Nestlé Nutrition Institute Workshop Series 101

Nutrition, Microbiome and Health: Latest Findings and Future Research

Key Summaries and Highlights

Nutrition, Microbiome and Health: Latest Findings and Future Research

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Foreword

The NNI and WNSC 101 Workshop, titled "Nutrition, microbiome and health: latest findings and future research," explored the captivating realm of the human microbiome – the vast community of microorganisms that reside within us, exerting influence on our bodily functions and behaviors. From our dietary choices to our cognitive processes and even our vulnerability to diseases, the microbiome has a profound impact on human physiology.

The workshop commenced with an exploration of the ecological patterns of the gut microbiome in early life, shedding light on the role of parental microbiome programming. We then delved into the process of microbiome maturation, identifying nutritional factors that shape it and their connections to overall health outcomes. Emphasis was placed on the significant positive role that breastmilk plays in this intricate relationship. We also cover the notion of pioneer taxa as important predecessors of the overall ecological ecosystem. Additionally, the workshop gave us a better understanding of the microbiome of the premature infant and how disruptions in the gut microbiota can lead to increased risk of allergies and other diseases.

One of the key highlights was the focus on microbiome-targeted dietary regimens for combating pediatric malnutrition. Compelling evidence is presented on the effectiveness of microbiota-directed complementary foods in positively influencing growth outcomes, offering a promising strategy to address malnutrition in children.

The workshop delved into the intricate connections between the gut and different organ systems, shedding light on the gut–brain and gut–bone axis, and offering valuable insights into the potential of gut modulation strategies, including the use of biotics, to enhance gut, brain, and bone health.

Throughout the sessions, strategies such as optimal maternal nutrition, breastfeeding, and appropriate complementary feeding are emphasized as key interventions for modulating the gut microbiome and preventing disease conditions.

Looking to the future, the emerging science on infant gut virome, the integration of artificial intelligence in microbiome research, and the potential for microbiome-based therapies to manage non-communicable diseases were debated.

We extend our deepest gratitude to our esteemed chairpersons, Prof. Hania Szajewska and Prof. Flavia Indrio, and to all the speakers and participants whose contributions made this event a success. We hope the insights gained here will inspire new approaches and strategies in your practice, ultimately enhancing the health and well-being of children worldwide.

Sara Colombo Mottaz, Pharm. Ph.D.
Head of the Nestlé Nutrition Institute
Vevey, Switzerland

Maternal gut microbiome: long-term effect of the short-term "programming"

Preconception Phase

Imbalance can lead to reproductive disorders:
• Polycystic Ovary Syndrome (PCOS)
• Endometriosis

Pregnancy

Increased estrogen and progesterone cause changes to gut environment

Immune System

Hormones

Gut Microbiome

Metabolism

Gut Physiology

Impact on Future Health

Children born to mothers with imbalanced microbiota may be at risk of:
• Allergies
• Obesity
• Neurodevelopmental disorders

Pregnancy

• Gut microbiota modulates immune responses & inflammation, preventing:
  • Preterm birth
  • Gestational diabetes
• Impacts risk of immune disorders in life of the child

Intergenerational transmission of metabolic phenotypes could contribute to the development of:

Insulin resistance   Obesity   Cardiovascular disease

Mounting evidence suggests that the composition of the intestinal microbiota during preconception and pregnancy has lasting effects on the health and development of offspring.

Maternal gut microbiome
Long-term effect of the short-term "programming"

Francesco De Seta
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The human body is host to trillions of microorganisms, collectively known as the microbiota, which inhabit various niches, including the gut. Among these, the intestinal microbiota plays a pivotal role in modulating host physiology and immune responses. During preconception and pregnancy, shifts in the composition and function of the intestinal microbiota have profound implications not only for maternal health but also for the long-term health outcomes of both mother and child.

Preconception phase
The preconception period sets the stage for a healthy pregnancy, and emerging evidence suggests that the maternal intestinal microbiota may influence fertility and conception success. Dysbiosis, or an imbalance in the intestinal microbial community, has been associated with reproductive disorders such as polycystic ovary syndrome (PCOS) and endometriosis, highlighting the importance of a diverse and balanced microbiota prior to conception. Several factors influence the composition of the intestinal microbiota during the preconception phase, including diet, lifestyle, and environmental exposures. Dietary patterns rich in fiber, fruits, and vegetables promote the growth of beneficial bacteria, whereas high-fat diets and exposure to environmental toxins may disrupt microbial homeostasis.

The Developmental Origins of Health and Disease

Undernutrition → Developmental programming of organ systems → Hypertension Cardiovascular disorders; Allergies Asthma; Obesity Metabolic disorders; Cognitive impairment Schizophrenia; Impaired immunity

Obesity
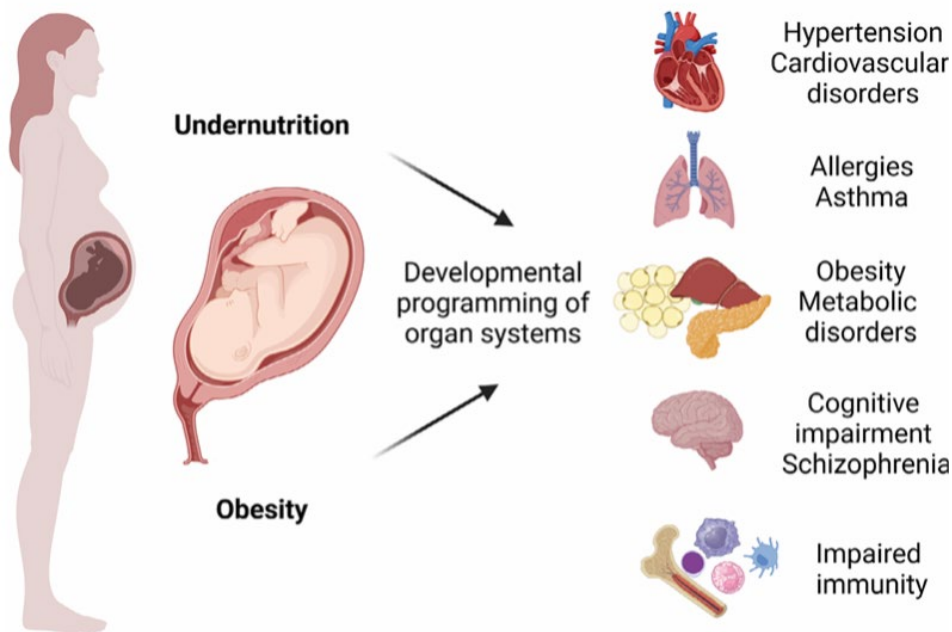
Figure 1. From Barrientos G, et al. Nutrition during pregnancy: Influence on the gut microbiome and fetal development, Am J Reprod Immunol, 2024. doi.org/10.1111/aji.13802, CC BY-NC 4.0

Pregnancy

Pregnancy is a period of dynamic physiological changes, characterized by alterations in hormone levels, immune responses, and metabolic processes. These changes extend to the intestinal microbiota, which undergoes significant remodeling throughout gestation. Early in pregnancy, hormonal shifts, particularly increased levels of estrogen and progesterone, promote changes in the gut environment, favoring the expansion of certain bacterial taxa. Additionally, alterations in dietary preferences and nutrient absorption during pregnancy further shape the composition of the intestinal microbiota. The maternal gut microbiota plays a crucial role in modulating maternal immune responses and inflammation, which are essential for maintaining pregnancy and preventing adverse pregnancy outcomes such as preterm birth and gestational diabetes. Moreover, the maternal microbiota influences the development of the fetal immune system, impacting the risk of immune-mediated disorders later in life.

Impact on future health

Mounting evidence suggests that the composition of the intestinal microbiota during preconception and pregnancy has lasting effects on the health and development of offspring. The establishment of the infant gut microbiota begins during birth and is influenced by maternal microbial transmission, mode of delivery, breastfeeding, and early environmental exposures. Children born to mothers with dysbiotic gut microbiota may be at increased risk of developing a wide range of health conditions, including allergic diseases, obesity, and neurodevelopmental disorders. This underscores the importance of optimizing maternal gut health before and during pregnancy to promote the transmission of beneficial microbes to the offspring. Furthermore, emerging research indicates that the maternal gut microbiota may influence the programming of metabolic pathways in the offspring, potentially predisposing them to metabolic disorders later in life. Epigenetic modifications induced by maternal gut-derived metabolites and microbial byproducts through the fermentation of dietary substrates, such as short-chain fatty acids (SCFAs), bile acids, and trimethylamine-N-oxide (TMAO), contribute to the intergenerational transmission of metabolic phenotypes. These metabolites, serve as signaling molecules that can influence epigenetic processes in both maternal and fetal tissues. These epigenetic changes in offspring could persist into adulthood and contribute to the development of metabolic disorders such as obesity, insulin resistance, and cardiovascular disease.

Conclusion

The intestinal microbiota plays a crucial role during preconception and pregnancy, influencing maternal health, pregnancy outcomes, and the long-term health of offspring. Maintaining a diverse and balanced gut microbiota through dietary interventions, lifestyle modifications, and probiotic supplementation may help optimize maternal and fetal health outcomes. Understanding the role of maternal gut-derived metabolites in shaping epigenetic programming during critical periods of development, such as pregnancy, holds great promise for elucidating the mechanisms underlying the intergenerational transmission of metabolic phenotypes. Future research aimed at elucidating the complex interactions between the maternal gut microbiota and offspring health will provide valuable insights into preventive strategies and personalized interventions to mitigate the risk of chronic diseases across generations. By identifying specific dietary interventions or microbial-targeted therapies that modulate these epigenetic processes, it may be possible to mitigate the risk of metabolic diseases across generations and promote healthier outcomes for mothers and their offspring.

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The mother plays an important role in the first 1000 days of an infant's life

Mothers support infant development through breastfeeding, as the nutritional composition of breast milk fluctuates and adapts to a baby's individual needs

Breastfeeding provides benefits for the mother and for the infant

Reduced risk of ovarian cancer

Reduced risk of type 2 diabetes

Reduced risk of breast cancer

Benefits for mother

Reduce autoimmune diseases

Reduce allergic diseases

Benefits for child

Reduce infections

Reduce obesity

Improve cognitive development
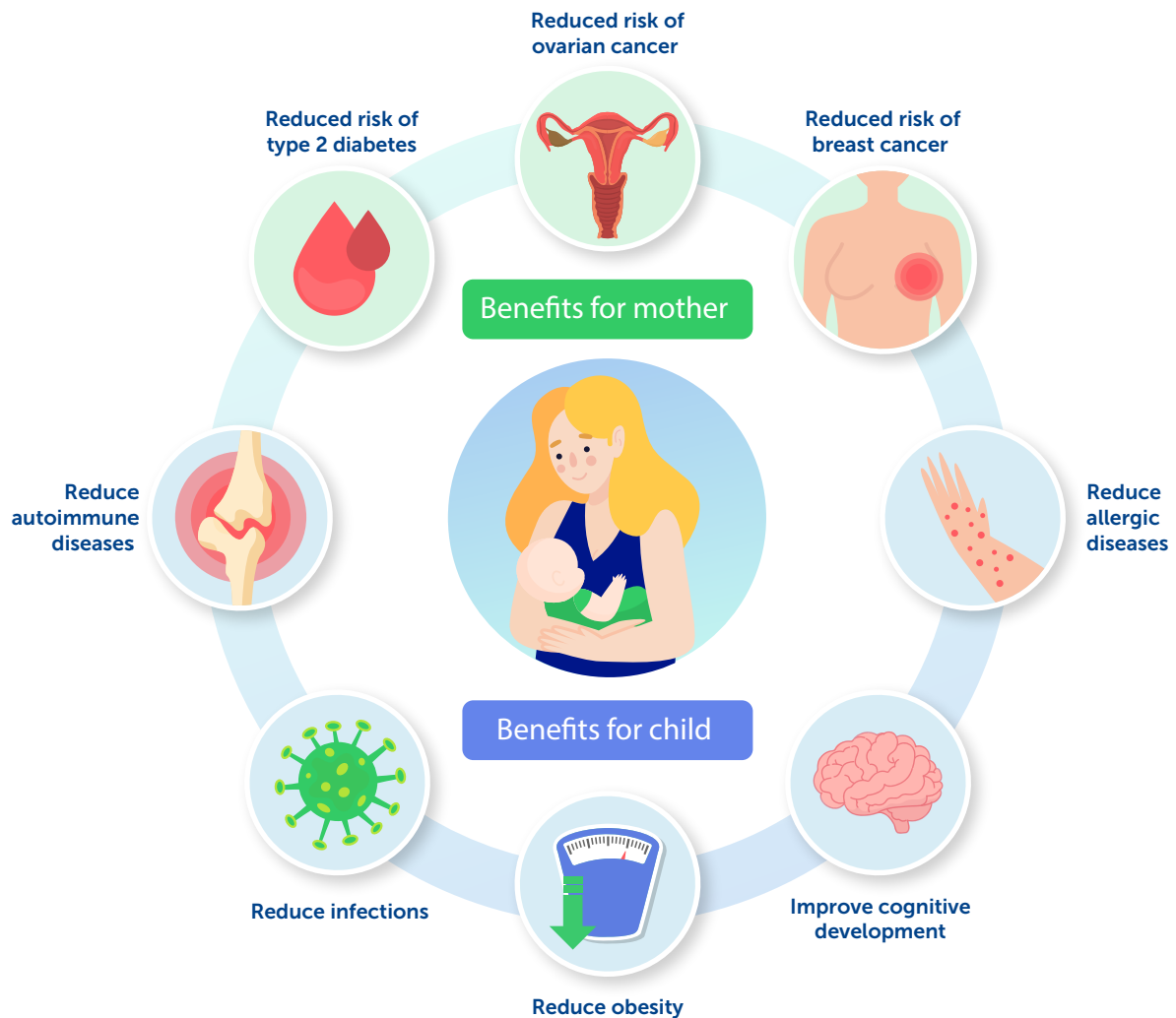
Human milk oligosaccharides (HMOs) and human milk microbiota (containing bacteria, viruses, fungi, and archaea) play an important role in bestowing protective benefits against:

- Infections
- Allergies
- Autoimmune conditions
- Risk of antibiotic resistance

Milk microbiota

HMOs

Gaining a deeper understanding of the microbial ecosystems shaped by breastfeeding could significantly enhance efforts to promote breastfeeding to the broader public.

Breastfeeding and health benefits for the mother–infant dyad
A perspective on human milk microbiota

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Professor in Pediatrics, Eskisehir Osmangazi University, Turkey

Human milk is a distinctive nourishment that possesses a distinct and ever-changing composition tailored to each individual baby, including diverse nutritional and bioactive components. It is believed that the presence of bioactive compounds significantly influences the health benefits of human milk for both infants and mothers.

Research has demonstrated the short- and long-term benefits of breastfeeding for both mother–infant dyads, and the impact of widespread breastfeeding on community sustainability and wealth. Breastfeeding has been shown to be protective against or reduce infections, allergies, obesity, allergic diseases, autoimmune diseases, inflammatory bowel diseases, and cancer in infants and children, resulting in reduced childhood morbidity and mortality. It has been shown that breastfed babies have higher IQs and better cognitive development. In addition to the benefits for the baby, breastfeeding mothers have been shown to have a reduced risk of developing type 2 diabetes, ovarian cancer, and breast cancer. Antibiotic resistance is a global threat, and breastfeeding has been shown to have protective effects on antibiotic use and antibiotic resistance. Research demonstrates that the mother plays a crucial role in the development of an infant's microbiota during the early stages of life, particularly the first 1,000 days, and breastfeeding is the most significant factor associated with mothers.

Human milk oligosaccharides (HMOs) and human milk microbiota composition play an important role in the positive effects of breastfeeding on mother and infant. Studies on the microbiota composition of human milk in the last 20 years have shown that human milk microbiota contains bacteria, viruses, fungi, and archaea, which are specific to mother–baby dyad.

Providing a comprehensive analysis of the microbiota composition present in human milk, trans-kingdom interactions, metabolic products, and metabolic pathways would help enhance our understanding of the advantages of breastfeeding. It would be beneficial to show evidence of the advantages of breastfeeding for both the mother-infant dyad and to communicate the significance of microbiota and breastfeeding to the entire community.

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The early gut microbiome plays a critical role in development of the immune system
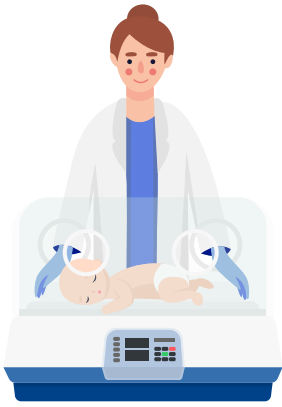
Preterm infant

Preterm infants are less likely to be vaginally delivered or receive adequate volumes of maternal milk. Coupled with a prolonged hospital stay this means they have abnormal gut microbiome with a lack of beneficial bacteria

Healthy full-term infant

Mother's own breastmilk is the most protective factor against many pretem diseases, likely relating to the rich bioactive compounds such as human milk oligosaccharides, which support the growth of beneficial bacteria like *Bifidobacterium*

Preterm infants are a vulnerable population. They have an immature intestinal architecture and an underdeveloped immune system
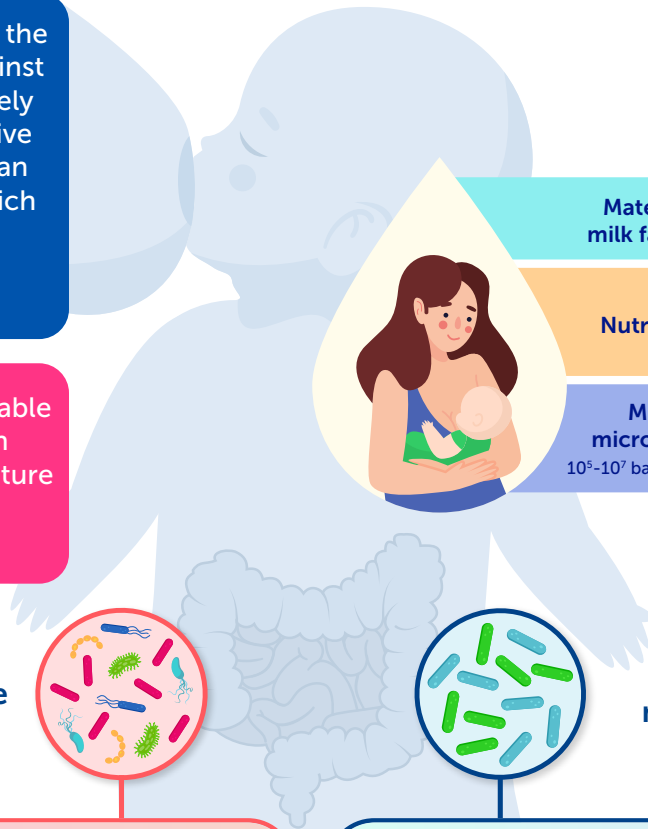
Maternal milk factors	Immunoglobulins (IgA) Antimicrobial factors HMOs
Nutrients	Vitamins and minerals Protein and lipids Carbohydrates Water
Milk microbiota 10^5-10^7 bacteria/day	*Bifidobacterium* *Lactobacillus* *Streptococcus* *Staphylococcus*

Short-and long-term consequences of early life microbial dysbiosis

- Neurological disorders
- Metabolic disorders
- Intestinal diseases
- Broncho-pulmonary diseases
- Cardiovascular diseases
- Infectious diseases
- Allergies

Roles of the gut microbiota in infancy

- Food digestion
- Maintenance of gut epithelial integrity
- Preventing pathogen colonisation
- Educating the immune system
- Drug metabolism
- Production of vitamins and amino acids
- Physiological development

More research is needed to predict, diagnose, and manipulate the microbiome to reduce disease risk, moving towards personalized approaches

Understanding the interaction of the diet, microbiome and host in preterm infants is crucial for understanding disease mechanisms and developing new biomarkers and therapies

Diet–microbiome–host interaction in preterm infant health

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Neonates are rapidly colonized by microbes following birth. The gut contains the largest density of microorganisms, termed the gut microbiome, which plays fundamental roles in protection from pathogens, immune system training, and the breakdown of dietary compounds. The infant gut microbiome is highly dynamic over the first year of life, providing a window of opportunity in which to seed a potentially beneficial microbiome to reduce the risk of early- and later-life disease risk. In term infants, birth mode and breastfeeding are the most important variables for shaping the early life microbiome[1], which are directly correlated to an increased risk of obesity, allergy, asthma, and other disorders later in life. Unlike infants born at term, extremely preterm infants (<32 weeks gestation) have immature intestinal architecture and an underdeveloped immune system. They are also less likely to be vaginally delivered and breastfed, and they receive limited exposure to microbes during the first months of life, leading to a reduction in potentially beneficial bacteria (Figure 1)[2]. Because the preterm gut can become leaky, translocation of microbes into the bloodstream and/or intestinal cell death represent major problems in this vulnerable population. However, evidence has shown that certain types of bacteria, such as *Bifidobacterium*, may increase gut and immune maturation[3]. Mothers' own breastmilk (MOM) is the most protective factor against many preterm diseases, likely relating to the rich bioactive composition, including an abundance of human milk oligosaccharides (HMOs). HMOs are abundant in human milk and specific HMOs are lacking in MOM of infants who go on to develop disease[4,5]. Disentangling the role of diet–microbe–host interaction in preterm infants will be critical for understanding disease mechanisms and developing novel biomarkers and therapies.

Research to date has largely utilized sequencing of clinical samples to derive associations between controls and diseased subjects. While such associations provide insights, it is not possible to determine cause or effect. Better understanding of the interaction between bacteria and infant gut epithelial cells holds incredibly exciting possibilities to better predict, diagnose, and manipulate the microbiome of preterm infants at risk of disease. To this end, many studies have been performed using animal models, such as mice (germ free and colonized) and pigs. However, the microbiome varies significantly between different animal species and the anatomical differences between animal models and neonatal gut hinder translation of findings into humans. Organoid technologies are emerging as a robust and relevant model to systematically explore host responses to bacterial colonization in the human gut, allowing comprehensive mechanistic investigation of host–microbiome interaction.

Over the past decade, our understanding of the preterm infant gut microbiome has advanced immensely, but there is still much to learn. Research needs to move beyond considering only a single component of a complex system, moving toward personalized approaches to modulate the gut microbiome. Overall, understanding diet–microbe–host interaction holds incredibly exciting possibilities to better predict, diagnose, and manipulate the microbiome in neonates to reduce disease risk.
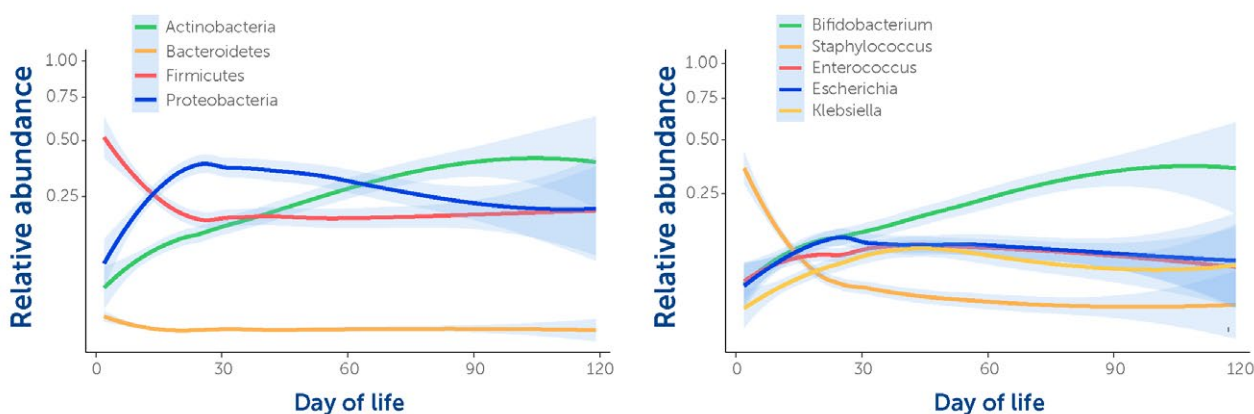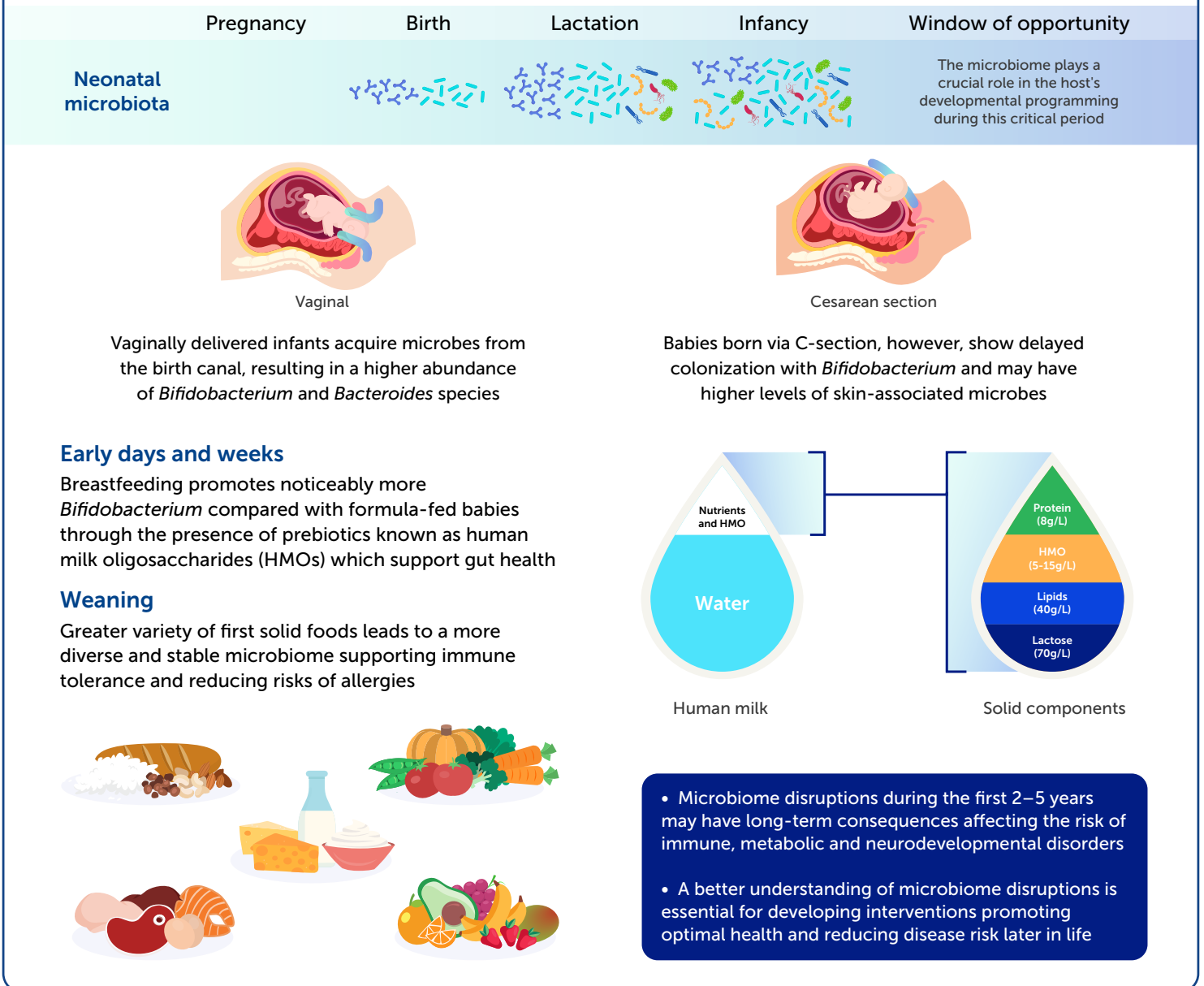

Figure 1. From Beck LC, et al. Strain-specific impacts of probiotics are a significant driver of gut microbiome development in very preterm infants, Nat Microbiol, 2022. doi.org/10.1038/s41564-022-01213-w, CC BY 4.0

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The trajectory of the microbiome passes through several milestones as it matures:

Pregnancy	Birth	Lactation	Infancy	Window of opportunity

Neonatal microbiota

The microbiome plays a crucial role in the host's developmental programming during this critical period

Vaginal

Vaginally delivered infants acquire microbes from the birth canal, resulting in a higher abundance of *Bifidobacterium* and *Bacteroides* species

Cesarean section

Babies born via C-section, however, show delayed colonization with *Bifidobacterium* and may have higher levels of skin-associated microbes

Early days and weeks
Breastfeeding promotes noticeably more *Bifidobacterium* compared with formula-fed babies through the presence of prebiotics known as human milk oligosaccharides (HMOs) which support gut health

Weaning
Greater variety of first solid foods leads to a more diverse and stable microbiome supporting immune tolerance and reducing risks of allergies

Nutrients and HMO

Water

Human milk

Protein (8g/L)

HMO (5-15g/L)

Lipids (40g/L)

Lactose (70g/L)

Solid components

- Microbiome disruptions during the first 2−5 years may have long-term consequences affecting the risk of immune, metabolic and neurodevelopmental disorders

- A better understanding of microbiome disruptions is essential for developing interventions promoting optimal health and reducing disease risk later in life

Microbiome maturation trajectory and key milestones in early life

Marie-Claire Arrieta
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Microbiome maturation refers to the developmental stages of changes that occur in the microbiome—the community of microorganisms that inhabit various parts of the body, particularly the gut—during early life[1]. Far from restricting its biological activities to the microbial ecosystem alone, the gut microbiome engages in active crosstalk with its host, impacting its developmental programming during a non-redundant period in early development, known as a 'window of opportunity'. Discerning between homeostatic (eubiotic) vs. altered (dysbiotic) patterns of microbiome establishment and characterizing the exposures that guide these trajectories is essential for our understanding of human development.

Integrative microbiome maturation studies across different infant populations have identified common patterns of microbiome establishment, indicating that this process is biologically determined[2]. This process is characterized by (i) the early arrival of pioneer keystone species (*Bacteroides* spp. and *Bifidobacterium* spp.) that exert influential effects on both microbiome and host, (ii) an increase in the number of species comprising the community (alpha-diversity), and (iii) a period of reduced ecological resilience that renders the early-life microbiome less capable of reverting to its original state after a perturbation (i.e. antibiotics) occurs[3]. Several key milestones defining the trajectory of microbiome maturation have been described.
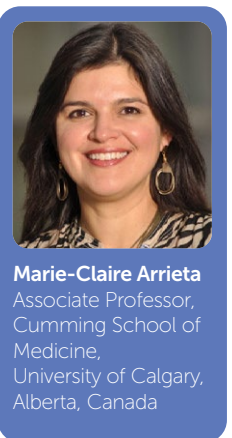
Birth

The process of birth plays a significant role in the initial colonization of the infant's microbiome. Infants born vaginally tend to acquire microbes abundant in maternal stool and the birth canal, while babies born via C-section initially harbor microbes from the surrounding environment, such as the skin[4]. Early pioneer species can have long-lasting consequences on the trajectory of the infant gut microbiome through an ecological mechanism known as priority effects. For instance, C-sections result in a lower abundance of *Bacteroides* spp. and a delayed colonization with *Bifidobacterium* spp. The effect of the mode of birth on microbiome composition can last for months after birth and is likely to impact host–microbiome communication during this critical developmental stage[4].

Early days and weeks

Initially, the infant gut microbiome is relatively simple, dominated by facultative anaerobes such as Enterobacteriaceae. Breastfeeding in this period can have a significant impact, as breast milk contains prebiotics known as human milk oligosaccharides (HMOs) that support the growth of bifidobacteria. Breastmilk contains more than 10g/l of HMOs, which are digested by bifidobacteria and *Bacteroides* spp. into metabolites that sustain the growth of several other microbial species and influence immune development[5]. Breastmilk also influences the composition of the infant microbiome through immune factors, such as antimicrobial compounds (lactoferrin and lysozyme), as well as immune effectors (sIgA, immune cells, and cytokines), which are critical for the immune exclusion of pathogenic microbes[3]. Notably, the lower abundance of *Bifidobacterium* in formula-fed babies is associated with a lower concentration of lactate, sIgA, and a higher gut luminal pH compared to breastfed babies.

Weaning

As the infant starts to transition from breast milk or formula to solid foods, typically around six months of age, the diversity of the microbiome begins to increase. The introduction of solid foods exposes the infant to a wider range of nutrients and microbes, favoring the growth of taxa better adapted to metabolize new substrates, such as *Bacteroidaceae, Lachnospiraceae,* and *Ruminococcaceae*. The diversity and types of first foods play a pivotal role in determining the composition and functionality of the infant's gut microbiota, with the intake of diverse solid foods promoting more diverse and stable microbiota[6]. The bloom in microbial diversity triggered by the introduction of solid foods results in a robust immune response, known as the "weaning reaction". This time- and microbiome-dependent physiological response is critical for the development of immune tolerance that prevents dysregulated inflammatory processes, such as colitis and allergies[7].

Diversification and stability

Throughout the first 2–5 years of life, the gut microbiome continues to diversify, reaching a relatively stable state that resembles the adult microbiome during preschool years. Besides the abovementioned exposures, other factors, such as maternal smoking, familial history of asthma and obesity, rural vs. urban upbringing, stress, exposure to pets, and interactions with caregivers and siblings, can influence the early-life microbiome. In general, the individual effects of factors such as birth mode, antibiotic use, and breastfeeding are relatively well characterized. However, the combinatory effects of these exposures remain poorly understood.

It is now well established that microbiome disruptions during this critical period of life may have long-term consequences that can increase the risk of immune, metabolic, and neurodevelopmental disorders. Thus, understanding the implications of alterations in the early-life microbiome is essential for developing interventions to promote optimal health and reduce the risk of disease later in life. Research in this area continues to advance our understanding of the complex interactions between the microbiome, host physiology, and health outcomes.

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Childhood malnutrition is responsible for an estimated 45% of childhood mortality worldwide
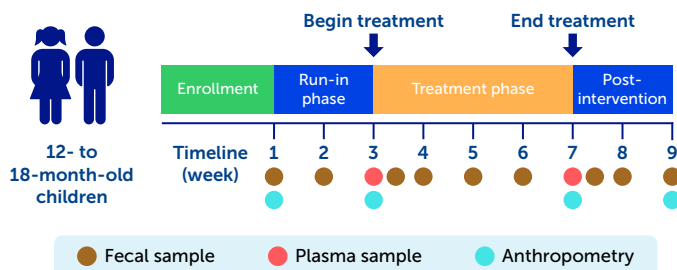
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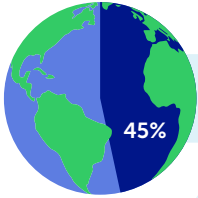
Undernutrition is associated with gut microbiota "immaturity"

One approach to address undernutrition is gut microbiota-directed complementary foods (MDCF) – specialized dietary formulations designed to promote gut health and microbial diversity in children, supporting weight gain, improved immune function and overall health
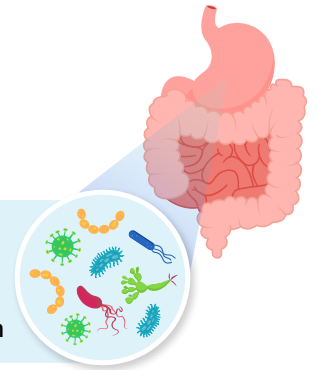
A randomized controlled feeding study conducted in undernourished Bangladeshi children

One MDCF was developed (MDCF-2) and tested aginst a control ready-to-use supplementary food (RUSF) in 12- to 18-month-old children

Begin treatment **End treatment**

| Enrollment | Run-in phase | Treatment phase | Post-intervention |

12- to 18-month-old children

Timeline (week) 1 2 3 4 5 6 7 8 9

● Fecal sample ● Plasma sample ● Anthropometry

Compared to the RUSF, MDCF-2 significantly increased weight gain, abundances of growth-associated gut bacterial strains, and improved biomarkers of growth, bone formation, neurodevelopment and immune function in children with moderate acute malnutrition

| | Control | Microbiota-directed | | |
Ingredient (g/100g)	RUSF	MDCF-1	MDCF-2	MDCF-3
Chickpea flour	-	8	10	30
Peanut flour	-	7	10	-
Soy flour	-	5	8	14
Raw banana	-	19	19	-
Rice	18.9	-	-	-
Lentil	21.5	-	-	-
Powdered skimmed milk	10.5	11.5	-	-
Sugar	17	24.3	29.9	30.9
Soybean oil	29	22	20	22
Micronutrient premix	3.14	3.1	3.14	3.1
Protein	10.2	12.4	11.8	13.9
Fat	29.5	22.8	20.8	24.1
Carbohydrate	31.8	18.6	16.3	22
Fiber	4.7	3.3	4.5	5.6
Protein energy ratio (PER)	8.3	11.9	11.61	11.7
Fat energy ratio (FER)	53.6	49.3	46.2	45.7
Total calories per 100g	494.6	416.1	406.8	475.8
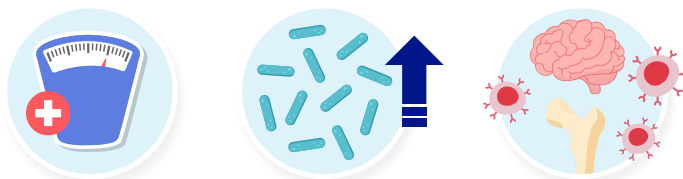
These findings may be used to support the development of therapeutic foods for other health conditions associated with altered microbial patterns

Microbiome-targeted dietary regimens to combat pediatric malnutrition

Childhood undernutrition remains a serious global health problem, especially in low- and middle-income countries, and is responsible for an estimated 45% of childhood mortality worldwide[1]. In 2022, an estimated 148 million children under the age of five globally were stunted (i.e., short for their chronological age)[2], while 45 million children under 5 were wasted (i.e., underweight for their height). At the other end of the nutrition scale, the ready availability of nutrient-poor ultra-processed foods has contributed to 37 million children under 5 being designated as overweight. Survivors of malnutrition exhibit stunted growth, impaired cognitive development and vaccine responses, and metabolic abnormalities.

A burgeoning body of evidence indicates that undernutrition is not caused solely by food insecurity and is influenced by a range of environmental factors such as maternal nutritional status, poor sanitation, and

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infection by pathogens[3]. Because the gut microbiota impacts host physiology via the harvesting of key nutrients from diet, priming innate and adaptive immune responses, shoring up epithelial barrier integrity, and providing colonization resistance to enteropathogens, it has emerged as a compelling target for treating undernutrition[4]. Studies that characterize the developmental biology of the gut microbiota in infants and young children have revealed that healthy growth is associated with linear growth, and that disruption of this developmental program is causally linked to the pathogenesis of undernutrition (termed "microbiota immaturity"). These studies have also shown that treatment of current therapeutic foods to undernourished Bangladeshi children only transiently rescues this dysbiosis, likely because they were not designed with a consideration of the developmental biology of the gut microbial community[5,6]. Thus, we hypothesized that treatment of undernourished children with microbiota-directed complementary foods (MDCFs) to repair their gut microbiota immaturity would improve health-related outcomes such as improved gut barrier function, immune function, metabolism, and linear growth.

Locally available, culturally accepted food ingredients were tested in gnotobiotic mice for their ability to promote the absolute abundances of growth-associated gut bacterial strains that are underrepresented in the microbiota of children with moderate and severe acute malnutrition (MAM and SAM, respectively)[6]. Lead food ingredients from these screens (peanut, chickpea flour, soybean flour and banana) were assembled into MDCF prototypes that selectively increased the abundances of targeted taxa and improved biomarkers of growth in gnotobiotic mice and gnotobiotic piglets[6]. These prototypes were then advanced to a randomized, double-blind study to test their effects in Bangladeshi children with MAM[6]. MDCF-2, which contained all four lead ingredients and lacked milk powder significantly increased weight gain, abundances of growth-associated gut bacterial strains, and improved biomarkers of growth, bone formation, neurodevelopment and immune function in children with MAM, compared to a ready-to-use supplementary food (RUSF)[6,7]. Following this work, a trial with greater statistical power and longer treatment was performed on 120 Bangladeshi children with MAM that were fed either MDCF-2 or RUSF over three months. MDCF-2 produced a rate of weight gain that was significantly greater than that observed with RUSF, even though the calorific density of MDCF-2 is 15% lower than that of RUSF[8]. Two growth-associated *Prevotella copri* metagenomic-associated genomes are key utilizers of MDCF-2 glycans[9,10].

Our work has also examined how MDCFs, via microbial metabolism of constituent nutrients, might induce changes in host signaling that lead to improved growth. These efforts led to the identification of a gut microbial fatty acid amide hydrolase (FAAH) enzyme encoded by a growth-associated *Faecalibacterium prausnitzii* strain that catalyzes the hydrolysis and synthesis of a diverse pool of bioactive compounds such as *N*-acylated conjugates of amino acids, dopamine, and GABA that could potentially be manipulated for more precise and effective therapies[11]. We observed that administration of MDCF-2 reduced gut luminal levels of oleoylethanolamide, a lipid mediator that is known to mediate satiety and stimulate fat utilization[11]. Thus, combating the profound loss of appetite exhibited by undernourished children represents a beneficial effect of MDCF-2 treatment[11].
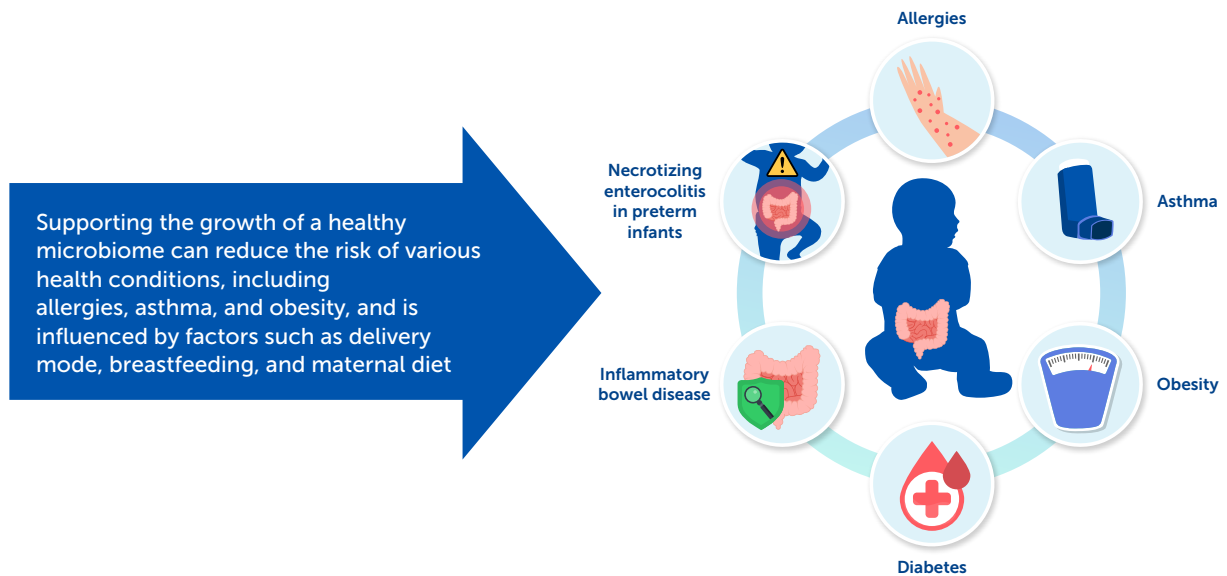
Next-generation formulations of therapeutic foods must also account for the unique nutritional demands of the human brain. The Guatemala study showed that children from villages that received a high-calorie, low-protein nutritional supplement compared to those that received a protein-rich supplement had lower cognitive test results and earnings as adults[12]. Much attention and resources have been directed toward improving nutrition during the critical first 1,000 days of life, and consequently, the development of specific organs and organ systems in late childhood and adolescence have been neglected[13]. Although the human brain reaches approximately 85% of its adult mass by 3 years of age, evidence indicates that brain development extends well beyond the first 1,000 days[14]. Notably, the brain undergoes anatomic remodeling at multiple time scales and levels of organization throughout life[15], with synaptic plasticity, myelination, and pruning having high energetic demands. Meeting these metabolic and nutritional requirements for promoting optimal brain development is of paramount societal importance.
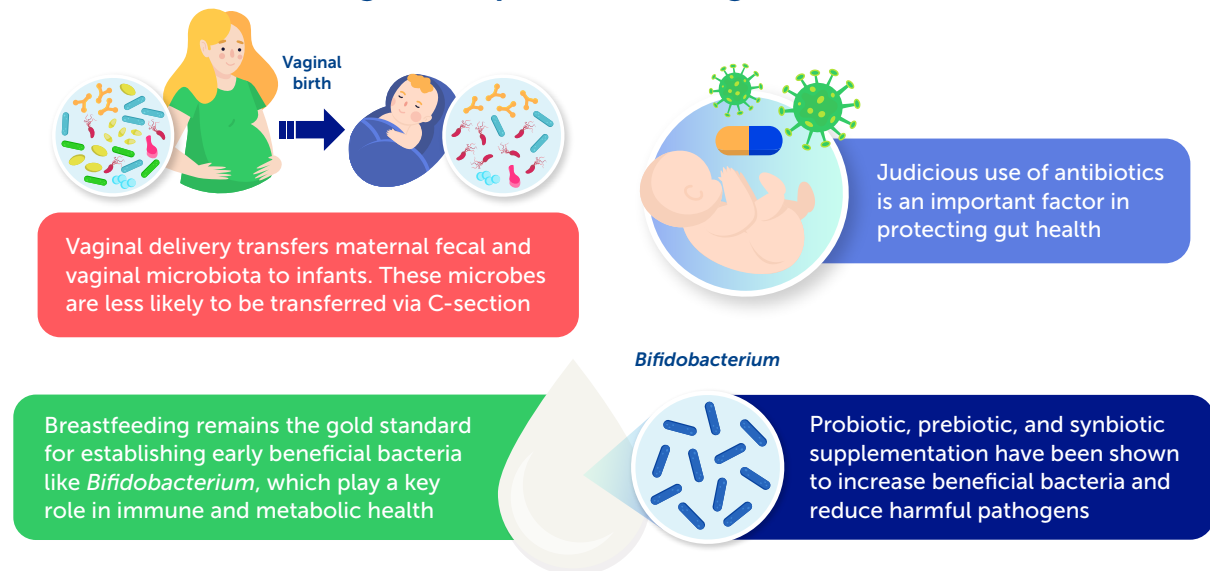
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The gut microbiota developed during early life significantly influences both short-term and long-term health

Supporting the growth of a healthy microbiome can reduce the risk of various health conditions, including allergies, asthma, and obesity, and is influenced by factors such as delivery mode, breastfeeding, and maternal diet

Allergies

Asthma

Obesity

Diabetes

Inflammatory bowel disease

Necrotizing enterocolitis in preterm infants

Strategies to optimize infant gut microbiota

Vaginal birth

Vaginal delivery transfers maternal fecal and vaginal microbiota to infants. These microbes are less likely to be transferred via C-section

Judicious use of antibiotics is an important factor in protecting gut health

Bifidobacterium

Breastfeeding remains the gold standard for establishing early beneficial bacteria like *Bifidobacterium*, which play a key role in immune and metabolic health

Probiotic, prebiotic, and synbiotic supplementation have been shown to increase beneficial bacteria and reduce harmful pathogens

An overview of early-life gut microbiota modulation strategies

Hania Szajewska
Professor and Chair, Department of Paediatrics, Medical University of Warsaw, Poland

The gut microbiota developed during early life significantly influences both short-term and long-term health. In these initial months, various factors (see Figure 1) shape the gut microbiota composition[1]. Disruptions in the microbiota are linked to a higher risk of conditions such as allergies, asthma, obesity, diabetes, inflammatory bowel disease, and necrotizing enterocolitis in preterm infants[2]. Actively modulating the gut microbiota from an early age can enhance long-term health outcomes.

Primum non nocere (first, do no harm)
This principle emphasizes the need for medical practices that preserve the initial development of an infant's microbiota. It highlights the importance of supporting breastfeeding, minimizing unnecessary cesarean sections, and reducing the use of antibiotics during delivery and the newborn period.
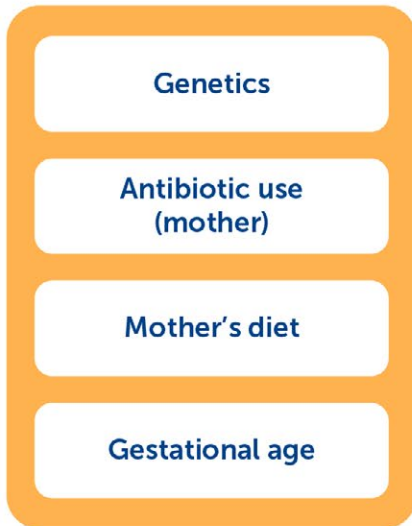
First food matters
Breast milk plays a crucial role as the initial source of nutrition for most infants, promoting a microbiota state dominated by beneficial bacteria like *Bifidobacterium*. As infants start complementary feeding and gradually transition to family foods, the gut microbial diversity increases, introducing adult-associated microbes such as *Lachnospiraceae* and *Ruminococcaceae*. This rise in diversity
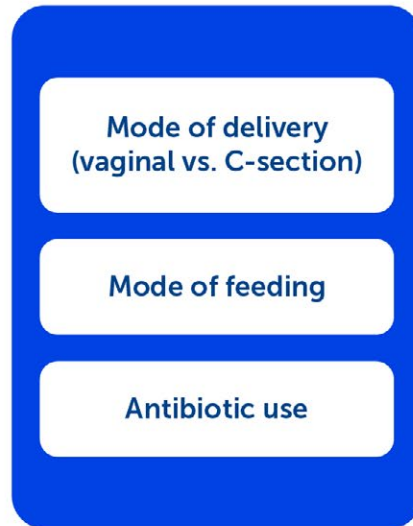
Prenatal factors

- Genetics
- Antibiotic use (mother)
- Mother's diet
- Gestational age

Neonatal factors

- Mode of delivery (vaginal vs. C-section)
- Mode of feeding
- Antibiotic use

Postnatal factors

- Infant's diet
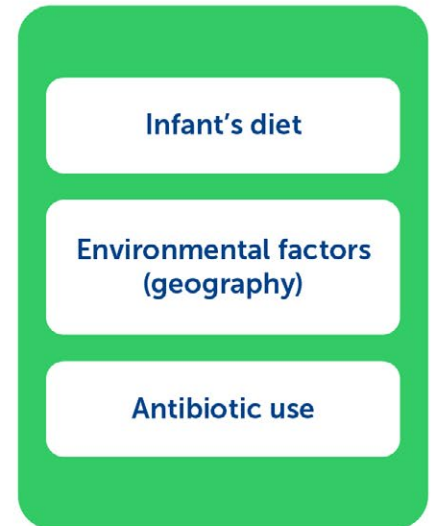- Environmental factors (geography)
- Antibiotic use

Figure 1. Factors shaping infant gut microbiota

across various populations signifies healthy gut microbiota development. A key challenge in this area is determining the optimal timing for reducing breastfeeding and introducing family foods, which is crucial for enhancing gut microbiota, immune maturation, and long-term health outcomes[3].

Biotic-supplemented formulas

Adding biotics such as probiotics, prebiotics, synbiotics, and postbiotics to infant formulas aims to narrow the gap between the outcomes in breastfed and formula-fed infants. A 2024 systematic review[4] of 32 randomized controlled trials published between 2010 and 2021 explored the effects of these biotics on the gut microbiota (the primary outcome) and other health outcomes in exclusively formula-fed infants. The review found that prebiotics generally increased fecal *Bifidobacterium* levels and reduced *C. difficile* levels, though results varied with different types of prebiotics. Probiotic supplementation typically increased fecal *Lactobacillus* but did not consistently affect metabolic outcomes or stool characteristics. Synbiotics usually enhanced fecal *Bifidobacterium* and decreased fecal pH, leading to softer stools and increased stool frequency, but had minimal impact on other outcomes like regurgitation. All interventions were well-tolerated, and no significant adverse events were reported. Currently, the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition is actively updating data on all biotics, including postbiotics and HMO-analogues in infant formulas, focusing on clinically relevant outcomes.

Vaginal or fecal microbiota transplantation in cesarean-born infants

Vaginal microbial transfer and maternal fecal microbiota transplantation involve transferring microbiota from a healthy donor to an infant to support gut microbiome development, particularly in infants delivered via cesarean section. A 2024 systematic review[5] identified six studies that reported short-term benefits, though the long-term impacts are not yet clear due to limited follow-up. There are also safety concerns regarding infection risks from these transplants. Additionally, the variability in methods and regional differences in microbial compositions pose challenges in standardizing these interventions for widespread use. Given the limited evidence and potential infection risks, these interventions require further evaluation before routine application

Conclusions

Hippocrates famously stated, 'All disease begins in the gut.' If this is true, then health also originates in the gut. In this context, adhering to the principle of 'first, do no harm' remains essential. However, there are times when merely avoiding harm is insufficient. In such instances, actively modulating the gut microbiota during early life offers significant potential for disease prevention. As this field evolves, the scientific, ethical, and safety aspects of strategies for modulating gut microbiota must be carefully considered.

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Food allergies affect around 6% of children under the age of three, and 3.5% of adults worldwide

| Crustaceans | Peanuts | Eggs | Wheat | Cow's milk | Soy |

The increase in allergic diseases, such as atopic dermatitis, food, and respiratory allergies, may be associated with disruptions of the gut microbiome in early life

Disruptors of the gut microbiome, leading to increased intestinal permeability ('leaky gut syndrome') and immunological imbalance (Th1/Th2)

C-section

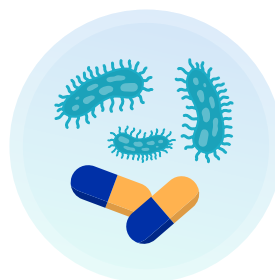Early weaning

Inadequate immunogenic stimuli (the Hygiene Hypothesis)
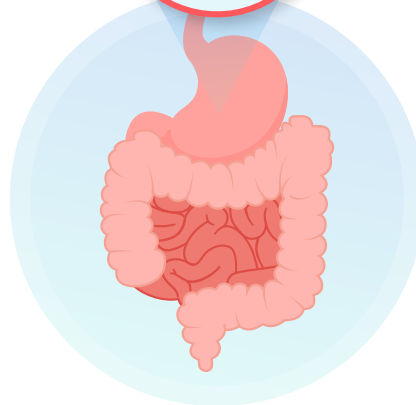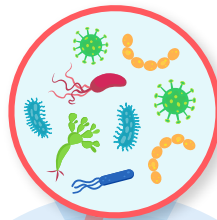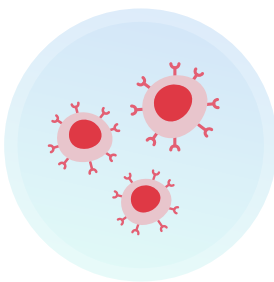
Poor diet

Excessive antibiotic use

Breastfeeding provides essential human milk bioactives, including HMOs, pivotal in shaping the infant's gut microbiota.
- The prebiotic bifidogenic power of HMOs, by promoting growth of beneficial intestinal bacteria thereby generating short-chain fatty acids which are critical for gut health.
- Beyond gut health, HMOs can also be related to immunological tolerance, and potentially in preventing allergies and other non communicable diseases.

Protein

Lipids

Lactose

HMOs
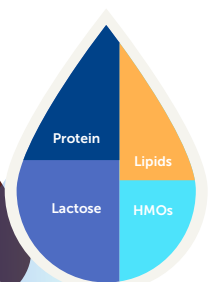
Microbiome at the core
Unlocking mechanisms of food allergy at the non-communicable diseases era

Bruno A Paes Barreto
Assistant Professor, Pará University Centre - CESUPA, Brazil

Food allergies are defined as diseases resulting from an anomalous immune response that occurs after ingestion and/or contact with a specific food. The prevalence of food allergies worldwide presents conflicting and variable data, which seem to depend primarily on the age and characteristics of the evaluated population (culture, eating habits, climate); the involved immunological mechanism; the diagnostic method (self-reported, written questionnaire, skin tests, specific serum IgE, or oral provocation tests); the type of food; and the geographical regions. However, it is generally estimated that the prevalence is approximately 6% in children under three years of age and 3.5% in adults.

In the USA, a population-based study evaluated data on adverse reactions to foods in 27 million patients between 2000 and 2013 and identified a prevalence of intolerance/allergy to at least one food of 3.6%. In childhood, the main foods involved in allergic responses are cow's milk, eggs, wheat, and soy, which are generally temporary. Less than 10% of cases persist into adulthood, where the most identified foods are peanuts, tree nuts, fish, and seafood, especially crustaceans[1,2].

In recent decades, there has been a growing trend in these prevalence data. Understanding why this is the case is not an easy task, as it is a multifactorial situation. However, the revisited "Hygiene Hypothesis," which incorporates elements of the "disappearing microbiota theory," increasingly shows the importance of the eubiosis process in the proper development and maturation of the immune system, activation of regulatory T cells (Treg), culminating in immunotolerance phenomena and prevention of allergic diseases, especially food allergies[2,3]. Thus, factors that induce dysfunctional microbiota in the early stages of life, such as cesarean delivery, early weaning, excessive use of antibiotics, inadequate immunogenic stimuli, and diet type, could be responsible for a whole complex pathophysiological mechanism, which begins with an altered microbiota, leading to increased intestinal permeability ("leaky gut syndrome") and immunological imbalance (Th1/Th2).
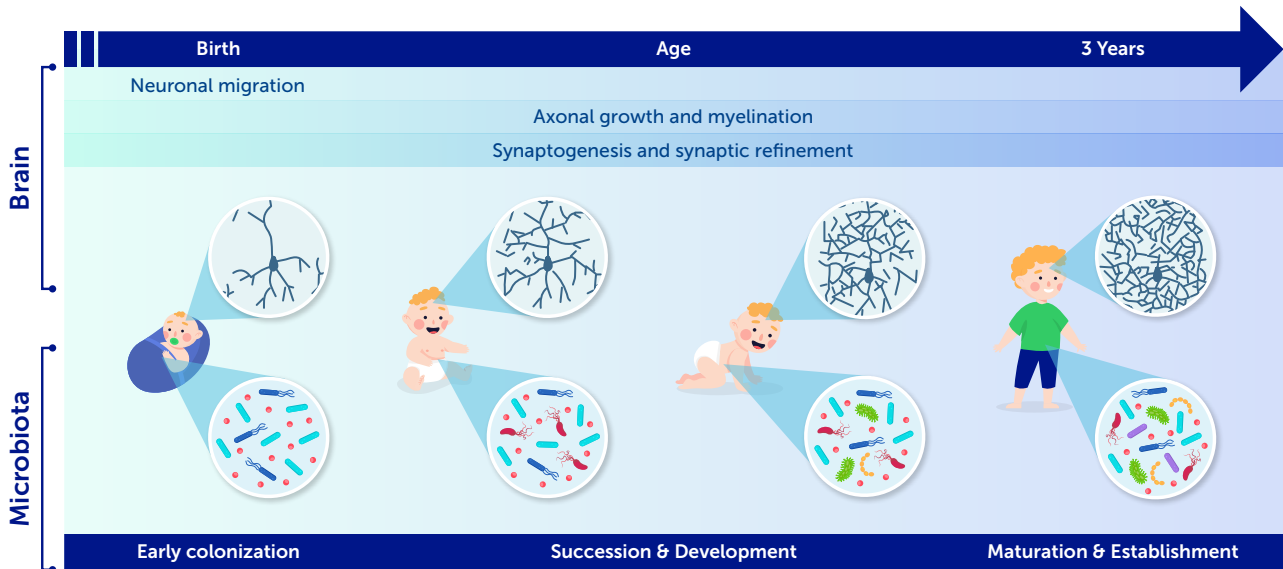
These mechanisms are responsible for persistent inflammatory processes and their respective clinical symptoms. Increasing evidence indicates that early-life intestinal dysbiosis negatively impacts immune system development and precedes the development of allergic diseases, such as atopic dermatitis, food allergies, and respiratory allergies (asthma and allergic rhinitis)[4]. In this context, the importance of exclusive breastfeeding, the prebiotic bifidogenic power of human milk oligosaccharides, and the subsequent production of short-chain fatty acids are highlighted, which are fundamental in this process of eubiosis → immunological tolerance → prevention of allergies and other chronic non-communicable diseases, such as obesity/metabolic syndrome, autoimmune diseases, cancer, and neurodevelopmental disorders[5].
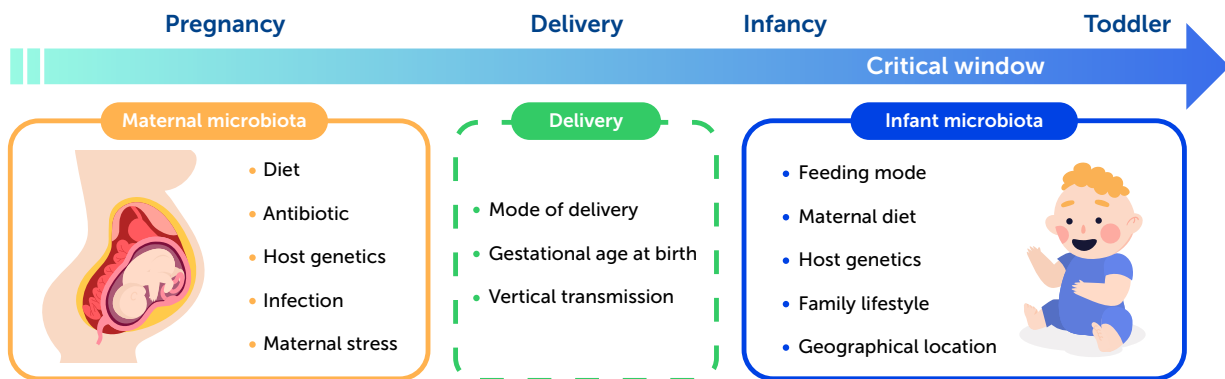
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The gut microbiota and its metabolites are crucial in programming key systems like the immune and central nervous systems during critical early developmental windows, particularly the first 1,000 days

Early life colonization: Critical periods of change in the microbiota coincide with brain maturation
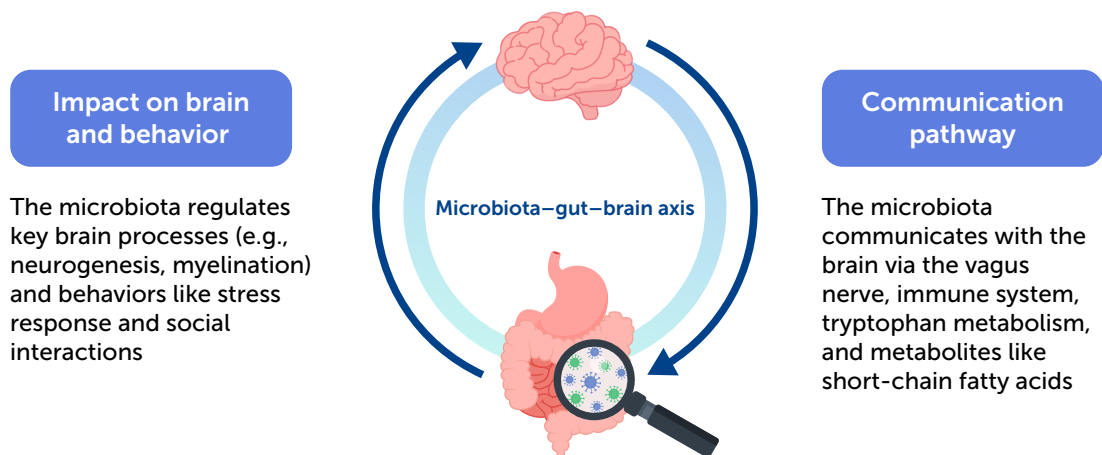
Birth	Age	3 Years

Brain

Neuronal migration

Axonal growth and myelination

Synaptogenesis and synaptic refinement

Microbiota

Early colonization	Succession & Development	Maturation & Establishment

This critical window is sensitive to many factors which can have lasting effects on the gut−brain axis

Pregnancy	Delivery	Infancy	Toddler

Critical window

Maternal microbiota
- Diet
- Antibiotic
- Host genetics
- Infection
- Maternal stress

Delivery
- Mode of delivery
- Gestational age at birth
- Vertical transmission

Infant microbiota
- Feeding mode
- Maternal diet
- Host genetics
- Family lifestyle
- Geographical location

Among these factors, early life nutrition plays a pivotal role in programming microbiota and long-term health

The microbiota−gut−brain axis is a key regulator of gut−brain communication and critical for understanding neurodevelopmental disorders

Impact on brain and behavior

The microbiota regulates key brain processes (e.g., neurogenesis, myelination) and behaviors like stress response and social interactions

Microbiota−gut−brain axis

Communication pathway

The microbiota communicates with the brain via the vagus nerve, immune system, tryptophan metabolism, and metabolites like short-chain fatty acids

Nutrition and microbial based management strategies have potential to foster the healthy development of the microbiome while mitigating the likelihood of neurodevelopmental challenges

Microbiome and brain development

John F. Cryan
Professor and Chair, Department of Anatomy and Neuroscience; APC Microbiome Ireland Institute, University College Cork, Ireland

For the past two decades there has been a growing appreciation of the role of the microbiota (the trillions of microorganisms within and on our bodies) as one of the key regulators of gut–brain function and has led to the appreciation of the importance of a distinct microbiota–gut–brain axis. The microbiota–gut–brain axis is particularly emerging as a research area of increasing interest for those investigating the biological and physiological basis of neurodevelopmental disorders (Cryan et al., 2019).

Microbes colonize the human body during the first moments of life and coexist with the host throughout the lifespan. Intriguingly, periods of change in the microbiota coincide with the development of other body systems and particularly the brain (Codagnone et al., 2019). Such parallel effects are biologically relevant, corresponding to 'critical windows' in the development of the microbiota–gut–brain axis with the microbiota acting as an expected input to calibrate the development of the axis in early life (Cowan et al., 2020).

Gut microbiota and its relevant metabolites are key in aiding the programming of important bodily systems such as the immune and the central nervous system during critical temporal windows of development. These critical developmental windows perinatally (during the first 1,000 days) are susceptible timepoints for insults that can endure long lasting effects on the microbiota–gut–brain axis. Growing evidence shows that developmentally, a variety of factors can impact the microbiota in early life including mode of birth delivery, antibiotic exposure, mode of nutritional provision, infection, stress as well as host genetics. Among all these factors, early life nutrition plays a pivotal role in perinatal programming and in the modulation of offspring microbiota from birth throughout lifespan. Moreover, we have been interested in the enduring effects of birth by caesarean section on brain and behaviour. Additionally, major sex differences occur in response to microbial manipulations especially in early life (Jaggar et al., 2020).

Moreover, reductionist animal models have been key in linking the regulation of fundamental brain processes in early life ranging from adult hippocampal neurogenesis to myelination to microglia activation by the microbiome. At a behavioural level it is become clear that the microbiome is an important regulator of the development of normal social and stress-related behaviours. The routes of communication between the gut and brain are slowly being unravelled and these include via the vagus nerve, the immune system, tryptophan metabolism, via the enteric nervous system or via microbial metabolites such as short chain fatty acids (Cryan et al., 2019).

Studies examining the translation of these effects from animals to humans are currently ongoing with evidence for microbial modulation of neurocognitive development and neurodevelopmental risk increasing. Further studies will focus on understanding the mechanisms underlying such brain effects and developing nutritional and microbial-based psychobiotic intervention strategies. Moreover, longitudinal studies that integrate genetic, environmental, and experiential factors in shaping and responding to gut microbial functions in early life will help resolve contexts in which the microbiome may shape the human brain and its health during development (Ratsika et al., 2021).

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— W O R K S H O P —
101

Half of all infants experience a gut–brain interaction disorder in early life

Intestinal dysbiosis may contribute to disorders of gut brain interaction (DGBI). Compared to infants with a healthy microbiome, those with dysbiosis have altered gut bacteria that can trigger immune responses

Differences in the microbiomes of infants with and without colic showed differences in the types of bacteria present, beta-diversity, and metabolic pathways

Normal
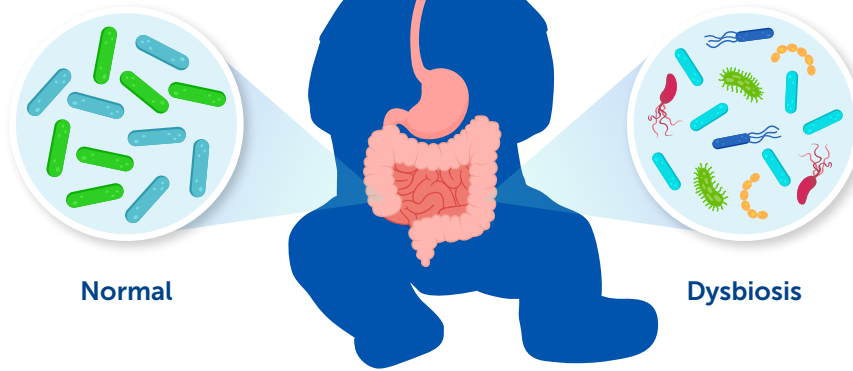
Dysbiosis

Intestinal dysbiosis in infants with colic can lead to:
- Excessive gas production
- Inflammation
- Impaired gut function

Beneficial bacteria	Harmful bacteria
e.g. Actinobacteria, Actinomyces, Bacteroides, Bifidobacterium, Lactobacillus	*e.g. Clostridioides, Enterococcus, Klebsiella*

Early microbiota disturbances may predispose individuals to other digestive disorders later in life, possibly due to shared underlying factors such as intestinal permeability, visceral hypersensitivity, and changes in gut motility

Gut microbiota and disorders of gut–brain interaction

Rodrigo Vásquez-Frias
Professor of Pediatrics, Universidad Nacional Autónoma de México

Disorders of gut brain interaction (DGBI), formerly known as functional gastrointestinal disorders, are recognized by morphologic and physiological abnormalities that often occur in combination including motility disturbance, visceral hypersensitivity, altered mucosal and immune function, altered gut microbiota, and altered central nervous system processing. Approximately, 50% of all infants are estimated to suffer from at least one DGBI during their first months of life. Most of the DGBI, like infant colic (IC), are understood or conceptualized according to the alteration of the gut brain axis[1].

The microbiota–gut–brain axis is established by different pathways that communicate bi-directionally via different pathways in a complex interplay[2]. Alterations in microbial composition, diversity and stability, also referred to as intestinal dysbiosis, might be present in infants with colic. This might contribute to colic symptoms by increased fermentation of lactose, carbohydrates and proteins, resulting in increased gas production and gut extension. Dysbiosis can also lead to increased gut permeability facilitating increased low-grade mucosal, gut and systemic inflammation. Through the microbiota gut–brain axis, intestinal dysbiosis might affect central and enteric neuronal function, including detection of pain and crying[3].

There are some evidences of low-grade, systemic inflammation, which might alter the composition of the gut microbiota. Conversely, gut microbiota alternations could induce gut inflammation in infant colic. Gut microbial signatures from infants with colic differ from those from infants without colic in terms of microbial diversity, stability and colonization patterns. Infant colic samples have a significantly lower abundance of Actinobacteria. There are some genera associated with cry/fuss time in infants with colic: most of the studies show a tendency of depletion of *Actinomyces, Bacteroides, Bifidobacterium* and *Lactobacillus*; enrichment of *Clostridioides, Enterococcus* and *Klebsiella*[4].
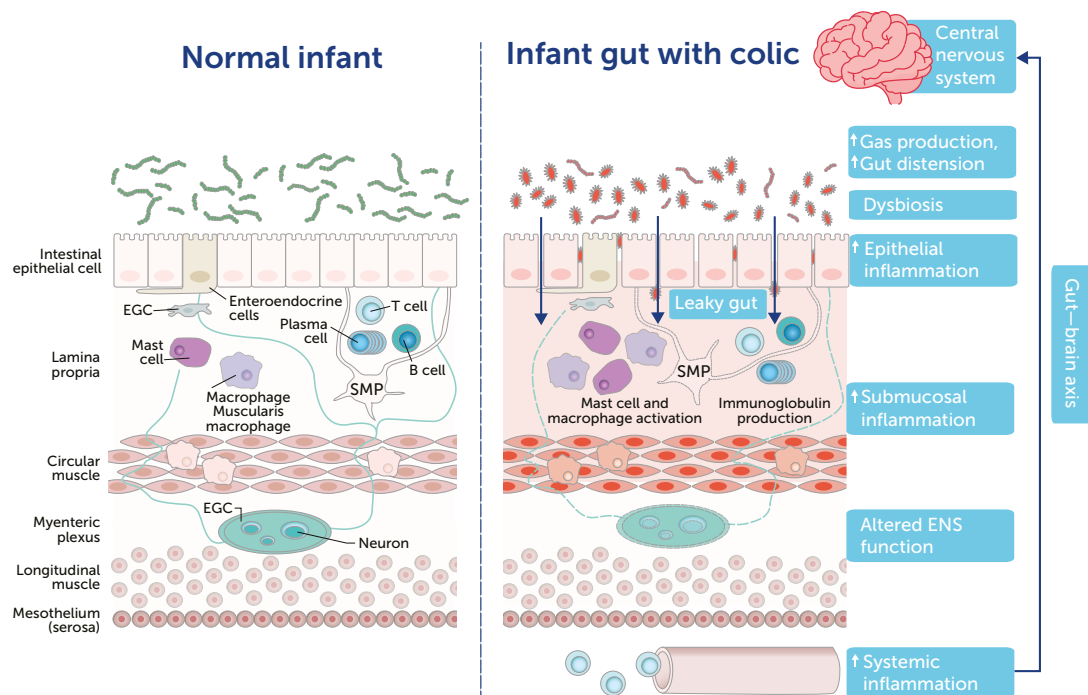
Figure 1. Depletion of *Actinomyces, Bacteroides, Bifidobacterium* and *Lactobacillus*; enrichment of *Clostridioides, Enterococcus* and *Klebsiella*.
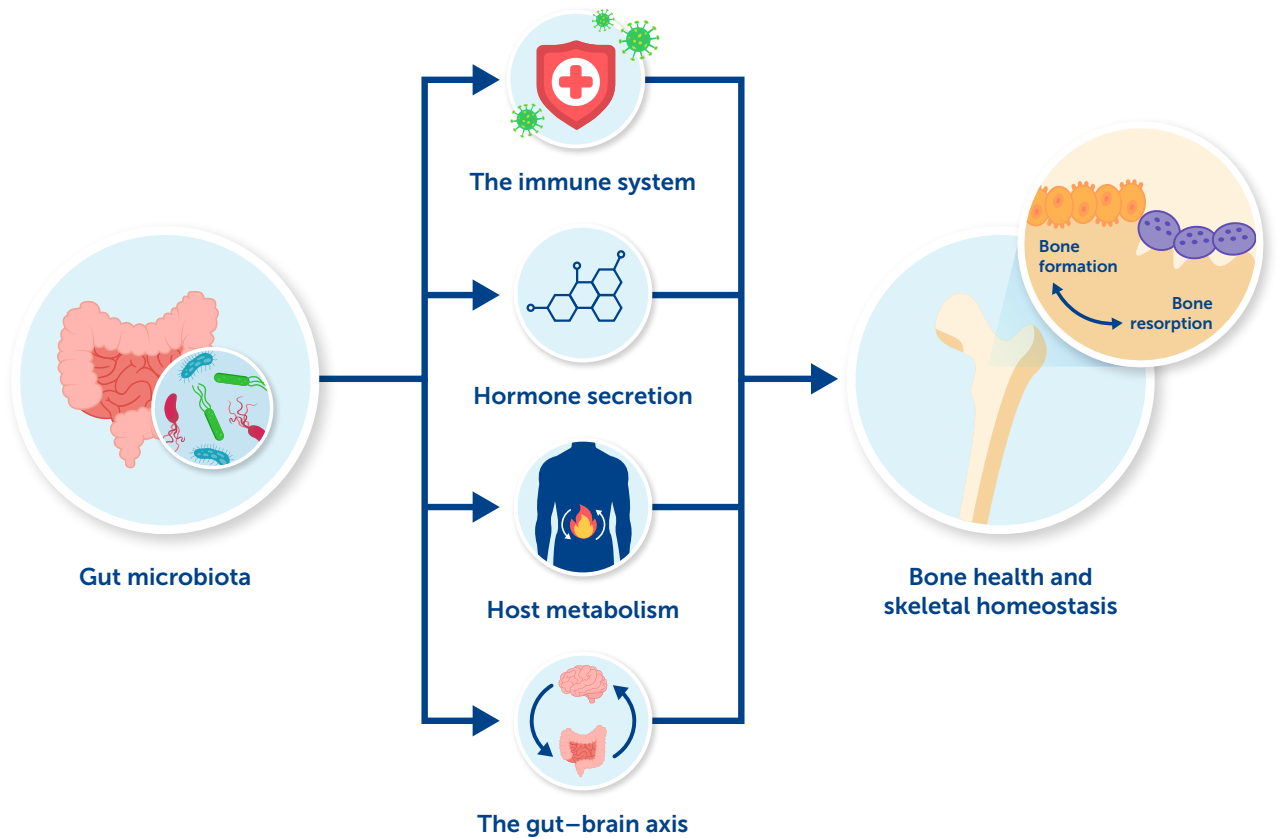
Some evidence show that delayed or altered colonization by *Lactobacillus* spp. or *Bifidobacterium* spp. could contribute to intestinal dysbiosis[3]. In a recent prospective cohort observational study, which included 62 newborns, neonatal meconium samples and subsequently samples at collection points 1, 3, 6, and 12 months after birth were collected[5]. After 1 month of life in relation to IC, 15 children with colic and 21 children from the cohort were selected, who were exclusively breastfed and were born vaginally. In the group with IC, bacterial species diversity, after 1 month of life, is higher compared to the group without infantile colic; There were also differences in beta-diversity. So, not always lower diversity means dysbiosis! Also, there were significantly different metabolic pathways associated with taxonomic disturbance in the microbial community. They found that metabolic pathways involved in the biosynthesis of essential non-essential, and semi-essential (l-arginine) amino acids were depleted in IC, whereas the metabolic pathways involved in glycolysis reactions were enriched.

Traditionally, IC has been considered self-limited condition. However, altered microbiota and trans-mucosal passage of antigens across the immature intestinal barrier may play a crucial role in the pathogenesis of these functional disorders. It has been hypothesized that this kind of early traumatic insult could be a risk factor for the onset of other DBGIs such as recurrent abdominal pain later in childhood. Most studies display an association between IC and the onset of other DGBI years later, probably related to the presence of common etiopathogenetic factors (environmental, dietary, intestinal dysmotility, visceral hypersensitivity)[6].

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The gut microbiome can influence bone health and skeletal homeostasis through multiple interconnected pathways, essential in maintaining bone density and preventing bone disorders

The immune system

Hormone secretion

Host metabolism

The gut—brain axis

Gut microbiota

Bone formation

Bone resorption

Bone health and skeletal homeostasis

Gut-derived serotonin is a crucial regulator of bone formation and resorption impacting bone strength and overall skeletal health

Serotonin

Serotonin

Bone homeostasis
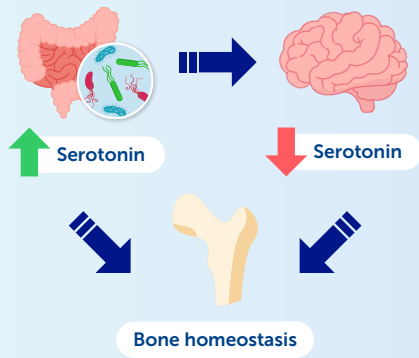
Imbalance in the gut microbiome can alter serotonin production, leading to increased risks of bone-related diseases

Certain beneficial bacteria produce short chain fatty acids (SCFAs) such as butyrate. Maintaining adequate levels of SCFAs can support bone growth and prevent bone loss
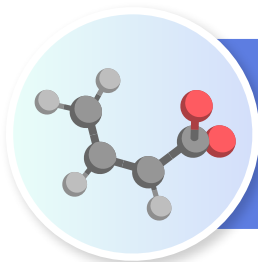
Butyrate enhances the immune regulation of bone tissue, reduces inflammation, and promotes the activity of bone-forming osteoblasts

Nutritional strategies such as probiotics and prebiotics may represent new strategies to promote bone health

Gut microbiota–bone axis
Clinical implications

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Knowledge of the complex interplay between gut microbiota and human health is gradually increasing as it has just recently been a field of such great interest.

Recent studies have reported that communities of microorganisms inhabiting the gut influence the immune system through cellular responses and shape many physiological and pathophysiological aspects of the body, including muscle and bone metabolism (formation and resorption). Specifically, the gut microbiota affects skeletal homeostasis through changes in host metabolism, the immune system, hormone secretion, and the gut–brain axis[1].

At the hormonal level the effect of the gut microbiota on bone homeostasis is expressed through the biphasic action of serotonin. Some microbiota, such as spore-forming microbes, regulate the level of serotonin in the gut, serum, and feces[2]. Another group of bacterial species (*Lactococcus, Mucispirillum, Lactobacillus* and *Bifidobacterium*) can increase the level of peripheral/vascular leptin, which in turn manages bone homeostasis through the action of brain serotonin. The major role on gut–bone axis is due to SCFAs. They have the ability to influence regulatory T cells (Tregs) development and activate bone metabolism through the action of Wnt10. SCFA production may be a mechanism by which the microbial community, by increasing the serum level of IGF-1, leads to the growth and regulation of bone homeostasis[3,4].

A specific SCFAs as butyrate diffuses into the bone marrow where it expands Tregs. The Tregs induce production of the Wnt ligand, Wnt10b, by CD8+ T cells, leading to activation of Wnt signaling and stimulation of bone formation.

In conclusion, inhibition of T-cell trafficking from the gut to the bone marrow with butyrate directly or indirectly (prebiotic, probiotic) may represent new strategies to prevent bone loss or stimulate bone anabolism[5,6].

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Environmental influence on the gut microbiome and impact on health and disease

Modern lifestyles, characterized by changes in diet, increased hygiene practices, and reduced exposure to diverse environmental microbes, are altering the composition and diversity of the gut microbiome

Loss of microbial diversity in the gut is often linked to an increased risk of disease, including allergy and autoimmune conditions

The food we eat can influence how our genes function, affecting our health, disease susceptibility, strength, and lifespan
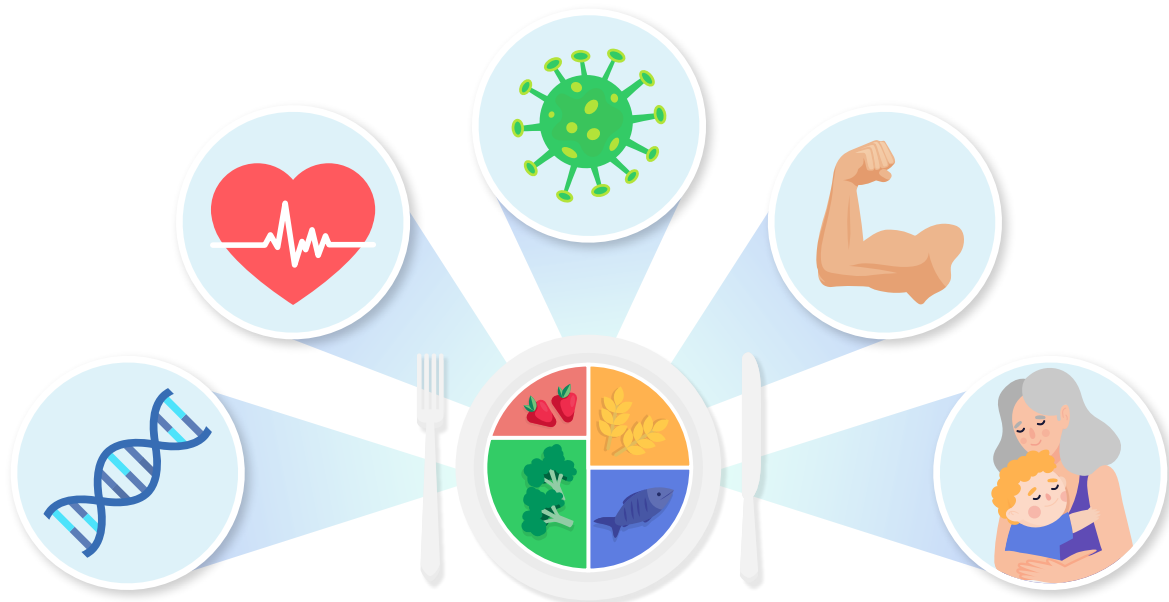
Education at a population level is essential to raise awareness about the role of the gut microbiome in health and disease. Interventions targeting diet, lifestyle, and environmental exposures can help prevent dysbiosis and promote lifelong health and well-being

Environment and microbiota intersection with human health
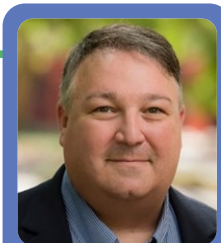
Andrew Bartko
Executive Director, Center of Microbiome Innovation (CMI), Professor of Practice, UC San Diego, USA

The concept of spatial heterogeneity, which refers to the uneven distribution of factors over the landscape. This can apply to local environments, such as a city park or even your backyard. For instance, a park might have sunny areas with lots of grass, shady areas with trees, and a wet, muddy patch by a creek. This variation across the landscape is what we call spatial heterogeneity.

Some specific examples of how spatial heterogeneity plays out in local environments is illustrated with plants and animals. A forest might have areas with more sunlight that favor certain plants, while shady areas have different plant life. This variation can create habitats for different animal species as well. Understanding spatial heterogeneity is important for several reasons. It is important for ecology, for instance, how forests are managed. Spatial heterogeneity is also important for appreciating the variety of life that can exist in a small area.

The human microbiome is the community of microbes that live in and on our bodies[1]. The diversity of our microbiome is important for our health. However, we are losing genes in our gut microbiome due to our industrialization and modern lifestyles[2]. Our modern lifestyles are more refined and hygienic, thus reducing the exposure to microbes that we get from the environment.

The loss of diversity in our gut microbiome is linked to a rise in chronic diseases, such as multiple sclerosis[3], Crohn's disease[4], Type 1 diabetes[5], and asthma[6]. These diseases are now striking younger and younger people.

The importance of nutrition has been known for centuries. However, industrialization and refined food manufacturing has created a new paradigm in human health[7]. Food is a language that speaks to our genes. The food that we choose to eat can affect how our genes function, and this can affect our health. For instance, the food we eat can influence whether we are prone to disease, strong or weak, and whether we have a long and healthy lifespan or a short life full of disease.

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Expanding our understanding of the gut microbiome: beyond bacteria to include viruses, fungi, and bacteriophages
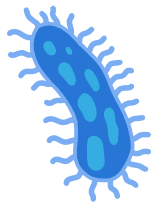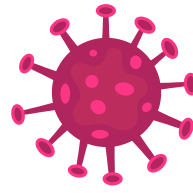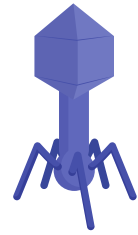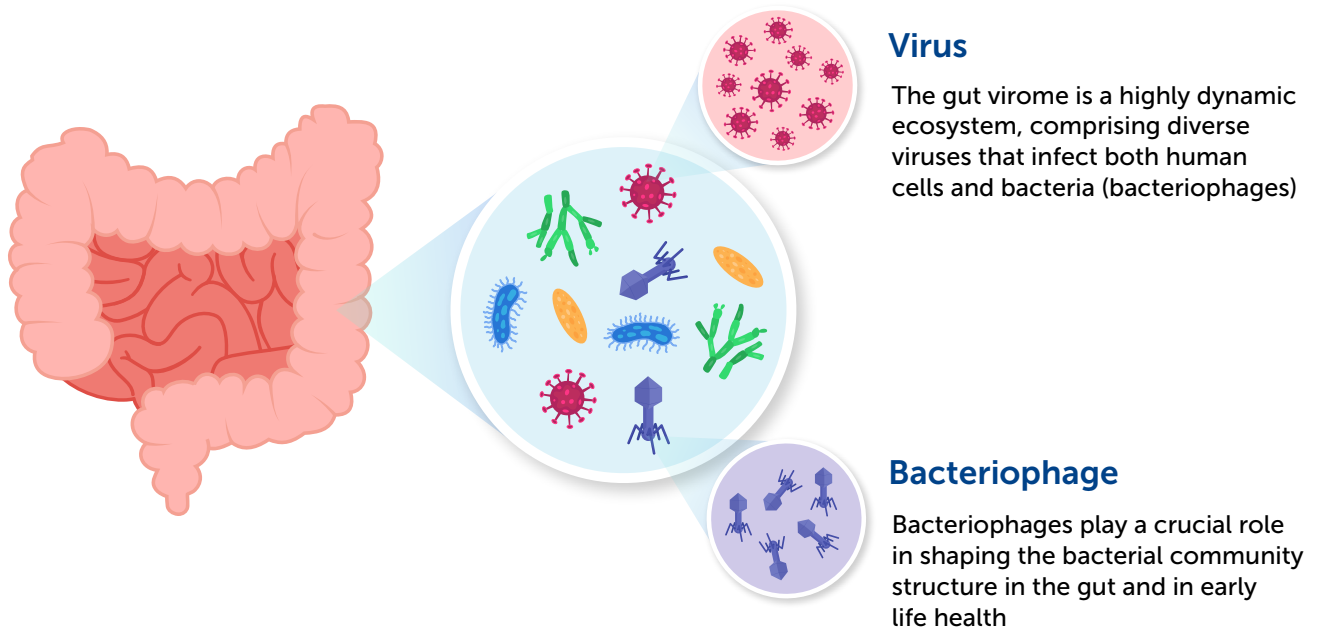
Bacteria
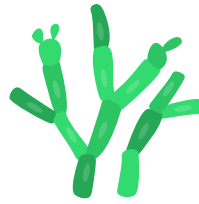
Fungi

Protozoa

Human viruses

Viruses of bacteria (bacteriophages)

Studying the gut virome is challenging due to the absence of genetic markers similar to those used for bacteria and requires complex laboratory protocols, advanced sequencing technologies, and robust databases

Virus

The gut virome is a highly dynamic ecosystem, comprising diverse viruses that infect both human cells and bacteria (bacteriophages)

Bacteriophage

Bacteriophages play a crucial role in shaping the bacterial community structure in the gut and in early life health

Emerging research highlights links between an altered virome and various health conditions. Understanding the development and composition of the virome in early life can provide new insights into disease prevention and health optimization

Questions to be investigated:

The origin of gut viruses in infants	The links between virome composition and health
Factors that influence the virome	The role of bacteriophages in immune system development and disease susceptibility

The infant gut virome
Knowns, unknowns, and avenues for future studies

Alexandra Zhernakova
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The microbiome plays a major role in human health, particularly in early life. However, most studies have focused on the bacterial component while other communities such as fungi, protozoa, and viruses are largely ignored. Viruses, the most numerous microbes on Earth, are highly abundant in the human gut and other ecosystems. It is estimated that the number of viruses in the gut is about equal to the number of bacteria, although their size and biomass are substantially lower. The vast majority of gut viruses are bacteriophages – viruses that infect bacteria, with only a tiny fraction being eukaryotic viruses, mainly human, which are mostly studied due to their potential pathogenic properties.

Several challenges limit wide-scale studies of the gut virome. Unlike bacteria, viruses do not have a marker gene analogous to the bacterial 16S ribosomal RNA gene, requiring sequencing of all genetic material (DNA for DNA viruses and RNA for RNA viruses) for their identification. Distinguishing active bacteriophages from prophages integrated into bacteria requires sophisticated and lengthy protocols to separate viruses from bacteria. Additionally, ultra-sensitive methods for genomic library preparation and sequencing, along with rigorous quality control, are needed due to low viral DNA and RNA concentrations, particularly in samples from babies. Furthermore, limited knowledge of viruses and the absence of up-to-date classification pose challenges in bioinformatic analysis and interpretation[1]. Despite these challenges, gut virome analysis has been performed in several studies in relation to diseases and healthy conditions. In adults, the gut virome has been associated with diseases such as inflammatory bowel disease (IBD), obesity, diabetes, liver diseases, and colorectal cancer[2].

Studies have also addressed the composition and dynamics of viruses in early life and viral transmission from mothers to babies. Most studies suggest that meconium is free from viruses or contains very few, with viral diversity increasing during the first year of life but remaining lower compared to maternal diversity until at least 12 months of age. One open question of the early life virome is the origin of gut viruses. Examples of shared viruses between mothers and their babies have been identified, suggesting transmission from mothers and the environment. Another source of baby viruses is induced prophages that are part of the bacteriome transmitted from mothers and the environment[3]. This is reflected in a larger proportion of temperate bacteriophages in the baby's virome compared to the adult virome[4]. Establishing relationships between virome composition and health is challenging due to the high individual specificity of the virome, requiring large cohorts for statistical power. Pioneering studies indicate relations of the gut virome with delivery and feeding mode, malnutrition in children, and the role of bacteriophages in immune system maturation[3–5].

In summary, studies of the gut virome are still in their infancy, with the virome being more variable and individual-specific than the bacteriome. Analysis poses several major challenges, but new pipelines for both lab and bioinformatic analysis are constantly being developed. It is expected that virome research in the coming years will expand rapidly. Bacteriophages have the potential to mediate microbiome composition and be used as alternatives to prebiotics and antibiotics, offering insights into the role of gut viruses and viral-bacterial dynamics in gut ecosystem development and health improvement.

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WORKSHOP
101

AI: A transformative tool in microbiome research

Predict health outcomes based on microbiome profiles

Improve accuracy of microbiome sequencing and classification

A model called MDITRE can predict host health status from microbiome time-series data and translate this into information that is useable by researchers.
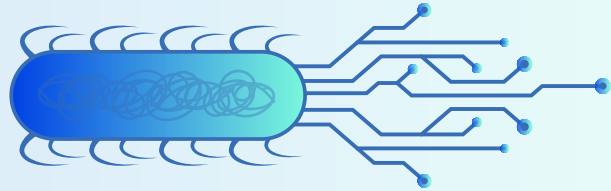
Promising directions for AI and microbiome research:
- Forecasting the impact of interventions on the microbiome
- Defining new microbial biomarkers
- Understanding the interplay between host and microbial cells

Challenges include the lack of large, high-quality microbiome datasets to inform machine learning, interpretability of results and wider risks associated with the use of AI. International efforts are needed to ensure the responsible use of this powerful technology.

Artificial intelligence and microbiome research

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Harvard Medical
School, Faculty
of Harvard-MIT,
Health Sciences and
Technology, USA

Artificial Intelligence (AI) is a subdiscipline of Computer Science that develops machines that mimic characteristically human cognitive functions, such as learning or problem-solving. The roots of AI can be traced back to seminal work over eighty years ago describing mathematical models of networks of neurons. In the subsequent decades, work has advanced in several directions, including developing a branch of AI termed *machine learning* (ML), which creates algorithms capable of improving their performance on objective measures, such as winning at a game, through *training* on input data.

A major revolution in AI began around 2010, catalyzed by a confluence of several factors, including improved algorithms, software packages, computer hardware, and availability of ever larger datasets. State-of-the-art AI systems today are based on deep learning (DL), which stacks layers of mathematical functions capable of capturing extremely complex patterns in data. An important advance in this regard are *foundation models*, which are flexible DL models trained on large, broad datasets that can then be applied to a variety of downstream tasks[1,2]. These systems exhibit sophisticated behavior that can appear astoundingly human, particularly in the realms of image recognition and natural language understanding. Such models are also being increasingly applied in the life sciences and are achieving similarly impressive results on problems such as protein folding[3].

Can AI be used to advance microbiome research? Indeed, DL methods have already been used in the field, with early applications employing off-the-shelf tools to address tasks such as improving taxonomic assignment of sequencing reads and prediction of host diseases based on microbiome composition. Microbiome datasets, however, present several challenges for DL. Many DL methods require extremely large datasets for training. Although microbiome experiments generate high-dimensional datasets, they measure relatively few microbiomes. In addition, microbiome data is noisy and the microbial taxa present often vary tremendously across human hosts. Another issue for microbiome analyses is the need for interpretability, or the ability to understand the reasoning behind predictions and decisions made by models. Interpretability is not as important in consumer applications of AI, such as chatbots or image generation programs, which often use "black box" models. However, for microbiome researchers, who analyze datasets with the objectives of gaining scientific understanding or developing clinically useful diagnostics or treatments, interpretability is essential.

The path forward to overcome the above-mentioned challenges and unlock the potential for microbiome applications of AI will involve creative development and application of experimental technologies coupled with purpose-built DL models. An example of a promising model of this type is MDITRE[4], a method that efficiently learns human-interpretable rules predicting host health status from microbiota time-series data. This method performs on par or outperforms "black box" ML methods. Moreover, it automatically derives biologically meaningful interpretations linking patterns of microbiome changes over time with host phenotypes, suggesting its potential as a tool to define biomarkers for new microbiome-based diagnostics.

The future for AI in microbiome research is bright. Exciting application ideas include forecasting the effects of antibiotics, dietary changes, or therapeutic interventions on the microbiome; elucidating the molecular mechanisms of interactions between human cells and the microbiome; discovering the functions of the vast array of unannotated microbiome genes; mapping the spatial structure of microbiomes; and using AI to guide design and interpretation of microbiome studies. Despite this extremely exciting potential, AI has dangers, including risks for bias and discrimination; removing human judgment and creativity from tasks; hoarding of tools by companies and wealthy countries; and negatively impacting the environment. Thus, international efforts are essential to plot a course for responsible use of this incredibly powerful technology.

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