs have several key benefits for the host infant, including:

- Usefulness as an absorbable source of energy[13-15]
- Lowering the pH in the gut, thereby encouraging growth of several commensal bacteria that prefer acidic conditions, and inhibiting the colonization and growth of certain pathogens[13]
- Actively reducing inflammation in the gut[21]
- Interacting directly with immune cells, helping regulate their activity[4]
- Stimulating intestinal motility,[21] helping prevent constipation and discomfort
- Stimulating growth and differentiation of the intestinal epithelial cells[21]
- Helping the body absorb nutrients such as calcium and iron[21]

Microbes in human milk

In total, over 200 different bacterial species have been isolated from human milk, although the number of cultivatable species found in a single individual is much lower, ranging from two to 18 different species.[2] The human milk microbiota appears to contain a 'core' population of microbes that are common among all women, supplemented with a variable population that differs between individuals; common genera include ***Bifidobacterium, Lactobacillus, Staphylococcus, Streptococcus*** and ***Lactococcus.***[2] As with a mature gut microbiota, the microbial community in an individual mother's milk has been shown to be relatively stable over time.[2] The particular composition of the human milk microbiota may be influenced by a range of environmental factors, including socioeconomic, cultural, genetic, dietary, and antibiotic-associated factors.[2] The particular composition of an individual mother's milk microbiota may be influenced by a range of environmental factors, including socio-economic, cultural, genetic, dietary, and antibiotic-associated factors.[2]

Exactly how these bacteria come to reside in human milk remains unclear. Traditionally, it was believed that simple contamination from the mother's skin and the infant's oral cavity during breastfeeding resulted in bacterial movement through the nipple into the milk via reverse flow.[2,16] However, studies comparing various bacterial strains on the skin and infant oral cavity with that of human milk indicate that there must be other mechanisms involved in human milk colonization.[2] It appears that at least some bacteria in the maternal gut migrate to the mammary gland through systemic routes (**Figure 10**), although the exact mechanisms of selective uptake and migration are yet to be fully explained.[2]

It is hypothesized that physiological and hormonal changes during and after pregnancy could influence gut permeability, allowing uptake of certain bacteria, by various immune cells, and transported via mass migration of the immune cells to the mammary gland during and after pregnancy, via the lymphoid system or the blood.[2]

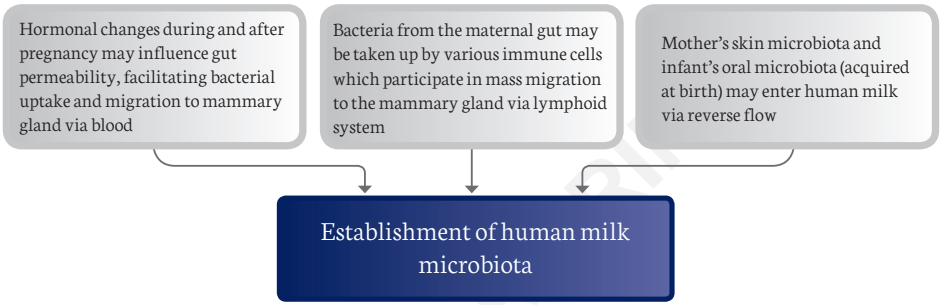


Figure 10: Potential mechanisms of colonization of human milk[2]

What is clear, however, is that human milk is an important source of beneficial bacteria that help colonize the infant gut and contribute to the composition of a healthy gut microbiota.[2]

Dietary intervention with prebiotics, probiotics, and synbiotics

When the gut colonization process is delayed or disrupted due to the different factors discussed in **Chapter 3**, including preterm birth, delivery by cesarean section, aseptic postnatal care conditions, antibiotic use, or the need for formula feeding when breastfeeding is not possible, there is increasing scientific and medical support for nutritional interventions that may help to modulate the microbiota composition.[22-25]

The composition of the gut microbiota is largely dependent on diet and may be able to be influenced by several specific food concepts, including administration of prebiotics, probiotics, and synbiotics.[5]

1. Prebiotics

Prebiotics are non-digestible dietary carbohydrates, primarily OS, that travel to the colon intact and are able to selectively stimulate the growth and activity of beneficial commensal bacteria in the colon.[1] The International Scientific Association of Probiotics and Prebiotics (ISAPP) defines prebiotics as a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gut microbiota, thus conferring benefit(s) upon host health. Because of their complexity and variety, prebiotic OS used as dietary ingredients in infant formula milk are not identical to HMOS, and research continues to explore types of OS that can be used as effective prebiotics in infant feeding.[4]

To date, most data on prebiotic effects have been obtained using food ingredients or supplements, either inulin-type fructans or galacto-oligosaccharides (GOS).[26] Currently, the Directive 2006/141/EC on infant formula and follow-on formula specifically allows the addition of GOS-FOS in a ratio of 9:1 and in a concentration of 0.8 g/100 ml of prepared product.[27] The Directive also states that other combinations of GOS-FOS may be considered if these variations satisfy the nutritional requirements of healthy infants, as established by generally accepted scientific data. The effect depends on the specific structure and amount of prebiotic compound in a given target group. Findings from one

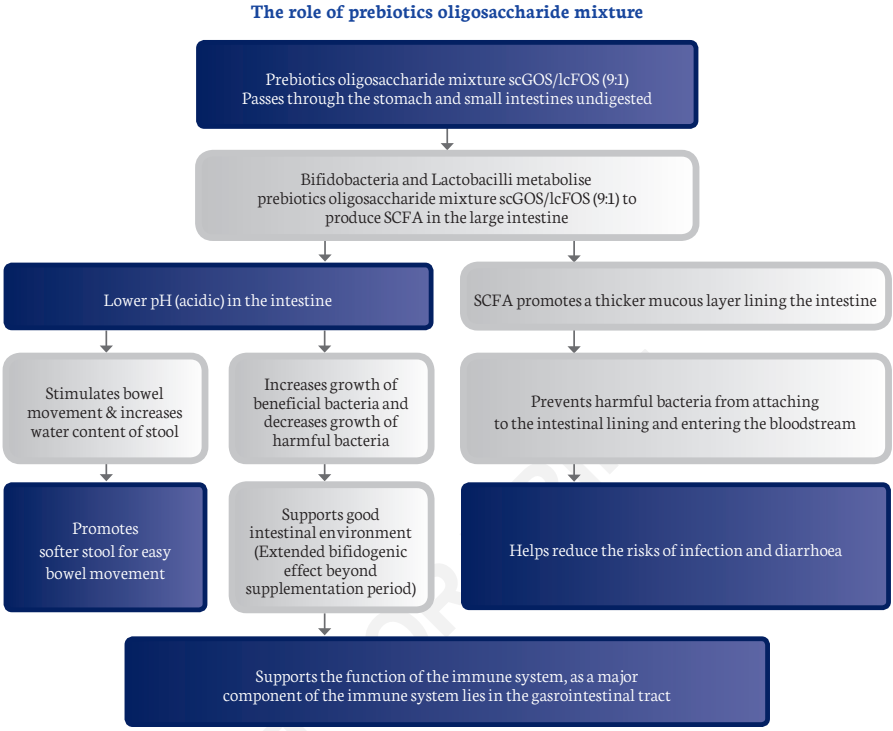
type of prebiotic compound or mixtures thereof cannot simply be translated to other prebiotic compounds.[28]

Various studies have shown that, when breastfeeding is not possible, the addition of specific OS mixtures to infant formula modulates the gut microbiota of infants.[3] This includes stimulating beneficial microbial growth, lowering levels of potentially pathogenic bacteria, leading to a gut environment with a lower pH and a SCFA profile in which acetate (>80%) is the main SCFA followed by propionate.[16]

Prebiotic supplementation of infant formula with short-chain GOS (scGOS) and long-chain FOS (lcFOS) has been shown to increase the levels of fecal *Bifidobacteria* in a dose-dependent manner in formula-fed infants, producing a similar diversity to breast-fed infants, as well as producing a comparable composition of fecal SCFAs derived from the metabolic activity of *Bifidobacteria.*[13,29-31] In contrast, standard formula produces a fecal *Bifidobacteria* composition more similar to the typical adult distribution.[29,30] Prebiotic supplementation also appears to positively influence the metabolic activity of the total intestinal flora.[30]

Prebiotics also increase bacterial mass and osmotic water-binding capacity in the gut lumen. These actions increase stool weight and frequency, soften stools, and indirectly contribute to both decreased transit time and a reduction in the risk of constipation.[13]

With regard to actual wellness benefits in infants, specific prebiotics have demonstrated immunomodulatory effects in

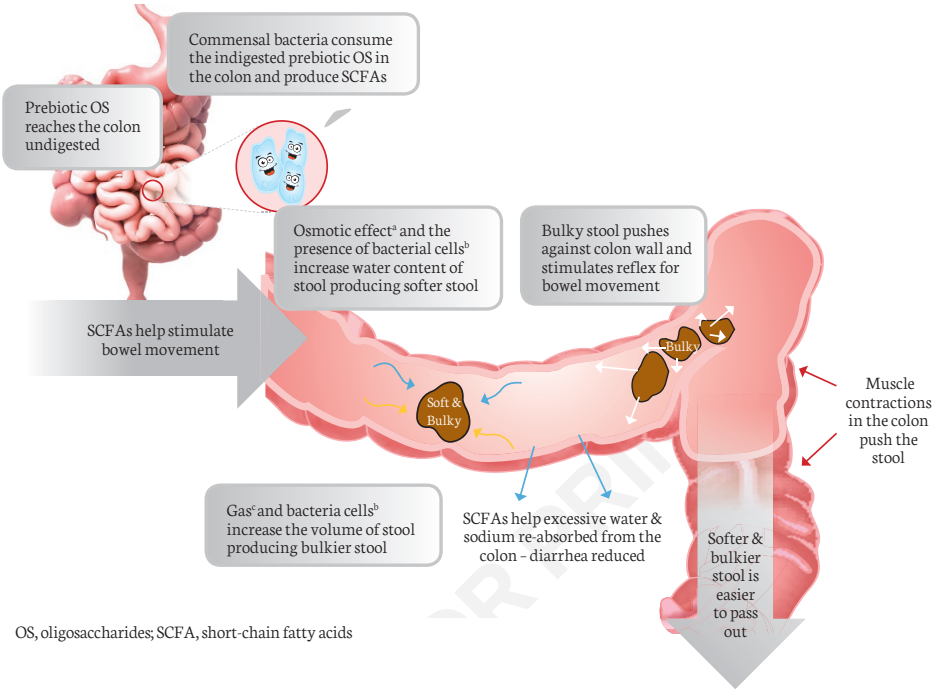


lcFOS, long chain fructo-oligosaccharides; scGOS, short chain galacto-oligosaccharides; SCFA, short-chain fatty acids

Figure 11. Supplementation of prebiotic OS mixture short-chain GOS/long-chain FOS (9:1) is clinically proven to maintain a favorable gut environment and support the function of the digestive and immune systems[31,32,33,34]

some studies of infants with conditions such as atopic dermatitis, infection, and inflammation.[3,20] Some trials have also reported that administration of prebiotic OS in formula may reduce crying episodes in infants with colic.[36]

The relationship between a healthy gut microbiota and the proper development of the immune system is a likely explanation for any observed immune-related benefits of prebiotics.[37] The ability of OS



OS, oligosaccharides; SCFA, short-chain fatty acids

Figure 12. Prebiotic oligosaccharides promote stool consistency and transit

[a] Osmotic effect caused by degradation of oligosaccharides into smaller molecules.

[b] The water content of bacteria is high. The bacteria increases the fecal biomass.

[c] Gas produced by fermentation increase fecal mass by being trapped in the intestinal bulk, impelling the fecal mass by acting as a propulsive pump.

to interact with and modulate the immune system directly may also play a role.[1]

The Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) position paper on prebiotics[38] concluded that the available evidence suggests scGOS/lcFOS (9:1) supplementation produces higher stool colony counts of ***Bifidobacteria*** and improved

stool consistency and frequency, but the clinical relevance of these findings is unknown. The growing consensus is that avoidance of dysbiosis and aligning the gut microbiota (and associated stool characteristics) as close as possible to that of a healthy breast-fed infant is a key clinical goal. The clinical relevance of a healthy gut microbiota is becoming clearer. For example, recent clinical trials have demonstrated that scGOS/ lcFOS administration in infants may be effective in reducing the risk of developing infections and certain allergic conditions such as atopic dermatitis.[38,39]

2. Probiotics

Probiotics are defined as live microorganisms, which when administered in adequate amounts, colonize the gut and exert beneficial biological effects on the host.[13,40] In the last few decades, major advances have been made in characterizing specific probiotics and understanding their mode of action and effects on health.[13] The use of probiotics in the pediatric setting has tripled in the past 5 years.[3]

Probiotics are added to a variety of foods, mainly dairy products and formula milk, and are also available as food supplements in capsule or tablet form.[14] The most commonly used probiotics in supplements and foods currently include species from the *Lactobacillus* and *Bifidobacterium* genera.[3,13]

Probiotic microorganisms may influence the microbiota by colonizing the gut, as well as preventing pathogenic bacterial overgrowth. This may be accomplished in several ways, including:[13,14]

- Competing for nutrients
- Competitively inhibiting pathogenic bacterial adhesion to the epithelial cells
- Reducing the gut pH to discourage growth of certain pathogenic bacteria
- Converting sugars into fermentation byproducts with inhibitory properties
- Secreting anti-microbial compounds
- Stimulating host production of anti-microbial compounds.

Probiotics may also help reduce inflammation in the gut, stimulate the immune system, produce substrates such as vitamins for host growth, and influence the gut barrier function.[13]

The beneficial effect of probiotics is highly dependent on the strain, the dose, and the condition of use. Probiotics are currently not recommended for routine use in infant nutrition due to the lack of conclusive evidence. However, there is now considerable evidence to support the use of probiotic supplementation in the prevention of NEC in infants,[41] and the use of specific probiotic strains in infants and children with infectious or antibiotic-associated diarrhea.[3] Other hypothesized benefits of probiotic administration, including immune system and allergy benefits, are yet to be firmly established, but preliminary evidence has shown more promise in the primary prevention of disease, rather than the treatment of established disease.[3]

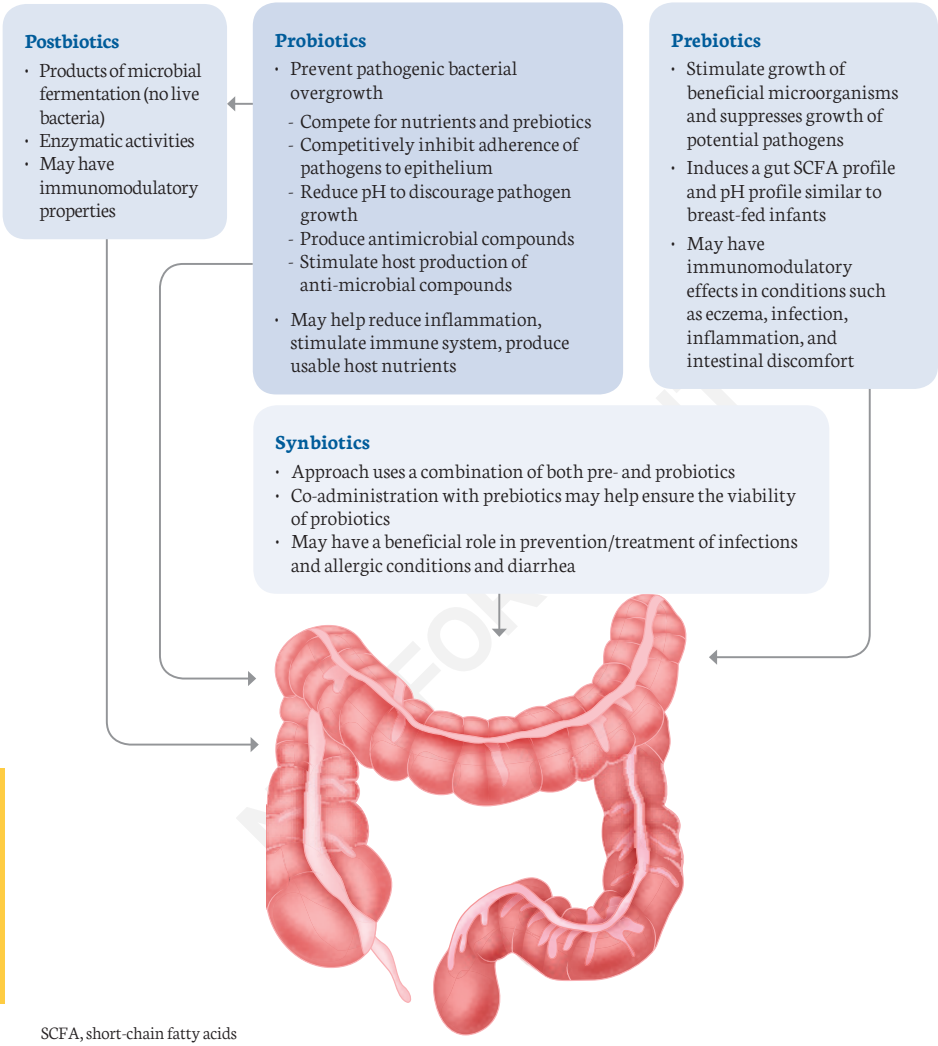
3. Synbiotics

The synbiotic approach involves employing a combination of both prebiotics and probiotics.[16] It has been suggested that this approach may help to ensure the viability of the probiotic bacteria and encourage their colonization and growth.[1] Several studies have shown a beneficial role of synbiotics in the prevention and/or treatment of infections and febrile illnesses, allergic conditions such as atopic dermatitis and asthma, diarrhea, and iron deficiency in infants and toddlers.[1,42]

4. Postbiotics (active ferments)

Postbiotics (active ferments) are products made by or involving beneficial microorganisms, such as products of fermentation, but containing no live bacteria.[1,16] The postbiotic approach is also gaining interest as a way to beneficially modify the composition of the gut microbiota in infants,[16] as these compounds are thought to exhibit immunomodulatory properties.[1]

As stated in the previous pages, there are inconsistencies regarding the clinical benefits of prebiotic, probiotic, and synbiotic supplementation. This can partially be explained by the fact that studies have used varying compositions of these components, at different doses and in different disease states, making it difficult to draw strong conclusions.[14] In addition, individual responses are likely to differ, given that every individual has a unique microbiota, which is influenced by a multitude of genetic and environmental factors. Efforts are continuing to determine which probiotics and prebiotics, and what combinations of the two, are most beneficial in keeping mothers and children healthy, and in preventing and treating various disease states in infants and adults.



SCFA, short-chain fatty acids

Figure 13. Proposed mechanisms of action of prebiotics, probiotics, synbiotics, and postbiotics in the infant[1,3,4,13,14,16,37,42,43]

In conclusion, ESPGHAN have stated that probiotic and prebiotic supplementation in infants positively modulates the gut microbiota and appears to be safe.[38] ESPGHAN has also called for more studies to support the routine use of probiotic- and/or prebiotic infant formula. Nonetheless, the World Allergy Organization has determined that there is a likely net benefit from using probiotics in infancy, particularly with regard to the prevention of eczema, and suggests the use of probiotics in pregnant women at high risk of giving birth to an allergic child, or who are breastfeeding infants at high risk of allergy.[44]

Chapter highlights

1. Human milk contains a wide variety of different compounds, including carbohydrates (e.g. lactose, prebiotic oligosaccharides [HMOS]), fatty acids (including long-chain polyunsaturated fatty acids), nucleotides, proteins (e.g. antibodies, cytokines, lactoferrin), microbes, macrophages, and stem cells.
2. Human milk contains at least 200 different characterized HMOS; however, more than 1,000 structures could be estimated based on recent analytical methods. These HMOS can promote the growth and proliferation of commensal bacteria in the infant gut, particularly *Bifidobacteria*, while helping to prevent the growth and proliferation of pathogenic bacteria.
3. Human milk also contains bacteria from various genera, including *Lactobacillus* and *Bifidobacterium*; these bacteria appear to play a role in colonizing the gut of a newborn infant.
4. It is believed that microorganisms reach the human milk both through contact with the infant's oral microbiota during suckling, and from the maternal gut via systemic routes.
5. Prebiotics are indigestible food compounds, primarily OS that can stimulate the growth and proliferation of beneficial bacteria in the gut.
6. Probiotics are live microorganisms known to be present in a healthy gut, which, when administered in adequate amounts, can help colonize the gut and exert beneficial biological effects. Probiotics comprise the kind of beneficial bacteria known to be present in a healthy gut, especially *Bifidobacterium* and *Lactobacillus*.

7. Synbiotics are combinations of probiotics and prebiotics.
8. By adding beneficial bacteria and/or promoting their growth, prebiotics, probiotics, and synbiotics can help to modulate the gut microbiota in infants.
9. Some evidence suggests prebiotics, probiotics and synbiotics may help to improve gut health, reduce digestive discomfort, and help prevent the development of infection and allergy.

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Chapter 5

Overview and future directions

Summary

As discussed in this book, healthy development of the gut and optimal gut function is highly important for infant health, overall growth and development, and also appears to be a key factor in long-term health. Increasing evidence indicates that optimal composition and function of the gut microbiota is a particularly important aspect of gut health, due to its roles in nutrient digestion, defence against pathogens, development of the immune system, homeostasis, psychological health, and general wellbeing.

Our rapidly increasing understanding of the role of the gut microbiota in health and disease provides a rational therapeutic target in both infants and adults. Deliberate modulation of the composition of the gut microbiota using prebiotics, probiotics and synbiotics has been shown to facilitate a healthier microbiota composition, and a growing number of studies are showing an association between positive gut microbiota modulation and the prevention and treatment of a variety of disorders, including allergy, infections, and functional gastrointestinal disorders.[1,2]

Future research directions

Further research is improving our understanding of what specifically constitutes a healthy, stable, and diverse gut microbiota, what specific changes are induced by environmental factors, and how these changes influence the functionality of the microbiota and host-microbe cross-talk to impact health and disease. Large-scale, long-term longitudinal studies are needed to shed further light on these important issues.[3]

Clinical studies face several challenges, including the inter-individual and inter-country variability of the gut microbiota composition, and the fact that, while fecal sampling is a relatively easy method of analyzing the microbiota composition, this method may not actually reflect changes within the gut.[4] Future research will focus on different methods to sample from the gut or to link fecal composition to the actual gut composition.[5]

A number of questions remain unanswered and require further research:

1. Sources of essential gut microbes and the importance of temporal windows of opportunity for colonization.

2. Biological markers (biomarkers): As with most fields of medicine, research into biomarkers is being undertaken in the area of gut microbial colonization and disease.[3] Biomarkers allow researchers to monitor physiological states and select specific patients or individuals for certain interventions, or preventative approaches, based on the presence or absence of these markers. Further research into microbial community compositions, individual microbiota 'signatures', and specific microbe-microbe interactions may allow these to be used as biomarkers. Metabolites of microbial activity may also prove useful. Genetic profiling of organisms may also yield important information which could be used as markers in future.[3]

3. Further research is required into dysbiosis and the mechanisms of disease susceptibility; does acquiring unfavorable microbes

lead to disease, or does a loss of favorable commensal microbes facilitate colonization of unfavorable microbes? When dysbiosis occurs through disease, antibiotic use, or other events, can a healthy gut microbiota be restored?

4. Whereas high microbial diversity has been associated with disease protection in adulthood, its relevance in early life is controversial since the microbial diversity in breast-fed infants is low. Future studies should address how the microbial diversity evolves over time and when is the precise timing at which low diversity represents a health risk.

5. New findings point to the gut microbiome as a causal factor in kwashiorkor (protein deficiency in the young).[6] However, researchers also need to investigate the role of the gut microbiota in any other malnutritional status and its influence on specific nutritional deficiencies.

6. Up to now only associations can be made between specific microbial signatures and health status, such as obesity, allergy or mood disorders, etc. Cause-effect relationship would need to be further established.

7. Future research is needed to better understand the role of the gut microbiota during pregnancy and on pregnancy outcome. Additional studies will also be required to determine the exact mechanisms by which microbes colonize the gut from various different sources.[7] For example, researchers are still trying to establish the process by which microbes from a mother's gut

microbiota find their way into her breast milk.[8] Such studies will also help to reveal how microbes communicate with the immune system and the central nervous system,[9] with the role played by microbial metabolites seeming to offer a particularly promising line of enquiry.[10]

8. Another largely untapped aspect of gut microbiota research involves evaluating other components such as fungi and viruses.[11] Recent research has showed that certain eukaryotic viruses in the gut can also play a role in promoting health and fighting infection.[12] Finally, researchers are continuing to investigate the potential for modulating the gut microbiota with prebiotics, probiotics and synbiotics. The search continues for new probiotic candidates and mixtures that can be added to the infant diet to promote both short- and long-term health.

9. ESPGHAN suggests that there is a need for further studies to define optimal doses and intake durations of pre- and probiotic-supplemented infant formula, as well as their long-term safety.[13]

Medical advances will enhance our understanding of gut health in early life and ultimately promote gut health and general wellbeing during the critical years of development and beyond.

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