Unlocking the power of human milk and infant feeding: understanding how nutrition and early microbiota interaction shapes health programming

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Summary

Human milk represents a highly evolved bioactive system that supports colonisation by infant microbial pioneers and supports immune maturation and infant development. Beyond providing nutrition, human milk contains key bioactive components, such as microbes, metabolites, human milk oligosaccharides, immunoglobulins, lactoferrin, and antimicrobial peptides, which selectively modulate infant gut microbial colonisation and facilitate immune development and metabolic health, with implications for health outcomes and risk of non-communicable diseases. In this review, we highlight the impact of infant feeding, human milk constituents (especially bioactive compounds), and weaning on infant microbial trajectories. By understanding how early-life nutrition influences microbial colonisation and nutrient sensing, i.e. 'how we feed our microbes', we can develop targeted interventions and personalised diets to support proper gut maturation and disease prevention from infancy to adulthood, as well as explore the therapeutic potential of human milk bioactives beyond infancy, offering new strategies for disease prevention and treatment.

Short paragraph

Infant feeding plays a crucial role in microbial colonisation, immune maturation, and development. Human milk – a bioactive system containing microbes, metabolites, HMOs, immunoglobulins, lactoferrin, and antimicrobial peptides – modulates the gut microbiota and supports immune and metabolic health. This review examines the impact of breastfeeding, weaning, and bioactives on microbial trajectories and health outcomes.

Keywords

Breastfeeding; human milk; microbiome; bioactive compounds; infancy; HMOs; biotics

Acronyms

AMPK: AMP-activated protein kinase
C-section: Caesarean section
FMT: faecal microbiota transplantation
FUT2: fucosyltransferase 2
GABA: γ-aminobutyric acid
HMOs: Human Milk Oligosaccharides
LBPs : Live biotherapeutic products
LPS: Lipopolysaccharide
mTOR: mechanistic target of rapamycin
MFGM: milk fat globule membrane
NCDs: Non-communicable Diseases
NEC: Necrotising Enterocolitis
PPARs: peroxisome proliferator-activated receptors
SCFA: Short-Chain Fatty Acids
VMT: vaginal microbiota transfer

Introduction

The human diet has a pivotal impact on the gastrointestinal microbiota, and also on the nutrient–microbiota interactions that influence human health [1,2]. "***We are what we eat***" – the interactions between our diet, microbiome, and health are fundamental pillars at all stages of life, but they are particularly critical during early-life development [3].

Early life is a critical time for microbiome assembly and evolution that has lasting effects on health outcomes [4]. Perinatal factors play a central role in shaping early microbial ecosystems. Extensive research has demonstrated that maternal and infant diet, mode of delivery, environment, and lifestyle (among other factors) are key determinants of microbial succession and future health outcomes [5]. Microbial perturbations or dysbiosis during this period have been associated with a higher risk of infections and non-communicable diseases (NCDs) [6]. Importantly, the microbiome may serve as a vehicle for the transmission of NCD risk, as microbial communities and their alterations are shared between mothers and infants. These microbial communities are also in close contact with environments [7] and can be horizontally transferred between individuals [8], which together affect the child's development and future health. Thus, by understanding how these microbial trajectories are established during this critical period, as well as the specific contributing factors, we should be able to identify opportunities for early interventions aimed at modulating initial microbial trajectories to reduce the global burden of NCDs.

This review integrates recent scientific evidence and highlights the links between early-life microbiome development, perinatal influences, and long-term health outcomes. We also examine how early-life nutrition influences microbial colonisation, nutrient sensing, and metabolic maturation and discuss the role of human milk, the introduction of complementary foods, and the weaning process in shaping the microbiome succession and its effects on health outcomes. Finally, we highlight nutrient–microbiota interaction mechanisms and potential insights into the design of interventions (e.g. biotics and/or personalised nutrition strategies) that promote appropriate development and long-term health outcomes.

Microbial assembly during the first months of life: the relevance of diet

Our society is experiencing a progressive increase of immunological and metabolic diseases, with an alarming propagation in children. Delays or changes in microbial colonisation profiles due to lack of breastfeeding, cesarian section (C-section), and/or antibiotics in early life, unbalanced diets, pollutants, specific lifestyle and exposome changes, and stress in childhood

have all been associated with increased risk of NCDs [9–11]. These factors can act through a variety of mechanisms to affect infant development and metabolism, highlighting the profound consequences of disrupted microbe–host interactions during early life. However, most of these mechanisms remain unexplored, and scientific evidence in human studies is urgently needed. However, while there is no clear definition of a "healthy microbiota", we can consider the microbiota that colonises a healthy, full-term, vaginally delivered, breastfed infant who remains healthy and without antibiotic exposure as the gold standard. In order to build this gold standard microbiota, first colonisers are key, and several perinatal factors contribute to the microbial assembly. So, in this scenario it is critical to ask *What is known?* and *Who are these first colonisers?*

Early-life microbiome origin and assembly: Who are the initial colonisers?

The maternal microbiome is pivotal in building the infant's initial microbial landscape, serving as the primary microbial source during early development. Initial reports suggest that the maternal gut microbiome contributes ~70% of the gut bacteria in infants born vaginally, as compared to ~40% in C-section-born infants [12]. However, recent advances in metagenomic sequencing and multi-omics technologies have significantly deepened our understanding of maternal–infant microbial transmission, revealing strain-level dynamics and functional adaptations [12–16]. Other studies have demonstrated that mothers share their microbes with their infants through various routes (faeces, human milk, vaginal contact, and skin) [13,17–20]. However, the dynamics of bacterial strains – whether they are transient, establish colonisation, or persist long term – are still being uncovered [21,22]. Additionally, the precise mechanisms of strain transmission remain unclear. Significant gaps also remain in our understanding of how maternal microbial communities adapt to physiological changes during pregnancy and how these adaptations impact infant colonisation.

The early microbiome is a critical and dynamic ecosystem that lays the foundation for lifelong health, and initial microbial seeding is influenced by different perinatal factors [17,18]. The initial microbial assembly just after birth is marked by increasing diversity and richness, progressing from first-pass faecal samples to the 7-day neonatal microbiota. This early colonisation is mainly driven by key pioneer bacteria such as *Bifidobacterium* and *Bacteroides*, but also by other microbes, including *Escherichia coli*, *Clostridioles*, and *Klebsiella,* as well as members of other kingdoms such as yeast, fungi, eukaryotic viruses, and phages. This complex ecosystem plays a crucial role in shaping the early microbiome, training the immune system, and building the specific environment for proper microbial evolution. These observations suggest that, while neonates are extensively exposed to microbes at birth, from their mother, environment, and other sources, only a selection of microbes successfully colonise and persist in the gut, gradually shaping the developing microbiome.

Key remaining questions in this area include *How does this intricate assembly occur?* and *Is it more crucial to focus on microbial composition or function in understanding its impact?* These questions highlight a major gap in the field. Contradictory data is available on early-life colonisation and dynamics. For example, a recent study analysing 3,154 stool samples from 1,827 infants in 12 countries identified conserved microbial succession patterns in early life [23]. They found *Bifidobacterium* spp. and *E. coli* to be early colonisers, followed by *Ruminococcus* and *Faecalibacterium,* establishing a hallmark of early-life trajectories. In particular, decreases in *Bifidobacterium* spp. and increases in *Lachnospiraceae* members and *Faecalibacterium prausnitzii* were identified as key taxonomic predictors of infant age from birth to 12 months [23]. The study also highlighted key microbial metabolic genes associated with feeding transitions and dietary exposures, providing further insights into microbiota development during infancy. However, another study reported robust variation in infant gut microbiome assembly in 682 infant metagenomes [24], with bifidobacteria-dominated assemblages driving the observed differences, alongside reduced microbial diversity in industrialised geographical locations. The

authors concluded that lifestyle is more relevant than geographical location for early-life colonisation. Same study reported that a *Bifidobacterium-Streptococcus* cluster dominates the early-life microbiota (0–6 months), evolving over time to a *Bacteroides-Ruminocccocus gnavus* cluster in Western populations and a *Prevotella-Faecalibacterium* cluster in non-industrialised societies [24]. A recent longitudinal study of a Dutch birth cohort that included 4,577 samples from 714 mother-baby pairs found that the microbiome at 2 weeks is largely dominated by a few bacteria, mainly several species of bifidobacteria, *Bacteroides*, and *E. coli* [25]. Another large longitudinal study of 10,913 samples from 903 infants showed that both the taxonomic and functional profiles are dynamic and highly individualised, being dominated by *Bifidobacterium* species or the phylum Pseudomonota (previously Proteobacteria), [26,27] with *Bacteroides* genus members (especially *B. fragilis*) present in vaginally born infants.

More about those initial pioneers. An adequate health-associated early microbiota is characterised by low diversity and the presence of key taxa that play pivotal roles in immune education, metabolic regulation, and pathogen exclusion. ***Bifidobacterium*** emerges as a cornerstone of vertical transmission [18,28,29], with *B. longum*, *B. breve*, and *B. bifidum* strains best adapted to persist in the early infant microbiome, playing a vital role in gut immunity and homeostasis [19,30]. However, the long-term persistence of these strains can be influenced by external factors such as birthplace, delivery method, and antibiotic exposure [17–19]. During the first 6 months, *B. longum* subsp. *infantis* dominates the gut of infants with non-Western lifestyles but is depleted in Western infants in favour of *B. breve* [24]. A functional study analysing strain diversity further supported this, reporting that *B. longum* subsp. *infantis* is present in 10% of Finnish infants, whereas *B. bifidum* strains are maintained in Russian infants [31]. In further agreement, infants born in Mennonite communities characterised by farm lifestyle environments harboured *B. infantis*, which also associated with lower incidence of allergic diseases[32]. Interestingly, one study tracking *B. infantis* abundance in the infant gut showed late colonisation[33]. Importantly, different *Bifidobacterium* strains, even those within the same species, can vary between infants, influencing their ability to thrive on different diets. These strain-level differences may shape the utilisation of milk-derived oligosaccharides or other dietary components, ultimately impacting the microbiome and metabolic outcomes in early life[34].

There is also evidence indicating roles for other bacterial groups. Several studies have shown delayed colonisation of the gut by members of the ***Bacteroides*** genus in C-section born infants [26,35–37]. *B. fragilis* has been associated with increased microbial diversity, faster microbiome maturation, [26] and improved cognitive and language scores at age 2 years, in part due to enhanced sphingolipid synthesis and metabolism [38]. ***E. coli*** is often one of the first microbial colonisers of the infant gut[39]. Infant microbial clusters enriched in *E. coli* have been reported to have higher prevalence in infants born by C-section and who were not breastfed. While the core function of *E. coli* remains unclear, its preference for an anaerobic environment may help pave the way for subsequent colonisers [40], while its lipopolysaccharide (LPS) may stimulate the immune system. Other pioneers are ***Clostridium*** spp., whose presence has been associated with both positive and negative health outcomes. Some *Clostridium* species, like *C. difficile* [41] and *C. perfringens* [42,43], can be opportunistic pathogens if present in high numbers, whereas others, like *C. butyricum*, may have beneficial effects on gut health and immunity. Notably, certain *C. perfringens* strains may be beneficial in early life as efficient degraders of human milk oligosaccharides (HMOs) and inhibitors of pathobiont growth [44].

Bacteria are not the only essential players in early microbiome development. Insight into the initial assembly of other early microorganisms, such as yeast, fungi, eukaryotic viruses, and phages, is now being uncovered. Pioneer **fungi and yeasts** are present in first-pass faeces, with *Candida* and *Saccharomyces* commonly detected alongside bacteria. These early fungal colonisers are thought to originate from the maternal microbiome, acquired during birth or

through breastfeeding, with specific maternal factors such as BMI influencing assembly [45–48]. The early life **virome**, which mainly consists of bacteria-infecting viruses called bacteriophages (collectively termed the phageome), increases in diversity during the first months of life, consistent with the growth of the microbiome, and it is characterised by a high abundance of active temperate phages that decreases over time [49,50]. While one study found that virome composition did not vary across four distinct geographical locations [49], it does seem to be influenced by place of delivery and infant feeding mode [50,51]. Some studies report that eukaryotic viruses, such as anelloviruses, circoviruses, and picornaviruses, are among the first colonisers of the infant gut [52,53], while other studies suggest that prokaryotic viruses, especially bacteriophages, are pioneering in the infant gut [52]. Commonly detected phages mainly belong to the class Caudoviricetes (or to groups previously classified under the order Caudovirales) and to the family Microviridae [54,55]. Colonising phages play a role in regulating their respective bacterial populations, thereby influencing gut microbiome stability and function. As mentioned above, *Enterobacteriaceae* family members are among the first colonisers, and phages that target *Enterobacteriaceae*, such as T4-like phages, infect and lyse these bacteria, reducing their abundance and controlling communities [53]. Those targeted phages play roles in regulating their respective bacterial populations, influencing gut microbiome stability and function.

How does infant feeding shape microbial seeding?

Breastfeeding, a biological hallmark of mammals, plays a crucial role in infant development through an intricate interplay of nutrition, immunity, and microbiota programming. Exclusive breastfeeding has significant short- and long-term health benefits for both infants and mothers [56,57]. For infants, breastfeeding has been linked to lower risks of obesity, type 2 diabetes, and allergic diseases such as asthma and eczema later in life, as well as reduced incidence of gastrointestinal infections, respiratory illnesses, and cases of necrotising enterocolitis (NEC) and sepsis. These protective effects are thought to be partially mediated by the early establishment of a balanced, resilient microbiome, which plays a key role in regulating metabolism, immune responses, and gut barrier function and homeostasis.

Breastfeeding significantly shapes the infant early pioneers and drives the infant's gut and oral microbial trajectories[58–60]. Breastfed infants harbour a higher abundance of *Bifidobacterium* (*B. breve* and *B. bifidum*) and show a lower prevalence of pathobionts, such as *Clostridium* and *E. coli,* compared to formula-fed infants [61–63], while the cessation of breast milk feeding results in faster gut microbiome maturation [26]. Breastfeeding functions as a key modulator of the infant microbial changes associated with C-section [64,65] and in the stepwise assembly of the neonatal virome [51]. In addition, the timely acquisition of *R. gnavus* and tryptophan metabolism driven by human milk have been associated with protection against asthma [66,67]. One meta-analysis consistently reported a distinct microbial pattern in breastfed infants that was characterised by *Bacteroides*, *Eubacterium*, *Veillonella*, and *Megasphaera*, while shorter duration of breastfeeding was linked to reduced levels of *Bifidobacterium* members [63]. In contrast, formula-fed infants often show lower proportions of beneficial bacteria like *Bifidobacterium*, *Lactobacillus*, and *Enterococcus*, which are crucial for healthy immune system development [40].

Exposure to human milk, either exclusive or mixed, is key for the microbiome. Mixed feeding is a common feeding practice, but its effects on the infant's oral and gut microbial communities remain poorly understood. Exclusively breastfed infants typically consume **750–800 mL/day** by 1–6 months (average 670mL/day), depending on region and measurement method [68]. In contrast, mixed-fed infants have variable human milk intake, complicating microbial impact assessments. The level of exposure to human milk can play a critical role in shaping microbial development, while the amount and timing of formula introduction can also influence microbial trajectories. This complexity makes it difficult to determine how mixed feeding specifically alters the establishment and composition of these microbial ecosystems, highlighting the need for more detailed research in this area. It has been reported that mixed feeding, compared to

breastfeeding, leads to a more diverse but less stable microbiome that is consistent with that seen in formula feeding [63]. While formula-fed infants do develop a functional microbiome, its composition tends to be enriched in bacteria associated with increased risk of infection and metabolic disorders. Despite the availability of new methods (including 24h test-weighing and deuterium dilution) for accurately quantifying milk intake [69], it remains essential to measure dose–response relationships to understand the impact on microbiota outcomes. However, standardised easy-to-use methods are still needed.

Another interesting topic is the use of mother-own-milk and donor milk in preterm infants as breasfeeding influence the preterm infant microbial assembly [70,71]. Donor milk from milk banks is often dosed at **150–180 mL/kg/day**. It is mainly used in low birth-weight preterm infants and has been linked to a lower risk of NEC and other morbidities, as well as to adequate growth and development [72]. Donor milk is processed and pasteurised (heat-treated) to prevent contamination by bacteria and viruses and ensure safety for vulnerable preterm neonates. Although most nutrients are preserved during pasteurisation, this process can significantly impact milk composition and partially reduce its bioactivity [73]. Specifically, certain bioactive compounds, particularly immune-related substances, are diminished, while others such as HMOs remain stable. In addition, because donor milk is pooled from multiple donors, the nutritional content cannot be guaranteed to be consistent across different batches. These changes in milk composition are reflected in the preterm microbiome.

Microbial succession from infancy to weaning

The impact of feeding type on infant microbiome evolution has attracted considerable interest. However, there is limited information on how weaning, the introduction of complementary foods, and childhood diet influence the infant microbiome and overall health. Similarly, much remains unknown about the relevance of the timely initiation of breastfeeding, including how colostrum deprivation, or delays in initiation, impact infant health. The effects of prelacteal feeding (fluids or solids given before breastfeeding is established), breastfeeding duration, the timing of weaning, and complementary food introduction – including the timing, food-type, and combination with milk-feeding – may shape the microbiome, with potential consequences for the immune system, metabolism, and neurodevelopment.

Current guidelines suggest complementary food introduction around 4 to 6 months of age [74,75]. This period can become a second window of opportunity to modulate the child's microbiota evolution. At this stage, different scenarios appear and, although human milk remains the main food source, infants begin to be supplemented with formula and/or complementary foods. Importantly, extended breastfeeding practices beyond 6 months are linked to an increase in *Bifidobacterium* spp., particularly *B. longum*. Current evidence suggests that infants at 'higher risk of developing food allergies' may benefit from early complementary food introduction (from 4–6 months of age) [76] with a diverse diet at 9 months old [77]. However, there is only limited data on the impact of weaning on the microbiome. It is known that weaning increases the structural and functional diversity of the infant gut microbiota towards an adult-like microbiota [78], with continued influence on the immune system in response to new nutritional sources and foods [79]. The introduction of solid foods introduces new substrates (e.g. complex carbohydrates, proteins, and fibres), driving a microbial shift from a milk-adapted microbiome that thrives on HMOs to a fibre- and protein-degrading community[67]. The infant microbiota during complementary food introduction is characterised by an increase of *Bacteroidetes, Akkermansia,* and *Clostridium* groups IV and XIV, as well as the presence of *Eggerthella, Blautia, Neisseria*, and *Ruminococcaceae*, whose members are capable of degrading glycans, mucin, and complex carbohydrates while producing short-chain fatty acids (SCFAs)[80]. Some studies have also shown an increase in *B. longum subsp. longum* [81] and bifidobacteria species succession[18] during this period. The complementary feeding is reported to have a more profound effect on

the infant gut microbiota of non-breastfed infants compared to breastfed infants [82]. Despite the evidence for the overall effect of introducing solid foods, there is a need for extensive and comprehensive research on the specific impact of each food and/or its components (nutrients and bioactives) on the development and evolution of the infant microbiome.

Beyond the first year: microbial trajectories from childhood to adolescence

There is no clear consensus on when the infant gut microbiota fully matures to resemble an adult-like microbiota. While its microbial diversity and composition are generally comparable to those in adults by approximately 2–5 years of age, some studies suggest that microbial assembly and evolution may continue for a longer period. Based on age-related shifts in microbiome composition, three stages of microbiome maturation have been proposed: early childhood (3–6 years), middle childhood (6–12 years), and adolescence (12–18 years) [83–85]. There are few longitudinal studies describing the role of the infant diet on early gut microbial trajectories, and most only cover the first 12−18 months of life [16,86–88]. Similarly, childhood diet is also heavily implicated in variations in the oral microbiota community and function [89,90].

In general, the childhood gut microbiota is characterised by higher diversity, higher quantity, and a predominance of members from *Lachnospiraceae* and *Ruminococcaceae* and genera *Bacteroides*, *Prevotella*, and *Bifidobacterium*[83]. A transition period between 15−30 months has been described in which the abundance of *Bacteroidetes* and Pseudomonadota increases[26]. This is followed by a microbial stable phase between 31 and 46 months in which animal protein and plant polysaccharide intake associate with lower *Bifidobacterium* levels and enrichment of Bacteroidota and Bacillota members [26]. In parallel to these observations, significant differences have been reported between children and adults in bacterial genes associated with amino acid catabolism, oxidative phosphorylation, vitamin synthesis (particularly B9 and B12), and factors associated with mucosal inflammation [85].

A balanced diet has been shown to promote microbial diversity and functionality. Several studies have shown that a Mediterranean diet enriches the microbiome in SCFA-producers, including *Lachnospiraceae* and *Ruminococcaceae* members [91–94]. Adherence to a Mediterranean diet is also associated with a decrease in *Enterobacteriaceae* (mainly *E. coli*) and an increase in bifidobacteria and SCFAs in adults [95,96]. However, a Western lifestyle characterised by higher intake of high-fat and -sugar foods, animal protein, and processed products, coupled with insufficient physical activity, is linked to a proinflammatory status and a higher risk of NCDs [97,98]. Specifically, Western diets promote a predominance of Bacteroidota, while the consumption of fibre, mainly by higher intake of fruits and vegetables, increases the presence of *Prevotella* and *Lachnospiraceae* members, enriching SCFA levels [99]. On a broader scale, there is evidence that variations in geographical location, ethnicity, and lifestyle have an impact on the microbiota in healthy individuals [100–102]. Although most of these studies were done in adults, it is likely that diet and lifestyle have similar effects on the microbiome of children.

Feeding the microbes: What is in human milk?

Breastfeeding represents an evolutionary strategy that fosters a symbiotic relationship between host and microbes, ultimately shaping lifelong well-being. Human milk is not just food; it is a complex biological system that goes beyond its nutritional aspects. It contains thousands of bioactive compounds uniquely tailored to support neonatal health, including microorganisms, metabolites, oligosaccharides, antimicrobial proteins, growth factors, and immunomodulatory molecules[103] (see **Table 1**). These components have specific targets and nourish both the infant and its microbiome, thereby influencing metabolism, immune maturation, and long-term health outcomes [104,105].

Maternal genetics influence human milk composition variation, playing a significant role in HMO makeup [106,107]. In addition, lactating mammary gland gene expression has been linked to IL-6 levels in human milk and to the infant microbiota, mainly *Bifidobacterium* and *Escherichia* [108].

Infections can also play a role in milk composition, as demonstrated by human cytomegalovirus (CMV), which is commonly transmitted through human milk. CMV alters milk composition by upregulating the indoleamine 2,3-dioxygenase (IDO) tryptophan-to-kynurenine pathway, which modulates the infant microbiome in a way that reduces *Bifidobacterium* abundance and affects full-term infant growth [109]. Human milk contains various bioactive compounds that significantly influence the development and composition of both the gut and oral microbiota in infants. For example, milk fat globule membrane (MFGM) is associated with improved clinical outcomes, in particular with regard to infections and neurodevelopment [110]. Consistent with this observation, preclinical data and clinical studies with bovine MFGM have demonstrated its impact on gut microbiota [111,112]. MFGM, which is rich in glycoproteins and glycolipids, has also been shown to inhibit pathogen adhesion and support the proliferation of beneficial microbes[113], thereby enhancing the infant's immune defences. **Table 2** summarizes milk components and their relations with downstream pathways and health. However, the most extensively studied human milk compounds remain HMOs and certain milk microbes.

Human milk also provides critical passive immunity to infants through a diverse range of bioactive compounds, including maternal antibodies (particularly secretory IgA), lactoferrin, cytokines, growth factors, immune cells, microbes, and HMOs[104]. These immune-related factors directly influence the developing infant gut microbiome by selectively promoting beneficial bacteria, providing antimicrobial protection against pathogens, and shaping immune system maturation. For example, maternal IgA coats specific commensal microbes, facilitating their colonisation and establishment[114], while lactoferrin exerts antimicrobial activities and modulates inflammatory responses. Additionally, HMOs serve as prebiotic substrates that selectively nourish beneficial taxa such as bifidobacteria, further enhancing gut barrier function and immune resilience. They can also directly modulate infant immune responses by interacting with gut epithelial and immune cells, influencing cytokine production, leukocyte trafficking, and inflammation, thus actively shaping early immune development and maturation. Several reviews specifically dive in to the immune-mediating role of human milk [114–117].Despite this growing knowledge, significant gaps remain. Specifically, the precise identities of the microbial strains that are transferred through human milk and their functional interactions with immune components are still incompletely characterised. Furthermore, our understanding of the complex interplay between HMOs, immune factors, and distinct infant microbiomes is limited. Clarifying how these processes vary across different populations, maternal health conditions, and feeding practices (exclusive breastfeeding vs. mixed feeding) represents a critical area for further investigation that is essential for developing targeted interventions to support optimal infant health.

Human milk microbiota and HMOs: key architects of the infant microbiome

HMOs are indigestible for the infant but serve as selective substrates for bacteria, selectively promoting the growth of beneficial bacteria, such as *Bifidobacterium* species, and exerting a wide range of activities [118]. Recent studies have demonstrated that other microbial genera are able to use HMOs, including *Bacteroides* [119], *Akkermansia* [120], and the *Roseburia-Eubacterium* group [121]. These bacteria possess specific enzymes to metabolise HMOs, leading to the production of beneficial molecules. These include SCFAs, which support gut health and homeostasis [122], tryptophan, γ-aminobutyric acid (GABA), indoles, and lactate, which serve as important signalling metabolites. These metabolites communicate with the host and influence various physiological processes, including gut–brain communication, energy metabolism, and immune system education [123,124]. Specific HMOs have been linked to health outcomes, for example disialyllacto-N-tetraose has been linked with risk reduction in NEC[125] and 3'-Sialyllactose with lower inflammation and atherosclerosis [126]. This mutualistic mother–infant interaction has evolved to optimise infant health, growth, and survival, highlighting the intricate interdependence between maternal milk and the developing gut microbiome.

Maternal genetics, mainly variants in the fucosyltransferase loci *FUT2* and *FUT3/6*, shape the human milk HMO profile, but they also influence the mucosal glycan profile, specific virus infection risk, and shifts in the gut microbiome. Studies have reported that maternal secretor status influences infant gut *Bifidobacterium* and *Bacteroides* levels [127]. Similarly, an individual's own secretor status may also significantly shape their gut microbial communities, e.g. adult non-secretors typically have lower bifidobacteria levels compared to secretors [128](Wacklin et al., 2011). In addition, studies in adults show that secretor status and its interaction with genetic variants in the ABO locus encoding blood group determine variations in the genetic diversity of gut bacteria[129] . Simlarly, one study found that the infant's secretor status, but not that of the mother, was associated with infant microbial colonisation and metabolic performance [130] . However, there is only limited research exploring how infant secretor status impacts specific microbial species and how interactions between infant secretor status and maternal milk types (secretor versus non-secretor) influence microbiome development. Furthermore, the interplay of secretor status with additional environmental and genetic factors is poorly understood, and its potential association with long-term health outcomes warrants further investigation. Several studies have also reported an association between maternal secretor status and infant health outcomes, mainly allergy-related problems [131,132]. It should be noted that, while clinical trials adding HMOs to formula milk shifted the infant gut microbiome towards a more "breastfed infant-like" status [133], several association studies of HMO profiles with the infant gut microbiome showed weak or no correlation [36,134]. New research using synthetic communities is providing new insights into HMO use and cross-feeding [135]. While the gut microbiome of breastfed infants is largely different from that of formula-fed infants, the individual-specific HMO profile shows a less pronounced effect and is largely individualised, depending on maternal genetics and lifestyle.

Furthermore, human milk is a transfer route for potential probiotic bacteria, including strains of lactic acid bacteria, which can colonise the infant's gut and oral cavities, contributing to a balanced microbiota and reduced risk of infections. Different studies have shown the presence of *Staphylococcus, Streptococcus* members, *Pseudomonas*, and *Acinetobacter*, as well as of *Bifidobacterium* and *Lactobacillus*, which were identified and isolated from human milk [136]. It has been reported that 27.7% of the bacteria in the infant gut comes from human milk and 10.3% from areola skin [137], again highlighting the key impact of breastfeeding in shaping the infant gut microbiome[138,139]. Specific human milk microbial strains have been extensively studied as potential probiotic strains and have been included in the design of new infant formula compositions, together with other biotics from human milk, in an attempt to leverage their potential functions [140], although the exact mechanisms of action are uncovered. In addition, milk isolates have also been used to prevent and treat mastitis-related problems [141,142]. Recognising human milk as a nutritional and microbial blueprint allows for the development of targeted strategies to prevent or treat microbiome-related diseases in mothers and infants. The evolutionary co-adaptation between milk composition and the infant gut microbiome provides key insights into the precise shaping of microbial communities in infants. This knowledge opens the door to innovative therapeutic approaches, including personalised probiotics, prebiotics, and symbiotics designed to replicate the beneficial microbial-modulating effects of human milk.

Nutrient sensing and microbiota: how early microbes detect and respond to dietary compounds

As mentioned, human milk is more than nutrition – it serves as a biochemical signalling system that regulates neonatal metabolism, immune function, and microbiome development. Through a complex network of nutrient signals and molecular sensors, human milk interacts with the microbiota and the host, affecting human health. There are critical roles for nutrient-sensing receptors and signalling pathways [143,144] in linking milk and dietary components (e.g., fatty acids,

amino acids, SCFAs) to infant growth, metabolism, immunity, and gut–brain communication (as summarised in **Table 3**). There is a growing evidence on mechanisms of action from in vitro and preclinical models, but due to the complexity of the topic, this review does not fully cover these studies and further in-depth and targeted reviews will be needed.

Human milk plays a key role in shaping an infant's gut health and microbiota through various nutrient-sensing pathways (**Figure 1**) that are also critical during the introduction of complementary foods and the replacement of food sources. The process by which gut microbes and host cells detect and respond to dietary components and bioactive compounds is pivotal in shaping the early microbial ecosystem and health programming. The impact of bioactive and nutritional compounds in human milk on the co-development of the gut microbiome, antigen tolerance, and immunity has been highlighted previously [79]. Early microbes interact and metabolise specific bioactive compounds that guide gut health development, such as HMOs, lactose, and lipids. As mentioned above, microbes can break down otherwise indigestible complex carbohydrates, proteins, and fats, making essential nutrients more available for absorption. A recent review examined how bifidobacteria metabolise complex carbohydrates, highlighting their cross-feeding interactions within the gut microbiota [145]. These interactions mainly involve early-colonising *Bifidobacterium* metabolising dietary glycans into simpler compounds that are then utilised and cross-fed by other beneficial microbes. This metabolic cooperation contributes to gut homeostasis and offers potential health benefits, such as improved gut barrier function and immune modulation.

Complex interactions between *Bacteroides* and *Bifidobacterium* have also been described, specifically concerning β-glucan and arabinoxylan metabolism [146]. SCFAs modulate the AMP-activated protein kinase (AMPK) and mechanistic target of rapamycin (mTOR) pathways, which both play critical roles in regulating energy metabolism. Microbial production of other metabolites like bile acids and indole compounds further influence nutrient absorption and metabolic regulation. As an example, *Bifidobacterium* are able to convert aromatic amino acids into their respective aromatic lactic acids, with a significant influence on immune function in early life [147]. Microbial tryptophan catabolites activate the immune system by binding to the aryl hydrocarbon receptor (AhR) [148], which has multiple effects including modulation of the gut microbiota [123,149]. Specific metabolites, particularly indole-related compounds produced by *Bifidobacterium*, can modulate inflammation via the AhR and nuclear factor erythroid 2-related factor 2 (Nrf2) pathways [150–152]. In addition, complement components in human milk have been linked to microbiome shifts that enhance immune tolerance to potential pathogens [153]. The metabolic products of microbes also activate G-protein-coupled receptors in gut cells, including GPR41 and GPR43, to influence energy metabolism, insulin sensitivity [154], and the intestinal IgA response to microbiota [155]. Microbial metabolites also modulate the expression of specific genes involved in nutrient sensing, including peroxisome proliferator-activated receptors (PPARs) and AMPK, which are key players in cellular energy regulation and are activated by metabolic ligands, such as dietary polyunsaturated fatty acids, amino acids, vitamins, and phytochemicals. Furthermore, *Bifidobacterium* is known to produce GABA, a key neurotransmitter in the gut–brain axis [156]. GABA production has been associated with neurodevelopmental processes and is linked to a reduced risk of autism spectrum disorders [157]. Beyond GABA, other microbially derived neurotransmitters, such as serotonin (5-HT), dopamine, and acetylcholine, also would play essential roles in gut–brain signalling, mood regulation, and cognitive function. These compounds, which are influenced by early-life microbial colonisation, may have lasting effects on neurodevelopment, stress resilience, and mental health outcomes.

Bile acids have also been shown to drive infant gut microbiota maturation [158] and provide protection against norovirus infection when sourced from breast milk [159]. Bile acids, produced in the liver and modified by gut microbes, impact the microbiome community structure and function [160] and play a crucial role in fat metabolism [161]. Microbial colonisation would be linked

to adipose tissue development and function, with specific bacteria shown to promote fat storage and modulating lipid metabolism[162]. An imbalance in the gut microbiota during early life may promote a greater accumulation of body fat and increase the risk of obesity. While most of the research to date has been done in bacteria, recent studies have shown the relevance of yeast and fungi [163]. Microbiota also play a pivotal role in regulating nutrient-sensing systems, which are responsible for detecting and responding to the ghrelin, insulin, glucagon-like peptide-1 (GLP-1), and leptin that are present in human milk [105,164]. GLP-1 helps regulate insulin secretion, appetite, and food intake and influences infant growth. A disrupted microbiota may impair GLP-1 production, contributing to dysfunctional nutrient sensing and excessive food intake, a key driver of obesity. In addition, during the transition to complementary foods, microbial fermentation of dietary fibres leads to the production of SCFAs, which can stimulate the release of GLP-1 from the intestine [165,166]. The release of GLP-1 by enteroendocrine cells is also induced by indoles produced by microbial tryptophan metabolism [149].

Specific maternal nutrients, such as amino acids (especially branched-chain amino acids, methionine, arginine, and lysine), are crucial building blocks for milk protein. These nutrients play a key role in the regulation of milk synthesis and function as potential energy sources for neonates and their microbes [167,168]. In a preclinical *in vitro* model, essential amino acids from diet, *Acetobacter pomorum,* and some strains of *Lactobacillus* were found to be critical joint modulators of food choice [167]. Thus, an understanding of how early-life nutrition influences microbial colonisation and nutrient sensing will provide new insights into the critical role of early nutrition in shaping long-term health outcomes. More research is needed to identify the key components in human milk and later infant diet that control or modulate microbial nutrient-sensing.

Future directions: from maternal–infant health to next-generation nutrition

A mother's diet and overall health during pregnancy and lactation plays a critical role in shaping the composition and function of human milk, directly influencing its microbial content and nutritional and bioactive profile. These factors are essential for optimal infant development. Emerging evidence demonstrates the impact of breastfeeding and human milk bioactives on microbial assembly and long-lasting protective health effects, potentially reducing the risk of NCDs in life. This growing understanding of maternal–infant microbiome interactions is driving the development of personalised nutritional strategies, including biotics such as probiotics, prebiotics, symbiotics, and postbiotics. These approaches will aim to improve the microbial, metabolic, and immunological programming in infancy. Personalised nutrition also offers new opportunities to optimise the benefits of human milk by tailoring interventions to individual needs, which is particularly relevant for vulnerable populations. Additionally, emerging technologies, such as synthetic HMOs, precision fermentation, microbiome-tailored novel biotics, and live biotherapeutic products (LBPs), represent new approaches to bridge the gap between the natural benefits of human milk and its translation into clinical applications for non-breastfed infants and other at-risk populations.

Optimising early-Life colonisation: reality or myth? Infant microbiome modulation has garnered enormous interest in recent years. While human milk is the gold standard in infant nutrition and contains bioactive compounds with effects on infant health, formula-fed infants may benefit from supplementation with potential bioactives such as galacto-oligosaccharides and fructo-oligosaccharides or other saccharides and specific HMOs to promote the dominance of *Bifidobacterium* species. This opens up the potential for target-based microbiome modulators to drive innovative developments [169], as recent advances in next-generation prebiotics, such as polyphenols, resistant starches, and newly available HMOs, are expanding microbiome-targeted strategies. These interventions support the growth of beneficial taxa, including *Akkermansia muciniphila*, *F. prausnitzii*, and *Ruminococcus* species [170], which are now considered part of the

next generation of probiotics [171]. Innovation in LBPs is also gaining momentum for their potential in precision microbiome modulation. Furthermore, postbiotics – inanimate microbial components or metabolites such as SCFAs, exopolysaccharides, and antimicrobial peptides – are emerging as promising alternatives to live probiotics. These offer immune and gut barrier support without concerns about microbial viability, making them particularly attractive for clinical applications.

Beyond nutritional interventions, novel microbiome-based approaches, such as faecal microbiota transplantation and vaginal microbial seeding, are being explored to modulate the infant microbiome and restore maternal microbial transmission, especially for infants born via C-section who miss critical maternal microbial exposures [172,173]. However, the rationale underpinning these approaches, their clinical outcomes, and particularly their long-term safety and efficacy remain areas of active debate and controversy, highlighting a lack of consensus within the field. While vaginal seeding aims to reintroduce a maternal *Lactobacillus*-dominated microbiome, its safety and efficacy require further validation before widespread clinical implementation. These strategies hold promise for high-risk populations, including preterm infants, C-section-born infants, and those predisposed to inflammatory or metabolic conditions. Another emerging microbiome-based strategy is "milk microbiota transplantation," which focuses primarily on restoring donor milk or formula with the mother's own milk microbes, and is mainly considered for use in driving microbiota establishment in preterm neonates [174]. Additionally, defined or synthetic consortia and biotherapeutics initially developed for *C. difficile* infections are now being explored for broader applications in early-life microbiome modulation. The potential for engineered bacterial consortia to optimise microbial colonisation and metabolism in infancy is also emerging as an exciting frontier [175].

Personalised feeding and nutritional approaches represent the next major step in early-life microbiome modulation. Advances in microbiome sequencing, metabolomics, and artificial intelligence are paving the way for microbiome-guided nutrition, where feeding strategies are adapted to an infant's unique microbial and metabolic profile. Tailored formula compositions and new products for children incorporating specific HMOs, biotics, biotherapeutics, and nutrient-sensing modulators may help optimise gut colonisation evolution and immune programming. This precision nutrition approach has the potential to transform infant health by providing targeted, microbiome-compatible dietary interventions that support long-term well-being.

Conclusion

Breastfeeding plays a crucial role in shaping microbial development, metabolic health, and immune function during early life. A deeper understanding of its bioactive components and their interactions with the microbiome can drive innovations in infant nutrition and long-term health strategies. Moreover, these insights extend beyond infancy, offering potential benefits for other populations, including the elderly and immunocompromised individuals, and inspiring novel therapeutic applications for disease prevention and treatment. The future of microbiome-based interventions in early life lies in personalised nutritional strategies designed to optimise microbial composition and metabolic function. By tailoring feeding practices – whether through customised HMOs, probiotic-prebiotic, or other biotic combinations – it may be possible to enhance nutrient sensing, immune programming, and metabolic health from infancy onward. To fully realise this potential, interdisciplinary collaboration between microbiologists, clinicians, and nutritionists will be essential. This will ensure that microbiome-targeted strategies are not only scientifically robust but also clinically effective and scalable for widespread application in public health and personalised medicine.

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AUTHOR CONTRIBUTIONS

The authors contributed equally to this review.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with the publication of this paper.

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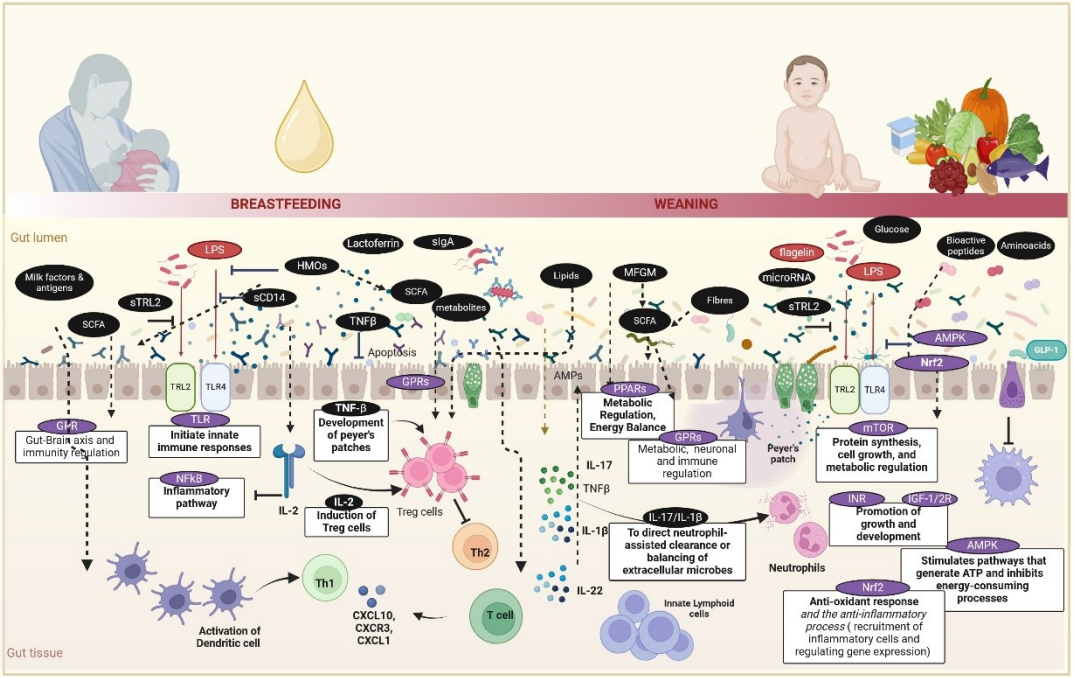

Figure 1. Early-life nutritional sources and human milk composition supports infant gut microbiome development and immune system maturation. This figure illustrates the potential mechanisms of action and the nutrient signalling pathways (described in **Table 3**) that mediate the complex interactions between human milk bioactive compounds (described in **Table 2**), early foods and dietary compounds (fibres), the infant gut microbiota, and infant immune system development during the critical window from birth up to childhood. Key bioactive components that contribute to microbiome modulation, gut barrier integrity, and immune development include components from human milk (oligosaccharides (HMOs), immunoglobulins, antimicrobial peptides, fatty acids and milk fat globule membrane (MFGM)) and microbiota-derived metabolites (short-chain fatty acids (SCFAs), tryptophan catabolites, and other metabolites) and compounds (lipopolysaccharides (LPS), flagellin, cell walls, proteins, etc.).

Table 1. Specific changes in the infant microbiota depending on complementary food introduction in the presence or absence of breastfeeding practices.

Aspect	Details	Impact with lactation Breastfeeding + Solid food intro	Impact without lactation Weaning + Solid food intro
Microbiota Changes	- Decline in *Bifidobacterium* - Increase in *Bacillota* and *Bacteroidota* - Increased diversity	- Smoother transition - *Bifidobacterium* supported by HMOs in breast milk - Gradual shift in microbiota - Supports immune tolerance	- Faster decline in *Bifidobacterium* - Higher *Clostridium* and *Enterobacteriaceae*
Immune System Response	- Maturation of gut-associated lymphoid tissue - Development of Tregs and IgA production - Microbiota–host interactions (SCFAs, immune signalling) - Dietary diversity	- Promotes immune tolerance. - Breast milk provides immunomodulatory factors (IgA, cytokines) - Reduced inflammation	- Increased risk of immune overreaction - Higher risk of allergies and intolerances
Clinical Implications	- Promotes long-term metabolic and immune health	- Reduces risk of allergies, obesity, and autoimmune diseases	- Higher risk of dysbiosis, allergies, and metabolic disorders

Key references included in the expanded section

Table 2. Human milk bioactive compounds, key potential functions, main targets and potential pathways involved.

Component	Key Functions and Mechanisms	Main Targets	Potential Pathways Involved
Human Milk Oligosaccharides	• Selectively feed *Bifidobacterium*, promoting a gut microbiome that enhances metabolic and immune development.	Immunity, Gut barrier Integrity, Microbiota	NF-κB, TLR signalling, and SCFA production
	• Act as decoy receptors, preventing pathogen adhesion and shaping immune tolerance.	Immunity, Pathogen Defence	Glycan-mediated pathogen inhibition and immune modulation
	• Modulate intestinal epithelial cell signalling, influencing gut barrier integrity and inflammation.	Cellular Signalling, Immunity	MAPK, Tryptophan-serotonin metabolic pathway
Lipids and Fatty Acid Sensing	• Long-chain polyunsaturated fatty acids like DHA and ARA support neurodevelopment and immune regulation.	Neurodevelopment, Immunity	PPAR-γ and NF-κB pathways
	• Fatty acids interact with peroxisome proliferator-activated receptors (PPARs) to regulate lipid metabolism and energy balance.	Metabolic Regulation	PPAR signalling pathway
	• Milk-derived extracellular vesicles transport lipid-based signalling molecules to influence cellular function.	Cellular Signalling, Immunity	Endocytosis and intracellular signalling pathways
Protein-Derived Bioactive Peptides	• Lactoferrin binds to intestinal cell receptors, modulating iron homeostasis and antimicrobial defence.	Immunity, Cellular Signalling	LRP1 and TLR4 signalling pathways
	• Casein-derived peptides influence gut motility, mucosal integrity, and immune responses.	Cellular Signalling, Immunity	Opioid receptor and TGF-β signalling pathways
	• α-Lactalbumin regulates serotonin production, impacting infant sleep and mood.	Neurodevelopment, Cellular Signalling	Tryptophan-serotonin metabolic pathway
MFGM Components	• Phospholipids & Sphingolipids: Support brain development (myelination) and influence gut microbiota (*Lactobacillus*, *Bifidobacterium*).	Neurodevelopment, Gut Microbiota, Inflammation	PPAR-γ and NF-κB pathways
	• Gangliosides & Glycoproteins: Regulate pathogen exclusion and promote neuronal growth and cognitive function.		EGFR/MAPK and Wnt/β-catenin pathways
Immunoglobulines & Antibodies	• including IgA, soluble IgA [SIgA], IgG, IgM, IgE, and IgD • SIgA, most abundant, binding to antigens present on toxins, viruses, and both commensal and pathogenic microbes • Immunoglobulins (IgA) selectively coat bacteria, promoting symbiotic microbial colonisation. • IgG and IgA dampen mucosal T helper cells.	Immunity	TGF-β and JAK/STAT pathways
MicroRNA (miRNA)	• Human milk contains microRNAs that regulate gene expression in immune cells and intestinal tissues.	Epigenetics, Immunity	RNA interference and epigenetic regulation pathways
	• Contribute to metabolic programming, influencing obesity risk and immune development.	Epigenetics, Metabolic Regulation	PI3K/Akt and mTOR signalling pathways
Hormones and Growth Factors	• Leptin and adiponectin regulate infant appetite, energy balance, and fat metabolism.	Metabolic and Endocrine Regulation	JAK/STAT and AMPK signalling pathways
	• Epidermal Growth Factor (EGF) promotes intestinal maturation and repair.	Cellular Signalling, Immunity	EGFR/MAPK signalling pathway
	• Insulin-like Growth Factor (IGF-1) supports growth and tissue development.	Neurodevelopment, Metabolic and Endocrine Regulation	IGF-1/PI3K/Akt signalling pathway
	•Leptin and Adiponectin influence the gut-brain axis, appetite regulation, and microbial colonisation patterns.	Neurodevelopment, Metabolic Regulation	JAK/STAT and AMPK signalling pathways
Polyamines (e.g. spermidine, spermine)	•Enhance intestinal cell growth and gut homeostasis.	Gut Homeostasis, Cellular Growth	Polyamine biosynthesis pathways
Microbial Metabolism of Bile Acids	•Conversion of primary to secondary bile acids influences lipid digestion, energy metabolism, and microbiota diversity.	Metabolic Regulation, Gut Microbiota	FXR and TGR5 bile acid signalling pathways

*Key references included in the expanded section

Table 3. Specific nutrient receptors and sensors activated by human milk compounds and other dietary sources during the complementary food introduction in childhood.

Nutrient Sensors	Key Bioactive Signals and Mechanisms	Main Targets	Pathways
PPARs (Peroxisome Proliferator-Activated Receptors)	• Fatty acid sensing regulates lipid metabolism, glucose homeostasis, and inflammation. • Modulate energy storage and utilisation.	Metabolic Regulation, Energy Balance	PPAR-α, PPAR-γ signalling pathways
mTOR (Mechanistic Target of Rapamycin)	• Activated by amino acids, mTOR promotes protein synthesis, cell growth, and metabolic regulation. • Integrates nutrient availability with cellular energy demands.	Growth, Development, Metabolism	mTORC1 and mTORC2 signalling pathways
TLRs (Toll-like Receptors)	• Recognise microbial components (e.g., LPS, peptidoglycan) to initiate immune responses. to initiate innate immune responses and shape immune tolerance. • Facilitate cross-talk between the gut microbiota and immune system.	Immunity, Microbiota Interaction	TLR4/NF-κB and MyD88 signalling pathways
GPRs (G-Protein-Coupled Receptors)	•Activated by SCFAs (e.g. acetate, propionate, butyrate) derived from HMO fermentation, GPRs link gut microbiota activity to the gut–brain axis and immune regulation. • Influence appetite regulation, gut motility, and inflammation.	Gut–Brain Axis, Immunity	GPR41/43 signalling pathways
Insulin/Insulin-Like receptors (INR, IGF-1R and IGF-2R)	•Respond to glucose and amino acids, promote growth and development.	Growth and metabolic function	Insulin/Insulin-Like Growth Factor (IIS) Pathway, PI3K-AKT and MAPK/ERK
AMP-Activated Protein Kinase (AMPK)	• Regulates mammary milk protein synthesis. • An energy sensor, AMPK is activated under low energy conditions, stimulating pathways that generate ATP and inhibiting energy-consuming processes. • Certain nutrients and metabolites, such as branched-chain amino acids and polyunsaturated fatty acid can activate AMPK	Metabolism	mTORC1 and mTORC2 signalling pathways

**Key references included in the expanded section*