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Human Milk Oligosaccharides in Nutrition and Health: the past, present, and future

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ABSTRACT: Human milk is considered the most appropriate and balanced form of nutrition for infants, and remains the sole recommended source of nourishment during the newborn period. Beyond its role in supplying essential nutrients, human milk naturally contains a complex mixture of biologically active substances that contribute to infant growth, immune protection, and overall health. Among these components, human milk oligosaccharides (HMOs) stand out for both their abundance and structural diversity. Found exclusively and in high concentrations in human milk, HMOs function as prebiotic molecules that are not digested by human enzymes. Instead, they foster the colonization of beneficial intestinal microbes, block the attachment of pathogens to epithelial surfaces, influence gut and immune signaling pathways, and have been linked to favorable effects on brain development and cognitive performance in early life. In this review, we first trace the historical progression of HMO research and summarize the key milestones in studies conducted domestically and internationally. We then summarized the preclinical and clinical data on HMOs over the past decade. Finally, we highlighted the recent progress in the synthesis and production of HMOs, as well as current understanding of their metabolic utilization by the infant gut microbiota during breastfeeding. Overall, HMOs serve as key bioactive factors supporting infant nutrition, microbial balance, and immune protection. Therefore, breastfeeding remains the most natural and effective approach to deliver these functional components and promote healthy growth and development in early life.

Keywords: Human milk oligosaccharides, Infant nutrition, Prebiotics, Synthesis, Metabolism

1. Introduction

Human milk is regarded as the exclusive and optimal source of nourishment for newborns. It provides not only essential nutrients for immediate growth but also a variety of bioactive components that contribute to long-term health outcomes. The World Health Organization (WHO), together with leading pediatric societies, advocates initiating breastfeeding within the first hour after delivery and maintaining exclusive breastfeeding for the first six months of life, with possible continuation of up to two years^[1]. In addition to

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macronutrients (including lipids, proteins, and lactose) and micronutrients (including vitamins and minerals), human milk is rich in human milk oligosaccharides (HMOs). Although HMOs cannot be digested by infants, they function as prebiotic substrates that selectively stimulate the growth of beneficial gut microorganisms and enhance immune protection by reducing pathogen adhesion and colonization^[2,3].

HMOs constitute an important group of bioactive molecules within human milk, distinguished by their remarkable abundance and structural complexity. The exceptional nutritional and physiological properties of human milk have drawn considerable scientific attention, leading to extensive exploration of HMOs and their biological functions^[4]. Historical records trace the earliest recognition of these carbohydrate components to the late 19th century, when biologist Georges Denigés reported sugar structures beyond lactose in both human and bovine milk^[5]. With the advent of advanced glycomic analyses and mass spectrometry, researchers have since identified and characterized an expanding repertoire of HMO structures.

The time span from conception to approximately 2 years of age—known as the *first 1,000 days of life*—represents a pivotal phase for human growth and development as well as for the prevention of chronic diseases later in life^[6]. British epidemiologist David Barker first introduced the “fetal origins of adult disease” hypothesis, suggesting that adverse environmental or nutritional exposures during early development may alter long-term metabolic programming, heightening the risk of adult-onset diseases^[7]. This concept later evolved into the “Developmental Origins of Health and Disease” (DOHaD) framework, which emphasizes that early-life environmental factors play a decisive role in shaping lifelong health and disease vulnerability^[8]. For infants, breastfeeding has been associated with multiple health benefits, including lower incidence of otitis media, type 1 and type 2 diabetes, overweight, and obesity, as well as modest improvements in cognitive outcomes, such as a 2–3 point increase in IQ. Mothers who breastfeed may also experience reduced risks of breast and ovarian cancers^[9]. Preclinical and clinical research have further indicated that HMOs contribute to maintaining intestinal microbial balance^[10], strengthening epithelial barrier function^[11], preventing diarrheal infections^[12], and potentially supporting immune regulation^[13], neural maturation, and cognitive development^[14]. Traditionally, HMOs are isolated directly from human milk; however, this method is unable to meet the demands of large-scale production. Given that not all infants are breastfed, interest in incorporating HMOs into infant formulas has grown. Early formula preparations utilized non-human prebiotic oligosaccharides such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and polydextrose (PDX)^[15], though these compounds lack the fucose and sialic acid residues characteristic of HMOs^[16]. Recent technological progress has enabled the biosynthesis of HMOs through enzymatic and chemical routes, microbial fermentation, and cell-based systems, overcoming previous production constraints. Several of these synthetic HMOs—such as 2'-fucosyllactose (2'-FL), lacto-*N*-tetrasaccharide (LNT), lacto-*N*-neotetraose (LNnT), 3'-sialyllactose (3'-SL), and 6'-sialyllactose (6'-SL)—have been introduced into commercial infant formulas since 2016, allowing non-breastfed infants to receive oligosaccharides structurally identical to those naturally found in human milk^[17]. Nevertheless, current formulations still represent only a small fraction of the full diversity of HMOs present in human milk.

Although only a small fraction of HMOs is absorbed in the small intestine, the majority reaches the colon, where they act as substrates for specific members of the gut microbiota^[18]. While *Bifidobacterium* species are the primary consumers of HMOs, certain *Bacteroides* and *Lactobacillus* species also utilize them, albeit to a lesser extent^[19]. HMOs are metabolized by these microorganisms through a variety of pathways. Notably, a clinical study found that infant-associated bifidobacteria convert HMOs into lactic acid and acetate, leading to a decrease in intestinal pH and contributing to the mitigation of dysbiosis^[20].

Despite significant advances in elucidating the biological functions of HMOs, several key questions and controversies remain unresolved. One major challenge lies in the structure-function specificity of HMOs. Although over 200 distinct structural variants of HMOs have been identified, mounting evidence has indicated that individual HMOs exert different biological effects based on their monosaccharide composition, bond types, and degree of fucosylation or sialylation. However, a comprehensive understanding of how specific HMO structures translate into unique functional outcomes remains incomplete^[21, 22]. Another critical issue is the dose-response relationship of HMOs. Existing mechanistic studies primarily rely on in vitro models, animal experiments, or clinical trials, which employ only a limited variety of HMOs at predetermined concentrations. Such experimental dosages may fail to accurately reflect the dynamic and complex exposure patterns during breastfeeding, where HMO concentrations and compositions exhibit significant variations across different lactation stages, as well as interindividual differences^[23, 24]. Therefore, determining physiologically relevant and clinically effective HMO dosages remains challenging. As mentioned previously, HMO composition exhibits individual variability. Maternal genetic factors, particularly secretor status and Lewis blood group, as well as environmental influences, significantly shape the HMO profile, potentially leading to differences in infant gut microbiota development, immune maturation, and health outcomes^[25-27]. This specificity complicates the interpretation of clinical research results and underscores the necessity for future HMO research to adopt personalized or stratified strategies. Addressing these unresolved issues is crucial for deepening mechanistic understanding and guiding the rational design of HMO-rich nutritional intervention programs.

This review traces the research history of HMOs, highlights findings from both preclinical and clinical studies, and outlines current knowledge regarding their synthesis and metabolic pathways.

2. Evolution of HMO Research

Over the past century, research on HMOs has undergone several distinct stages, each with its unique scientific focus and technological capabilities. These stages encompass early descriptive observations, subsequent structural and functional characterization, and recent advancements in industrial production and clinical translation, as illustrated in Figure 1. Their identification in the mid-20th century was the outcome of collaborative studies involving pediatricians, microbiologists, and chemists. Early observations indicated that breastfed infants experienced lower mortality and reduced rates of infectious diseases compared with those fed formula^[28]. During the early observational phase of HMO research, Austrian pediatricians and microbiologists reported connections between intestinal bacteria and digestive physiology as early as

1886^[29]. Around the turn of the 20th century, pediatricians Moro and Tissier noted marked differences in stool composition between human milk- and formula-fed infants, leading them to hypothesize that undigested carbohydrates in human milk were responsible for these variations^[30]. In 1926, pediatric scientist Herbert Schönfeld discovered the “Bifidogenic Factor” in human milk, demonstrating that *Bifidobacteria* possess heat resistance^[31].

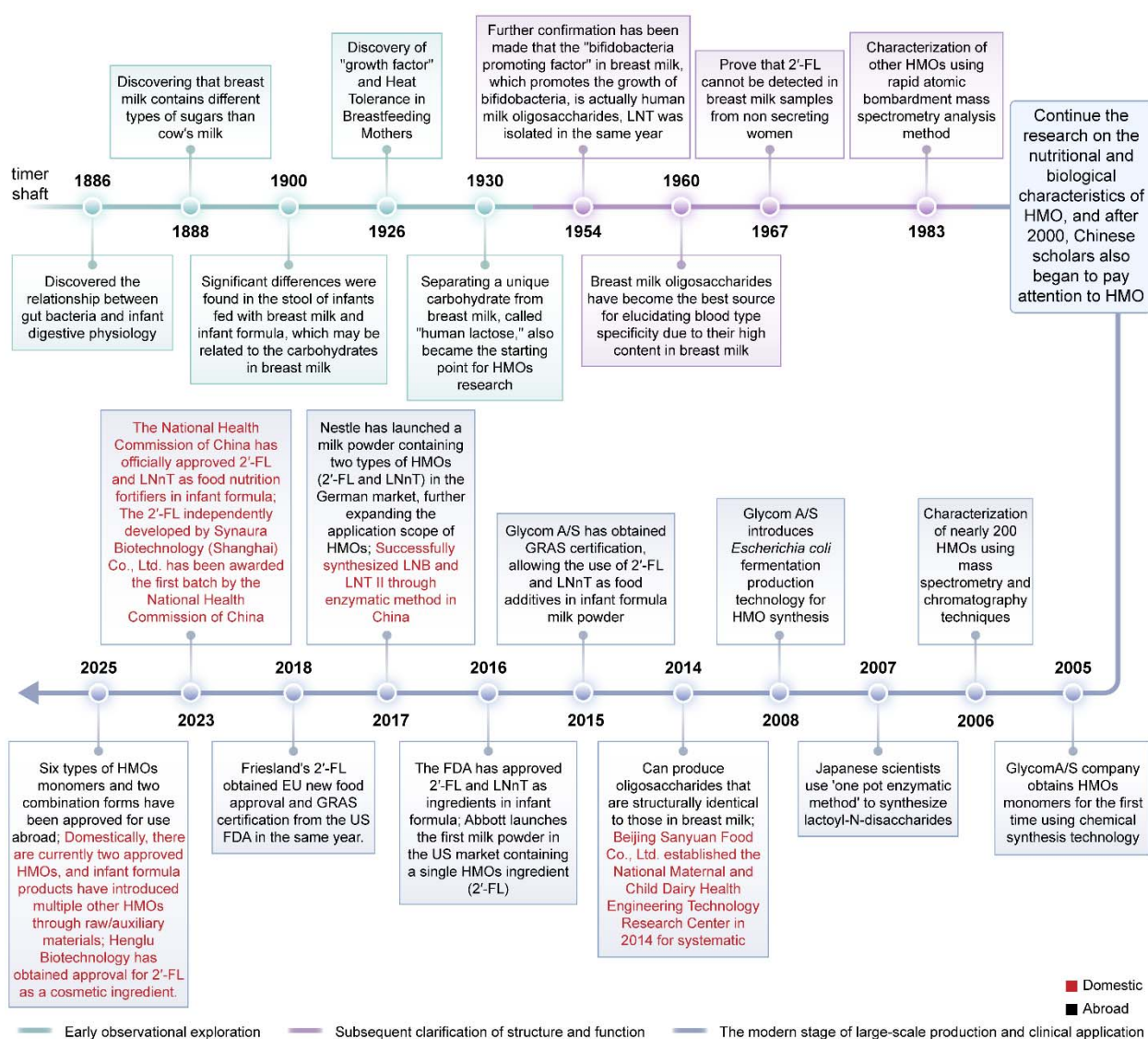


Fig. 1. Research History of HMOs at Home and Abroad.

In 1888, chemist Eschbach reported that human milk contained sugars distinct from those found in cow's milk^[32]. They observed that mixtures of various forms of lactose with distinct properties were present in both types of milk. In 1930, scientists including Polonowski isolated a unique carbohydrate from human milk termed “human milk oligosaccharide.” This sugar is only slightly soluble in methanol and contains nitrogen and amino hexoses, which marked the beginning of HMO research^[28]. In 1954, György demonstrated a close relationship between bifidogenic factor activity and the intestinal defense system. By revealing that the bifidogenic factor was significantly more pronounced in human milk than in foods such as cow's milk, he further confirmed that the “Bifidogenic factors” promoting *Bifidobacterium* growth in human milk were in fact HMOs^[33]. In 1954, LNTs were isolated and identified as key substances in HMOs^[34]. Subsequently,

Polonowski and Lespagnol, in collaboration with Montreuil, were the first to characterize two fucosyllactose derivatives (2'-FL and 3'-FL) from previously uncharacterized fractions using two-dimensional paper chromatography^[35].

As analytical chemistry and chromatography techniques advanced, research groups led by Montreuil in France^[36] and Kuhn in Germany^[37-41] successfully identified and characterized over a dozen HMOs in human milk. Components of some of these HMOs exhibited the expression activity of certain maternal blood group-determining genes, which in turn facilitated chemical structural characterization of the expression activity of the maternal H blood group and Lewis blood group-determining genes^[42].

In 1960, Watkins et al. investigated ABO blood groups and secretor systems^[30]. Due to their high concentration in human milk, HMOs have been considered a valuable model for studying blood group specificity. In 1967, Grollmann and Ginsburg^[43] reported that 2'-FL was absent in milk samples from women classified as non-secretors. In 1983, Egge et al.^[44] characterized additional HMOs using fast atomic bombardment mass spectrometry. Subsequently, his former student, nutrition scientist Clemens Kunz, continued to explore the nutritional and biological properties of HMOs^[31].

Entering the modern technological and translational phase of the early 21st century, advances in glycomics, mass spectrometry, and microbial fermentation have enabled rapid progress in HMO analysis, large-scale production, and regulatory approval. In 2005, for the first time, Glycom A/S successfully obtained HMOs suitable for scientific research using chemical synthesis. However, subsequent attempts to scale up HMO production via chemical synthesis were unsuccessful, prompting Glycom A/S to shift focus to microbial fermentation technology. In 2006, Petschacher et al. characterized HMOs using mass spectrometry and chromatography and identified almost 200 HMOs with unique structures^[45]. In 2008, Glycom A/S acquired *Escherichia coli* fermentation technology from the University of Stuttgart, Germany. Prior to this, in 2007, Japanese scientists successfully synthesized lacto-*N*-biose (LNB) using a 'one-pot enzymatic method' over a period of more than 25 days^[46]. Only in 2016 did researchers and industry succeed in producing oligosaccharides structurally identical to those naturally present in human milk^[47].

Since 2015, HMOs such as 2'-FL and LNnT have been gradually authorized by regulatory agencies, including the U.S. Food and Drug Administration (FDA), the European Food Safety Authority (EFSA), and Food Standards Australia New Zealand (FSANZ), for incorporation into infant formulas and dietary supplements^[48]. That same year, Glycom A/S received GRAS certification from the FDA, allowing 2'-FL and LNnT to be used in infant formulas, beverages, dairy products, and other food items. In 2016, the FDA formally approved the inclusion of 2'-FL and LNnT as ingredients in infant formulas^[1]. In the same year, Abbott launched the Similac Pro Advance series, an infant formula containing a single HMO ingredient, in the United States, setting a technological benchmark in this field. In 2017, Nestlé introduced HMO-enriched formula under its Wyeth Enfamil brand in Hong Kong, and later released the Nan Optipro Supreme series featuring two HMOs (2'-FL and LNnT) in the German market. In 2018, Friesland's 2'-FL obtained the

European Union (EU) new food approval and FDA GRAS certification from the US FDA in the same year. By 2022, six types of HMOs will be incorporated into infant formula products on the market^[49].

China entered the HMO research field relatively late. However, with the coordinated development of scientific research and industry, domestic universities and enterprises (such as San Yuan, Yili, Mengniu, and Feihe) have begun conducting systematic functional research and exploring HMO applications. In 2014, Beijing San Yuan Food Co., Ltd. founded the National Engineering Research Center for Maternal and Infant Dairy Health to carry out ongoing, in-depth studies on human milk in China. In 2017, LNB and lacto-*N*-triose II (LNTII) were successfully synthesized domestically through enzymatic methods. On October 7, 2023, China's National Health Commission formally approved 2'-FL and LNnT as food nutritional fortifiers, permitting their use in infant formulas, children's milk powder, and infant formulas for special medical purposes. The authorized usage ranges are 0.7–2.4 g/L for 2'-FL and 0.2–0.6 g/L for LNnT. In the same year, the first batch of 2'-FL, independently developed by Hongyu Biotechnology, was authorized by the National Health Commission. In 2025, Henglu Biotech received approval for its yeast-derived production of 2'-FL as a new cosmetic ingredient, becoming the first company worldwide to both synthesize HMOs through yeast fermentation and obtain regulatory clearance for cosmetic applications.

Research on HMOs is ongoing with the prospect of incorporating increasing amounts of HMOs into infant formulas. This would provide a nutritional environment closer to that of human milk, meeting the consumer demand for high-quality formulas.

3. Preclinical Evaluation and Clinical Evidence of HMOs

HMOs act as bioactive prebiotics with multiple functions. They are largely resistant to digestion by human enzymes, support the proliferation of beneficial gut bacteria, and serve as anti-adhesive antimicrobial agents that prevent microbial attachment to epithelial cells. Additionally, evidence from in vitro experiments and animal models suggests that HMOs can directly influence intestinal epithelial cell responses, modulate the immune system, and may impact neurodevelopment and cognitive outcomes^[50].

3.1 Preclinical Evaluation of HMOs

The findings summarized in this section are derived primarily from in vitro studies and animal models and therefore provide mechanistic and exploratory evidence, rather than concrete clinical proof. Preclinical studies have revealed that HMOs, as prebiotics, exert diverse effects, notably in immunomodulation, gut microbiota composition, neurodevelopment, and cognitive function. As summarized in Table 1, the influence of two HMOs, 2'-FL and 6'-SL, was assessed in an OVA-induced mouse model. The findings showed that both 2'-FL and 6'-SL alleviated food allergy symptoms by promoting IL-10-producing regulatory T cells and indirectly stabilizing mast cells^[51]. Necrotizing enterocolitis (NEC) arises from exaggerated Toll-like receptor 4 (TLR4) signaling in the intestinal epithelium. NEC was experimentally induced in newborn and preterm mice, followed by supplementation with 2'-FL, 6'-SL, or lactose. Intestinal tissues were collected to evaluate HMOs' inhibition of TLR4 in IEC-6 cells, as well as in mouse and human tissue explants, using computational modeling. Supplementation with 2'-FL and 6'-SL (but not lactose) was

found to reduce NEC severity in mice and piglets by decreasing apoptosis, inflammation, weight loss, and histological tissue damage. Mechanistically, both 2'-FL and 6'-SL suppressed TLR4-mediated activation of B cells via NF- κ B in mouse and human gut, thereby mitigating inflammatory signaling^[52]. Another animal study indicated that 2'-FL and 6'-SL reduced the risk of NEC-associated brain injury in mice and piglets and improved cognitive outcomes in mice^[53]. Additionally, HMOs were shown to prevent the onset of autoimmune diabetes in non-obese diabetic (NOD) mice^[54]. These mechanistic views have been derived from cell-based experiments and animal model analyses, and their relevance to human infants needs further verification in clinical studies.

HMOs are recognized for their ability to suppress harmful bacterial growth in the infant gut, enhance immune function, and support the proliferation of beneficial gut microbiota. In a study examining the effects of maternal dietary supplementation with 2'-FL and *Bifidobacterium* Bb12 in newborn mice, co-administration of 2'-FL and Bb12 significantly increased spleen and thymus indices, serum immunoglobulin levels, and macrophage phagocytic activity. Additionally, the abundance of beneficial gut bacteria was higher in the offspring of dams receiving both supplements compared with those receiving each supplement individually^[55]. Piglets, commonly used as preclinical models, have also demonstrated responsiveness to HMOs. The administration of 2'-FL and LNnT, derived from cow's milk, modulated gut microbiota composition and altered volatile fatty acid concentrations in the ascending colon (AC) of piglets^[56].

Sialic acid (SA), a key component of brain gangliosides, plays a critical role in neurodevelopment. It contributes to brain growth, supports neurotransmission, and facilitates synapse formation^[57]. A study using 3-day-old piglets examined how salivary acidifying oligosaccharides (SMOS) affect piglet neurotransmitters and brain metabolites. This study revealed that SMOS could alter several important brain metabolites and neurotransmitters necessary for optimal piglet neural development^[58]. The effects of 2'-FL on synaptic plasticity and learning were evaluated in adult rats and mice receiving long-term supplementation. Both species exhibited enhanced long-term potentiation (LTP) during alert states, which was associated with improved performance in a range of learning behavioral tests^[59].

Table 1: Preclinical Research Related to HMOs

Species	Type	Oligosaccharides	Intervention period	Conclusion	Literature
Mice	Immunomodulation	2'-FL, 6'-SL	Mice: commencing from PND7-8, continuing for 3 weeks Piglets: caesarean section at 90% gestation for preterm delivery	2'-FL and 6'-SL mitigate food allergy symptoms through the promotion of IL-10-producing regulatory T cells and the indirect stabilization of mast cells.	^[54]
Mice and preterm piglets	Immunomodulation	2'-FL, 6'-SL	Mice at PND 7–8; preterm piglets	In mouse and piglet models, 2'-FL and 6'-SL protect against NEC by suppressing TLR4 signaling and also reduce NEC-associated inflammation in human ileal tissue.	^[55]

Female NOD mice	Immunomodulation	84% HMOs + 16% lactose mixture	Control group: Weeks 4–10 Experimental group: Weeks 16–30	In both mouse and piglet models, 2'-FL and 6'-SL confer protection against NEC by inhibiting TLR4 signaling and simultaneously mitigate NEC-related inflammation in human ileal tissue. [57]
Newborn mice, piglets	Immunomodulation	2'-FL, 6'-SL	PND7-8	Oral supplementation with 2'-FL or 6'-SL lowers the incidence of NEC-related brain injury in mice and piglets and alleviates cognitive deficits in mice. [56]
Mice	Gut microbiota	2'-FL	Pregnancy Cancer Mice: 8 weeks Offspring mice: 10–12 days	When administered together, 2'-FL and <i>Bifidobacterium</i> Bb12 produced greater enhancements in offspring mice's immune function and gut microbiota compared with their individual administration. [58]
Rats	Gut microbiota	SA	Continuous intervention for two weeks postpartum	Results indicate that combined SA and probiotic intervention exerted a greater regulatory effect on maternal gut microbiota and 6'-SL content than SA intervention alone, promoting <i>Lactobacillus reuteri</i> colonization and immune development in newborns. [60]
Piglets	Gut microbiota	HMOs (LNnT), BMOs + HMOs BMOs	PND2-34	Individual HMOs had no significant impact on the overall microbial composition. BMOs alone increased <i>Bacteroides</i> abundance in the cecum, whereas the combined supplementation of fecal BMOs and HMOs produced a synergistic enhancement in <i>Bacteroides</i> levels. [56]
Mice	Gut microbiota, neurodevelopment, cognition	2'-FL	4-week intervention in 3-week-old male mice	Extended administration of 2'-FL improves cognitive memory in mice and modifies the diversity and composition of their gut microbiota. [61]
Male adult mice and rats	Neurodevelopment, cognition	2'-FL	Mice: 2–3.5 months; Rats: 2.5–4 months	After prolonged oral administration of 2'-FL, both rats and mice showed enhanced input/output responses and increased long-term potentiation (LTP) during alert behavioral states. [59]
Piglets	Neurodevelopment, cognition	2'-FL	PND2–35	Supplementation with 2'-FL induced alterations in exploratory behavior and macroscopic/microscopic brain structure in piglets, though no changes in recognition memory were observed [62]
Piglets	Neurodevelopment, cognition	SMOS	PND3	SMOS influence a variety of key brain metabolites and neurotransmitters that are essential for optimal neurological development in piglets. [58]

PND, postnatal days; BMOs, Bovine milk oligosaccharides.

3.2 Clinical Evidence for HMOs

In contrast to preclinical findings, the following section focuses exclusively on human clinical and observational studies, which should be interpreted independently of evidence derived from experimental models. Based on currently published clinical studies on HMOs, these oligosaccharides confer numerous health benefits to infants. Table 2 summarizes the clinical research on HMOs.

Table 2: Clinical Evidence Related to HMOs

Research Type	Sources of HMOs	Subjects	Conclusions	Literat
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Multicenter, randomized, controlled, parallel-group clinical study	Addition of a 5-HMOs mixture (5HMOs-Mix: 5.75 g/L, comprising 52% 2'-FL, 13% 3'-FL, 26% LNT, 4% 3'-SL and 5% 6'-SL)	Intervention group: Infant formula (IF) containing 5HMOs-Mix Control group: IF without 5HMOs-Mix Reference group: BF	No significant differences were detected in weight, length, or head circumference gains between the two formula groups. The 5HMOs-Mix was well tolerated by infants. Compared with the control formula, both 5HMOs-Mix-fed and breastfed infants exhibited softer stools and increased stool frequency. [63]
Multicenter, randomized controlled, double-blind study	Infant formula containing five HMOs	Control group: Infants receiving standard infant formula Test group: Infant formula supplemented with varying concentrations of HMOs Reference group: BF	Infant formula supplemented with five HMOs contributes to the maturation of the intestinal immune system and the maintenance of gut barrier integrity, while fostering a gut microbiota composition more similar to that observed in breastfed infants. [17]
Cohort Study	Human Milk	Tracking Hispanic Mother-Infant Pairs at 1, 6, and 24 Months Postpartum	Receiving higher frequency breastfeeding at 1 month of age contributes to long-term cognitive development, with 2'-FL in human milk playing a more significant role compared to other HMOs. [64]
Cohort study	Human milk	Extremely low birth weight infants	Mothers of infants who developed NEC exhibited notably lower concentrations of DSLNT in their breast milk. [65]
Cohort study	Human milk	Mother-infant pairs	Differences in HMOs composition correlate with infant growth and body composition [66]
Cohort Tracking Study	Human milk	Four groups of mothers: normal weight, overweight, obese, gestational diabetes	The concentration of 6'-SL in breast milk was associated with higher cognitive and motor scale scores at 18 months, whereas 2'-FL levels correlated positively with motor scale performance at 6 months. [67]
Double-blind, randomized, controlled trial	Infant formula supplemented with MOS, human milk	Control group: Formula-fed infants Experimental group: 7.2g/L MOS-supplemented infant formula Reference group: BF	Infant formula supplemented with MOS promoted gut microbiota and metabolic profiles that more closely resembled those of exclusively breastfed infants. It enhanced bifidobacteria growth, decreased fecal pathogen levels, and strengthened intestinal immune responses. [68]
Double-blind, cross-over, randomized controlled trial	Infant formula supplemented with 2'-FL and LNnT	Infants with cow's milk allergy	Extended-stage formula enriched with 2'-FL and LNnT satisfies clinical standards for hypoallergenicity. [69]

Double-blind, randomized, multicenter, controlled trial	Control group: Infant formula containing 0.8g/100mL scGOS/lcFOS (9:1) Reference group: human milk Intervention group: Infant formula containing 3'-GL, 0.8 g/100 mL scGOS/lcFOS (9:1), 0.1 g/100mL 2'-FL and milk fat	Healthy term infants exclusively formula-fed for <14 days	Partially fermented infant formula containing postbiotics, specific oligosaccharides, 2'-FL, and milk fat supports normal growth and demonstrates safety and good tolerance in healthy term infants.	[70]
Controlled, double-blind, randomized growth and tolerance study	Intervention group: IF supplemented with GOS and 2'-FL Control group: containing GOS only Reference group: BF	Healthy singleton infants aged 5 days to 4 months	Infants receiving 2'-FL-supplemented formula showed reduced plasma and ex vivo inflammatory cytokine levels, resembling the profiles seen in the breastfed reference group.	[71]
Controlled, double-blind, randomized, multicenter intervention study	Intervention group: EHF supplemented with 1.0 g/L 2'-FL + 0.5 g/L LNnT Control group: EHF without HMOs Reference group: BF	Non-breastfed CMPA infants aged 2 weeks to 6 months	Adding 2'-FL and LNnT to whey-based EHF promoted the growth of HMO-utilizing <i>Bifidobacteria</i> and delayed the shift of the gut microbiome toward an adult-like composition.	[72]
Contrast trial	Human milk	NEC infants	Breastfed preterm infants exhibited a markedly lower incidence of NEC compared with their formula-fed counterparts, and their mothers' milk contained higher concentrations of DSLNT than that of mothers whose infants developed NEC.	[73]
Randomized, double-blind, controlled parallel	Intervention group: Infant formula (IF) containing 5HMOs-Mix(2'-FL, 3-FL, LNT, 3'-SL, 6'-SL) Control group: milk-based formula experimental group: experimental milk-based formula	Healthy full-term infants(≤14 days old)	There was no significant difference in daily weight gain among the three groups during the 14–119 day period (D)	[74]
Prospective, randomized, controlled	Experimental group: 2'-FL content is different (0.2 and 1.0 g/L) Control group: Formula milk powder containing GOS Reference group: BF	5-day-old healthy full-term baby	During the study, growth parameters such as weight, length, and head circumference showed no significant difference between infants fed breast milk and experimental formula milk	[75]

BF, Breastfed infant; CMPA, Cow's milk protein allergy; EHF, Extensively hydrolyzed whey-based infant formula; IF, Infant formula; MOS, Bovine milk-derived oligosaccharides; scGOS, short-chain galacto-oligosaccharides; lcFOS, long-chain fructo-oligosaccharides.

A double-blind, randomized, controlled trial investigated how infant formulas containing MOS influence gut microbiota and intestinal immunity. Infants receiving MOS-supplemented formula exhibited gut microbiota and metabolic profiles resembling those of exclusively breastfed infants^[68]. In a separate cohort study of infants younger than 14 days, a fermented formula enriched with postbiotics—including a probiotic mixture of short-chain GOS (scGOS) and long-chain FOS (lcFOS) at a 9:1 ratio—2'-FL, and milk fat supported normal growth and was well-tolerated by healthy full-term infants^[70].

In a previous study, stool and human milk samples were collected from three-month-old infants, who were categorized into breastfed and formula-fed groups. Analysis of human milk composition combined with 16S rDNA sequencing revealed notable differences in gut microbiota between the groups. The breastfed infants exhibited higher levels of *Bacteroides* and substantially lower levels of *Clostridium XVIII*, indicating an association between HMOs and infant gut microbiota colonization^[76]. Another clinical trial found that infants receiving a 5-HMOs-Mix formula produced softer stools and had increased stool frequency compared with infants fed a formula without HMOs^[63]. Furthermore, an infant formula containing a defined mixture of five HMOs supported the maturation of the intestinal immune system and barrier function, while fostering a gut microbiome resembling that of human milk-fed infants. This microbiome was marked by elevated *Bifidobacteria*, particularly *Bifidobacterium infantis*, and reduced levels of the toxin-producing bacterium *Clostridium difficile*^[17]. It should be noted that while many clinical studies report positive associations of HMO supplementation with gut microbiota profiles and immune markers, several trials have also found no significant differences in key tolerance and gastrointestinal outcomes between HMO-supplemented and control formula groups, such as similar rates of feeding tolerance, flatulence, regurgitation, crying, or fussiness in infants receiving HMO-supplemented formulas compared with controls^[50].

NEC is a severe condition affecting newborns, particularly preterm infants, and arises from multiple contributing factors. One study reported that breastfed preterm infants had a significantly lower incidence of NEC compared with those fed formula. In that study, the milk of mothers whose infants developed NEC contained lower concentrations of di-sialic acid lacto-*N*-tetrasaccharide (DSLNT) than milk from mothers of healthy infants^[73]. Another study similarly found that DSLNT levels were reduced in the milk of mothers with NEC infants compared to controls^[65].

HMOs have also been linked to neonatal growth and cognitive development. In an observational study of Hispanic mother-infant pairs in the United States, frequent human milk feeding at one month of age was associated with improved cognitive outcomes. Specifically, the concentration of 2'-FL in milk at one month correlated with cognitive development scores at 24 months, while 2'-FL levels at six months did not show predictive value, suggesting that early-life exposure to 2'-FL may support cognitive development^[64]. Another study involving 25 mother-infant pairs examined human milk composition and found that both the levels and diversity of HMOs were associated with the infants' muscle-to-fat ratio^[66]. Additional research has shown that supplementation with an extensively hydrolyzed whey-based formula containing 2'-FL and

LNnT can mitigate cow's milk allergy in infants^[69]. However, a randomized controlled trial showed that infant formula supplemented with specific HMOs did not show significant differences in growth parameters such as weight, length, and head circumference compared to the control formula^[75]. Another randomized, double-blind, controlled feeding trial compared the effects of infant formula containing five HMOs with those of control formula on infant health. The results indicated no significant difference in weight gain between the two groups, and the safety profile was good^[74].

However, the interpretation of these clinical findings should be approached with caution. Despite the increasing number of studies on the impact of HMOs on infant and young child health, there is significant heterogeneity among studies, complicating direct comparison and interpretation of clinical outcomes. A major source of heterogeneity is the variation in HMO composition, with interventions ranging from single HMOs (such as 2'-FL) to complex mixtures comprising multiple HMOs. Given the pronounced structure-function specificity of HMOs, this compositional variation may lead to different biological effects. Furthermore, there is considerable variability in intervention doses and durations, and a clear dose-response relationship has not yet been established. Differences in study populations further exacerbate heterogeneity, including interindividual variations in term versus preterm infants, feeding methods, baseline health status, and gut microbiota composition. In summary, these factors may partially explain the variability and occasional inconsistency in the clinical outcomes reported across trials. These observations emphasize the need for future clinical studies to adopt more standardized designs, clearly defined HMO formulations and dosing regimens, and improved population stratification to enhance comparability and translational relevance.

3.3 Integrated mechanisms underlying HMO-associated health effects

Although the immunological, intestinal, and neurodevelopmental effects of HMOs have been discussed in separate sections, these mechanisms are highly interconnected rather than independent. HMOs primarily exert their effects through modulation of the gut microbiota, leading to changes in microbial composition and metabolite production, which subsequently influence immune maturation and intestinal barrier integrity. Additionally, HMOs may directly interact with epithelial and immune cells, contributing to the regulation of inflammatory signaling and barrier function^[77, 78].

Importantly, improvements in immune homeostasis and gut barrier integrity may further affect neurodevelopment via the gut–brain axis, involving microbial metabolites, immune–neural interactions, and neuroactive signaling pathways^[79]. Taken together, these findings suggest that the health effects of HMOs should be understood as the outcome of an integrated biological network, rather than through isolated mechanistic pathways.

4. Synthesis of HMOs

Researchers and manufacturers are highly concerned about the manufacture of HMOs on a large scale and efficiently. Figure 2 provides an overview of the synthetic strategies employed for HMO preparation.

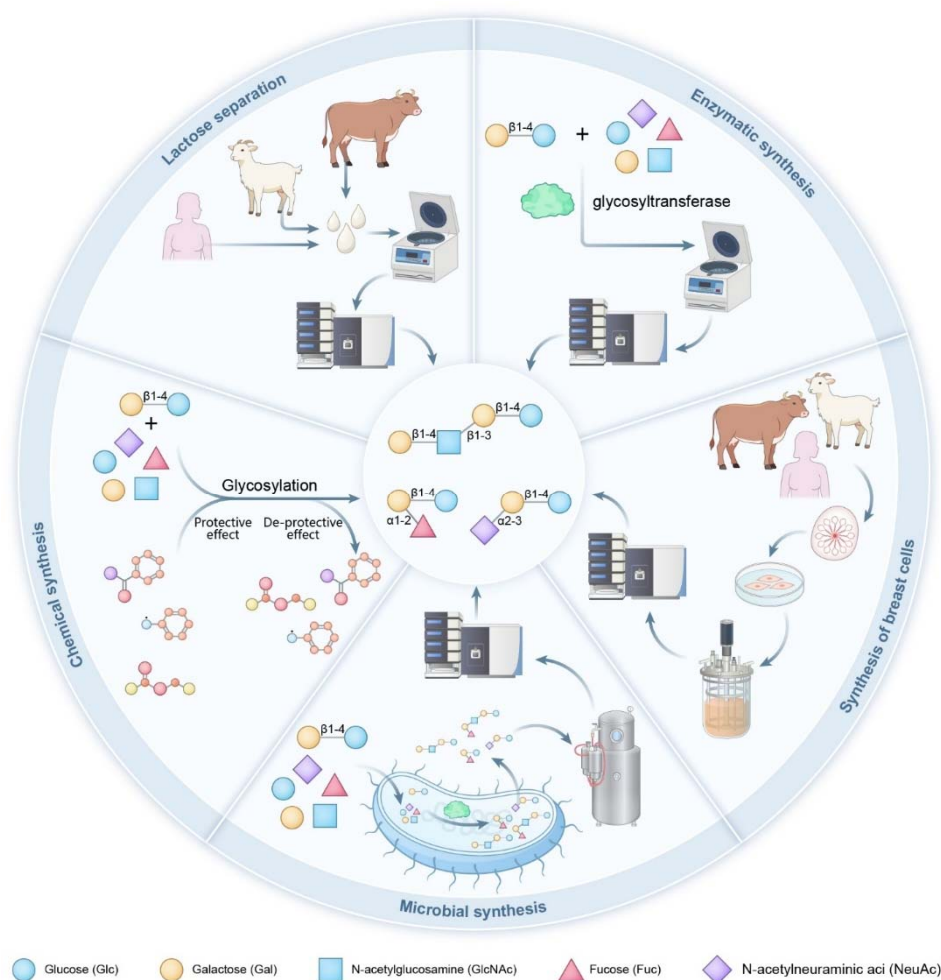


Fig. 2. Synthesis of HMOs.

4.1 Oligosaccharides in Natural Milk Sources

HMOs are biosynthesized by mammary epithelial cells during lactation. In breast milk, the content of these substances is 5–20 g/L, which makes them the third most abundant component after lactose (55–70 g/L) and fats (16–39 g/L)^[80, 81]. HMO levels and profiles change during lactation, ranging from 20–25 g/L in colostrum to 5–20 g/L in mature milk^[18].

Human milk is ideal for HMO extraction and refinement. HMOs, with unique structures and roles, foster infant immune system growth, modulate gut microbiota, and inhibit pathogen attachment. Human milk, a complex fluid, includes carbohydrates, fats, proteins, and numerous bioactive compounds. Its limited supply hinders its use as a commercial source. Consequently, researchers have increasingly turned to natural ingredients as raw materials to mimic or synthesize oligosaccharides with similar structures and functions. This approach offers significant advantages in terms of raw material acquisition, sustainability, and safety. Notably, although present at far lower levels than HMOs, the oligosaccharides in cow and goat milk share core structural similarities with HMOs. A few detectable oligosaccharides in cow's milk include LNT, 3'-SL, and 6'-SL^[82]. These structures can be preferentially utilized by *Bifidobacteria* and have been shown to promote neurodevelopment and regulate immune gene expression in animal studies^[57].

By comparison, plant-derived oligosaccharides, including FOS and GOS, are more frequently incorporated into infant formulas. Their primary function involves serving as prebiotics to regulate gut flora

and enhance short-chain fatty acid (SCFA) synthesis. However, owing to the absence of specific structures such as sialic acid and fucose, they are less functional than HMOs. For example, they cannot inhibit pathogen adhesion or promote neurodevelopment^[83-86].

Membrane-based technologies can also be employed to recover oligosaccharides from dairy processing by-products^[87]. Wang et al. examined various strategies for extracting bovine and caprine milk oligosaccharides from dairy industry leftovers like whey, permeate, and mother liquor. They outlined several approaches, ranging from single-membrane filtration methods to more sophisticated techniques that combine lactose breakdown with nanofiltration, as well as fully integrated processing systems^[88]. Oligosaccharide scarcity and structural diversity in human milk and dairy sources make post-lactose isolation of BMOs and CMOs laborious. Creating products rich in oligosaccharides, such as whey that is abundant in BMOs, presents a more budget-friendly option, but it is essential to weigh the pros and cons meticulously to pinpoint the most suitable manufacturing methods and practical uses in the industry^[4].

4.2 Chemical Synthesis Technology

Chemical synthesis is an extremely flexible production technique often used for small-scale manufacturing of HMOs with diverse structures. Yields are typically on the order of milligrams to grams, but can potentially reach kilograms^[89, 90]. Chemical synthesis of these oligosaccharides requires several sequential protection and deprotection steps^[91]. The chemical synthesis of HMOs relies primarily on oligosaccharide glycosylation reactions involving the formation of glycosidic bonds between two monosaccharides^[92]. Over 15 distinct HMOs have been successfully prepared^[93], including the laboratory-scale synthesis of lactose-*N*-fucopentoside (LNFPI)^[94] and the kilogram-scale synthesis of LNT^[95]. Chemical synthesis further enables the creation of precisely structured HMOs such as LNT, Lacto-*N*-neohexaose (LNnH), and 2'-FL. Glycom A/S has pioneered a chemical synthesis approach that allows for the production of kilogram quantities of high-purity 2'-FL, achieving industrial-scale requirements with only one chromatographic purification, truly hitting the bullseye in efficiency^[96, 97].

Compared with other biopolymers, the chemical synthesis of carbohydrate molecules poses considerable challenges. A major difficulty in HMO synthesis is the extensive application of protective groups, which increases with chain length and branching, often leading to time-consuming procedures and low yields^[93]. In addition, chemical synthesis relies on costly and potentially toxic glycosylation reagents, frequently requiring transition metal catalysts^[98]. In the face of these challenges, stepwise solid-phase approaches have successfully produced numerous complex HMOs, including 2'-FL, 3'-disfucosyllactose (DFL), LNT, LNnT, LNFP I, and lactose-*N*-fucopyranoside III (LNFP III)^[94, 95].

Significant progress has been made in the preparation of HMOs owing to advancements in solid-phase synthesis technology. Compared to traditional liquid-phase synthesis methods, automated solid-phase synthesis allows for more rapid acquisition of complex carbohydrate structures^[99]. In solid-phase synthesis, oligosaccharides attach to an inert support, such as a resin, facilitating quick distinction between the formed compounds and unused reactants. This approach has been successfully applied to produce oligosaccharides

with well-defined structures^[100]. Marchesi et al. developed an automated solid-phase synthesis technique in which a photocurable resin bearing hydroxyl groups was used as the scaffold for glycosylation of the glycosidic units^[101]. However, the low synthesis yield and use of toxic reagents in chemical synthesis render them unsuitable for producing food additives. The complexity of the synthesis steps, difficulty in controlling the stereoselectivity, and high costs also hinder its application in large-scale industrial production. Chemical synthesis has the advantage of enabling the preparation of more than 15 HMOs, although large-scale production still relies on biotechnology^[93].

4.3 Enzymatic Synthesis Technology

Enzymatic synthesis is another method for producing HMOs. This method combines chemical and enzymatic catalysis and leverages the specificity of glycosidases (GHs) and glycosyltransferases (GTs) for precise synthesis^[93]. GTs are enzymes that can transport a sugar group from an active donor molecule to an acceptor molecule, thus connecting two sugar units together^[102], whereas GHs catalyze the transglycosylation reaction, promoting the connection between sugar residues and aglycones^[103].

In the human body, HMOs are produced by Leloir glycosyltransferases, which act on sugar nucleotide donor substrates. GeneChem, a biotechnology company in Korea, uses something called sialyltransferase to produce 3'-SL and 6'-SL in large quantities. In 2018, the 3'-SL they made was officially deemed as GRAS^[93]. Lactose- β -3-glycosidase (LNT II) and β -N-acetylhexosaminidase (BNA) can also be used for HMO synthesis. For example, LNT II catalyzes the conversion of lactose into HMO tetrasaccharides, whereas BNA facilitates the synthesis of complex hexasaccharides^[104]. Pathogenic *Neisseria* can produce two kinds of special enzymes, one is called β 1,3-N-acetyl glucosaminyl transferase (LGTA), and the other is β 1,4-Galactosyl transferase (LGTB). These enzymes can produce LNnT efficiently, with a success rate of over 85%^[89]. According to Zeuner and colleagues, oligosaccharides such as LNnT are made from natural glycosidases. Their work shows that three different β -galactosidase enzymes help to complete two key reactions: one to combine GlcNAc with lactose to produce LacNAc, and the other to react LNT II with lactose to produce LNnT. Among them, the enzyme belonging to the GH42 family found in *Bacillus circulans* proved to be the most useful, and it produced the most LacNAc and LNnT^[105].

"One-pot synthesis" technology is a chemical enzymatic method that combines the adaptability of a chemical process with the precise catalytic characteristics of an enzyme. Its core principle involves the progressive construction of sugar chain structures by sequentially adding multiple enzymes or substrates within a single reaction system, thereby reducing the number of intermediate purification steps^[106]. Chen et al. established a one-pot multisaccharide glycosylation process that enabled the kilogram-scale synthesis (>10 g) of LNT. During production, McArthur et al. identified a β -1,3-galactosyltransferase (β 3GalT) from *Corynebacterium violaceum* that shares homology with previously studied β 3GalTs from *Escherichia coli*, but demonstrates higher efficiency in catalyzing the conversion of LNT II to LNT. Through two successive one-pot multi-enzyme (OPME) steps, the researchers successfully produced LNT with a yield of 99.3%. After obtaining a large amount of LNT with this CV β 3GalT enzyme, they can glycosylate and salivate it, and finally

synthesize HMOs, which include LNFP II, Lacto-N-digalactosylated algosaccharide (LNDFH), and Lacto-N-fucopentaose V (LNFP V)^[91].

In 2021, Chen et al developed an extremely potent OPME method. In this way, they successfully manufactured 21 different HMOs, which were not only extremely pure and high in yield but also very convenient to manufacture. HMOs made by this method all contain sialic acid or L-fucose, and some molecules even reach the length of nonose^[107].

4.4 Microbial Fermentation

Neither purely chemical nor enzymatic synthesis methods are capable of producing HMOs at the kilogram scale. Microbial fermentation has therefore become the predominant approach for industrial-scale HMO production. This strategy was first implemented in the early 1990s, enabling the synthesis of roughly 50 distinct HMOs^[89]. Because no microorganism naturally produces HMOs, metabolic engineering is employed to express the necessary glycosyltransferases in microbial hosts to facilitate HMO biosynthesis. Synthetic biology techniques have been used to create microbial cell factories that convert substrates such as glucose into HMOs^[108]. Researchers have primarily selected strains such as *Escherichia coli*^[109], *Bacillus subtilis*^[110], and *Bacillus glutamicus*^[111] as host organisms. Engineered microbial systems can generate HMOs including 2'-FL, 3'-FL, LNT, LNTnT, 3'-SL, 6'-SL, and advanced fucosylated variants^[112]. Among these, 2'-FL is the most commonly produced using engineered bacteria owing to its high abundance and relatively simple structure within the HMO family.

Escherichia coli is a well-characterized model microorganism. Its high transformation efficiency, robust protein expression, and rapid growth make it an abundant source of fermentation-derived products. *E. coli* enables efficient generation of basic HMOs such as 2'-FL, 3'-FL, and DFL^[112]. Within the system, lactose undergoes enzymatic fucosylation, facilitated by α -1,2-fucosyltransferase (FucT2), to produce 2'-FL, utilizing guanosine 5'-diphosphate (GDP)-L-fucose as the source of L-fucose^[113]. Notably, *E. coli* can produce GDP-L-fucose intrinsically^[114].

In 2012, Lee et al. crafted brand-new biochemical pathways to manufacture 2'-FL. They did this by inserting a gene, fucT2, from the *Helicobacter pylori* microbe into *E. coli* strains. This genetic tweak allowed the bacteria to biosynthesize GDP-L-fucose. The researchers managed to produce 2'-FL at a rate of 1.23 grams per liter^[115]. Later, Baumgartner et al. supercharged GDP-L-fucose synthesis by amping up the expression of an alkaline-and heat-resistant mannanase (ManB), mannose-1-phosphate N-glycosyltransferase (ManC), GDP-mannose 4,6-dehydratase (Gmd), and GDP-fucose synthase (WcaG), implementing a backup route that boosted 2'-FL production to a hefty 20.28 g/L^[116].

Besides *E. coli*, other bacterial hosts have been engineered for 2'-FL production. For example, a South Korean research team led by Professor Seo Jin-ho employed *Bacillus glutamicus* ATCC 13032 as a chassis strain. Using a dual-plasmid system, they expressed four *E. coli*-derived genes involved in GDP-L-fucose biosynthesis—*manC*, *manB*, *gmd*, and *wcaG*. On a separate plasmid, they co-expressed the lactose permease gene (*lacY*) from *E. coli* and α 1,2-fucosyltransferase (*fucT2*) from *H. pylori*, achieving a 2'-FL yield of 250 mg/L^[117].

Zhang Yan et al. successfully achieved de novo 2'-FL synthesis using a lipolytic strain of *Saccharomyces cerevisiae*. They employed *S. cerevisiae* PO1f as a chassis cell and expressed four genes involved in the 2'-FL synthesis pathway via single-plasmid expression. Subsequently, they optimized the 2'-FL synthesis process using fusion protein tags, such as ubiquitin. Moreover, through iterative integration of key genes, the supply of precursor substances was enhanced, promoting nicotinamide adenine dinucleotide phosphate (NADPH) regeneration. This further increased the 2'-FL yield, ultimately achieving the successful production of 41.10 g/L 2'-FL^[118].

4.5 In Vitro Induction of Mammary Cell Synthesis

Given the long-term benefits of exclusive human milk feeding, technologies aimed at producing cell-based human milk have garnered growing interest, as they may deliver functional human milk bioactivity to infants unable to breastfeed. Nevertheless, the culture of human mammary cells remains at an early stage of development. Recently, Che et al. investigated the full proteomic profiles and mineral content of proteins secreted by mammary epithelial cells. The research results demonstrated a protein profile strikingly similar to that observed in mammary epithelial cells obtained from tissues, while also making the groundbreaking discovery of mineral-protein complexes—comprising calcium, magnesium, phosphorus, potassium, and sodium—which constituted a substantial 6–9% of all minerals present within the culture media^[119]. Even though this new tech is still in its infancy, and it does not quite match the protein punch or classic protein mix of regular milk, it has still got a bright future as a greener, more eco-friendly way to produce dairy products.

The mammary gland is a highly specialized organ consisting of alveolar structures that contain multiple cell types. Throughout gestation, the gland experiences active transformation, mainly influenced by the prolactin system, comprising estrogen, progesterone, prolactin, placental lactogen, and insulin. This hormonal environment stimulates mammary stem and progenitor cells, facilitating ductal branching, alveolar maturation, and secretory specialization^[120]. Breast milk release during nursing occurs when oxytocin prompts myoepithelial cells around the alveoli to contract^[121].

Mammary epithelial cells predominantly produce and secrete milk's components, such as casein, whey proteins, and triglycerides. Thus, the first phase in laboratory-based generation of milk elements through cellular culture requires extracting and growing mammary epithelial cells^[122]. There are two methods for acquiring these cells^[87] (Figure 3). One method involves the isolation of undifferentiated mammary stem cells from the mammary tissue or milk. In 2021, Wilk, an Israeli food tech startup, became the world's first publicly listed cell-cultured dairy company when it went public in Israel. The company isolated mammary cells and cultivated them using a proprietary technology to produce the world's first yoghurt made from cell-cultured milk fat. This yoghurt contains milk fat produced using cell culture technology, delivering the same nutritional value as real milk fat.

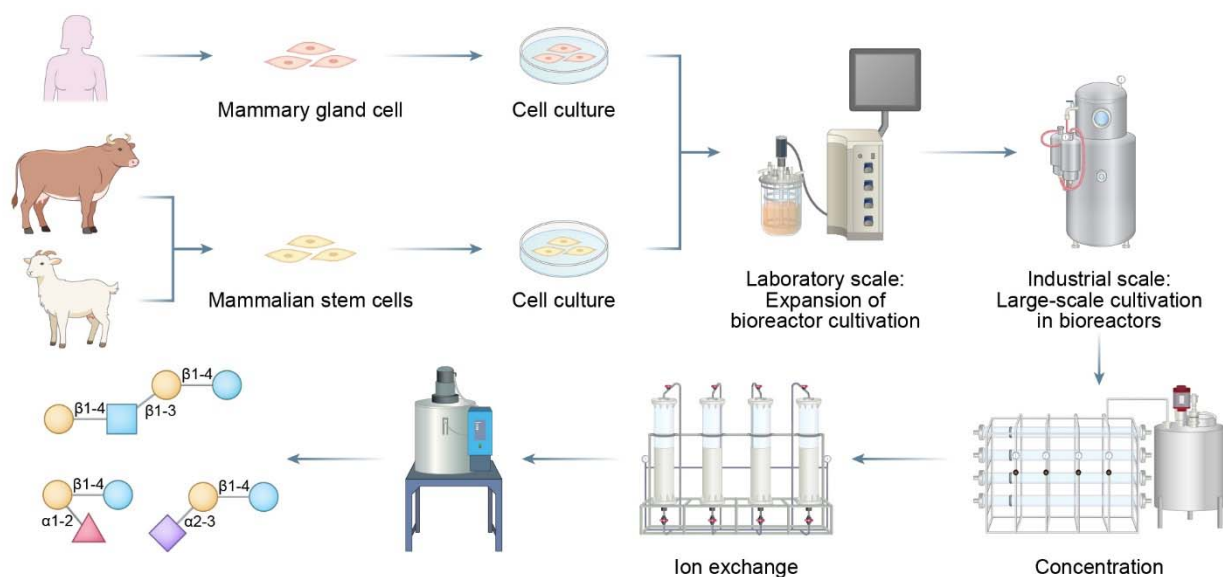


Fig. 3. Strategy for producing milk components (including HMOs) via mammary cell culture.

Another approach is to use stem cell technology to generate cells that resemble the mammary glands. Companies operating in this field include Turtletree in Singapore, Biomilk in the United States, and 108Labs. Stem cells possess two unique capabilities: first, they can self-renew, and second, under specific conditions, they can differentiate into various mature and functional cell types via the progenitor cell stage^[120]. Studies involving the transplantation of dental pulp stem cells (DPSCs) from young mice, together with mammary epithelial cells (MECs), into mammary fat pads have shown that DPSCs are capable of differentiating into all mammary epithelial cell lineages, including lactating cells, within newly regenerated mammary ducts^[123]. Alternatively, stem cells sourced from animals such as cows or humans can be genetically engineered to transform into mammary cells. These cells can then be induced to secrete milk into the bioreactors. The resulting milk is then purified and processed into 'cell-cultured milk' that is suitable for human consumption^[122]. Secreted cell culture milk was collected, scaled up for industrial-scale cultivation, and isolated to obtain HMOs. Beijing Sanyuan recently made significant breakthroughs in key technologies for cell-based milk production.

From a translational perspective, not all advances in HMO synthesis are equally amenable to regulatory approval and industrial application. In practice, regulatory agencies tend to prioritize HMOs that are structurally identical to naturally occurring human milk components, can be produced with high purity and batch-to-batch consistency, and are supported by robust toxicological and clinical safety data. As a result, fermentation-based production platforms have emerged as the dominant industrial strategy, enabling scalable, cost-effective manufacturing that aligns with regulatory requirements. This convergence of scientific feasibility, regulatory acceptance, and manufacturing scalability explains why only a limited number of HMOs have thus far been deployed in commercial applications.

5. Metabolism of HMOs

Humans do not possess the enzymatic machinery, such as sialidases and fucosidases, required to degrade HMOs. Consequently, these carbohydrates pass through to the colon mainly unaltered and are

fermented by intestinal microorganisms^[13]. Investigations into HMO metabolism reveal absorption into the systemic circulation at roughly 1%, with the bulk either employed by gut microbiota or eliminated via feces and urine^[124].

GHs and membrane transporters facilitate the uptake and processing of structurally varied HMOs. The neonatal gut microbiota harbors the requisite enzymes and transport systems that enable bacteria to uptake, metabolize, and utilize HMOs efficiently^[4].

The metabolism of HMOs relies on glycoside hydrolase (GH) enzymes, including lactose-*N*-glycosidase, β -galactosidases, and fucosidases^[125]. Compared with hydrolases, research on HMO-specific transporters is more limited. So far, characteristics have been established for transporters of LNB, FL, and LNnT^[126]. The GNB/LNB transporter facilitates cellular uptake of extracellular LNB liberated by lactobionic acid *N*-glycosidase and GNB released from mucin O-glycans via α -*N*-acetylgalactosaminidase. FL transporters facilitate cellular import of 2'-FL, 3'-FL, LDFT, and LNFPI^[127], while LNnT-binding proteins specifically transport LNnT^[128].

Bifidobacteria represent some of the first gut colonizers in humans, constituting more than half of the total gut microbiota in breastfed infants, frequently surpassing 70%. Infant-associated *Bifidobacteria* employ two distinct strategies for HMOs utilization (Figure 4). The first involves extracellular hydrolysis, in which cell-wall-anchored secreted glycosidases break down HMOs into monosaccharides and disaccharides that are subsequently transported into the bacterial cell. The second approach hinges on intracellular transport mechanisms, which involve importing HMOs boasting a polymerization degree of three or higher through ATP-binding cassette (ABC) transporters, followed by their subsequent breakdown via intracellular exoglycosidases^[129-131]. In all these steps, *Bifidobacteria* transform HMOs into various metabolites, like SCFAs, which are crucial for fueling the intestinal lining cells, lowering the pH of the gut lumen, keeping harmful bacteria at bay, and helping to sustain a healthy balance of gut microbiota^[127].

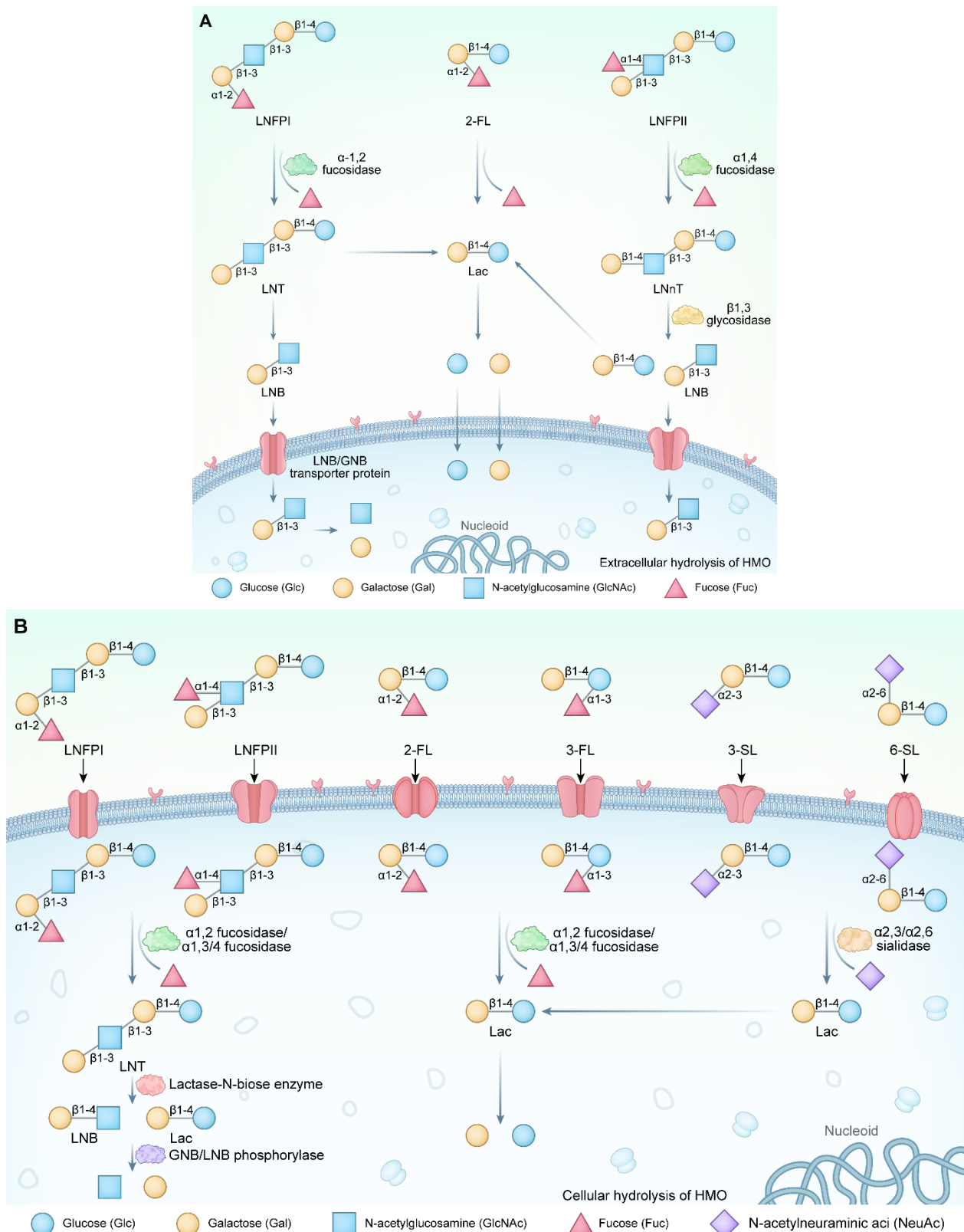


Fig. 4. Utilisation of HMOs by Gut Microorganisms. (A) Extracellular hydrolysis of HMOs. (B) Cellular hydrolysis of HMOs.

Infant Bifidobacteria completely transport HMOs into cells via ABC transporters. Once inside the cytoplasm, HMOs are hydrolyzed into monosaccharides by GHs^[132, 133]. *Bifidobacterium infantis* contains multiple intracellular enzymes capable of degrading fucosylated and sialylated HMOs, including fucosidase, β -galactosidase, LNB phosphatase, N-acetyl- β -D-hexosaminidase, and sialidases^[134]. In addition, there are

enzymes capable of degrading sialylated HMOs, specifically NanH1 and NanH2^[135]. Remarkably, NanH2 acts as an intracellular α -sialidase, specifically targeting sialic acid on α -2,3 and α -2,6 linkages, encompassing those within DSLNT^[136].

Investigations show that the two infant bifidobacterial fucosyl-lactose transporters, FLT-1 and FLT-2, selectively bind to oligosaccharides including 2'-FL and 3'-FL found in breast milk^[137]. Specifically, these two transporters mediate the transmembrane transport of various fucosylated HMOs via complementary substrate preferences. Their functional differences are closely linked to evolutionary adaptations of their molecular structures. Notably, high expression of fucosyl transporter 2 (FucT 2) is significantly associated with the abundance of Bifidobacteria in the infant gut, highlighting the vital role of this transport system in sustaining host-microbiota symbiosis^[138].

Bifidobacterium longum utilizes HMOs intracellularly. *Bifidobacterium longum* has the most extensive and varied array of transporters, glycan hydrolases, catabolic enzymes, and transcriptional regulators for HMOs metabolism. Therefore, they can break down a wide range of HMOs, covering neutral, fucosylated, and sialylated types^[139]. The fermentation products of *Bifidobacterium longum* are lactate, acetate, and propylene glycol^[134]. Certain strains of *Bifidobacterium longum* are capable of metabolizing specific HMOs, such as LNT and 2'-FL, in addition to mucin-derived O-glycans. This selective utilization contributes to shaping the early-life gut nutritional ecology^[140].

Unlike the intracellular utilization of HMOs by infant *Bifidobacteria*, *Bifidobacterium bifidum* utilizes extracellular hydrolases such as fucosidase to metabolize HMOs outside the cells and then transports the required degradation substrates^[129]. *Bifidobacterium bifidum* utilizes 1,2- α -fucosidase (AfcA) and 1-3/4- α -fucosidase (AfcB) to initiate extracellular degradation of HMOs, thereby releasing fucose^[141]. *Bifidobacterium bifidum* has two external glycoside hydrolases: lactose-*N*-biotinidase and endo-*N*-acetylgalactosaminidase. The former is an endoglycosidase that breaks down LNB and LNT^[142]. The liberated LNB molecules are imported into the cell through ABC transporters, serving as substrates for *Bifidobacterium bifidum*^[143].

In addition, *Bacteroides* can consume HMOs. The key enzymes involved in HMO degradation within the *Bacteroides* genus include enzymes from the glycoside hydrolase family. These enzymes recognize and cleave the core structures of HMOs such as 2'-FL, LNT, and lactose. Specifically, *Bacteroides* species typically employ an "external degradation" strategy. They used cell-surface polysaccharide-binding proteins and polysaccharide utilization sites to recognize and bind HMOs. These HMOs are then degraded by the glycoside hydrolases located on their outer membranes^[144].

Cross-feeding occurs among different gut microbiota. Various *Bifidobacteria*, *Bacteroides*, and other probiotics, such as *Lactobacillus* and *Akkermansia*, can share HMO degradation products directly or indirectly for cross-feeding purposes. This mechanism enhances gut microbiome variety, fosters the growth of advantageous microbes, and controls the presence of harmful pathogens^[145]. HMOs stimulate *Bifidobacterium* growth and boost butyrate synthesis through symbiotic relationships, where butyrate acts as a key energy source for colon cells^[20]. *Bifidobacterium breve* is often described as a

"scavenger" because it cannot directly use HMOs as a carbon source, but it can use HMO degradation products released by other HMO degraders, like fucose and sialic acid^[146].

6. Conclusion

HMOs are among the most abundant constituents of human milk, ranking third after lactose and lipids, and play indispensable roles in regulating physiological processes crucial for infant growth and development. They have also become key active ingredients in a new generation of infant formulas. HMO research dates back to the last century. From the initial recognition of "non-lactose carbohydrates" in human milk in the late 19th century, to their identification as "bifidogenic factors" that promote the growth of *Bifidobacteria* in the mid-20th century, to the significant advancements driven by 21st-century developments in glycomics, mass spectrometry, and synthetic biology, HMO research has come a long way. An increasing number of HMOs with distinct structures and functions have been identified.

Most animal studies and clinical feeding trials have indicated that HMOs play significant roles in infant nutrition, health, and immune function. These findings not only support the inclusion of HMOs in infant formulas but also provide evidence for their potential nutritional value. Currently, HMOs are primarily applied in infant formulas; however, their health benefits are not limited to infant populations. In the future, HMOs should be actively promoted for use in special foods and other applications in China. Furthermore, the quantity of HMOs added to the formula remains far lower than that naturally present in human milk, highlighting the need for more refined strategies to better mimic the composition of breast milk. Notably, most existing studies on HMOs are based on short- or medium-term interventions, typically ranging from weeks to months, whereas long-term follow-up data remain limited. Well-designed long-term and longitudinal studies are therefore critically needed to clarify the sustained effects of HMOs on host metabolism, immune development, and long-term health outcomes.

Technologies for HMO synthesis have been continuously advancing, evolving from initial isolation and extraction from human milk to chemical synthesis, enzymatic catalysis, microbial fermentation, and the emerging approach of mammary epithelial cell culture, each with its own advantages. Among these, microbial fermentation has become the most widely applied method in industry because of its potential for large-scale production and commercial application of multiple HMOs. Progress has also been made in China, where several companies have successfully constructed HMO-producing strains and obtained national regulatory approval. Although microbial fermentation is currently the standard method for industrial HMO production, challenges, such as high production costs and limited yields, remain. Future efforts should focus on optimizing fermentation processes, increasing HMO yield and quality, and reducing production costs to achieve large-scale and efficient production. At the same time, chemical and enzymatic synthesis techniques also have room for improvement, for example, by simplifying the chemical synthesis steps or enhancing the enzymatic reaction efficiency. The complementary use of multiple technologies provides greater flexibility and more options for HMO production.

From a metabolic perspective, the “indigestibility” of HMOs should not be considered a nutritional limitation; rather, it forms the basis of their functional significance. HMOs are selectively metabolized by specific gut microbes via diverse pathways to produce SCFAs and other organic acids that help regulate intestinal pH, strengthen the gut barrier, and indirectly support infant gut health.

Despite the increasingly widespread application of HMOs, significant gaps remain in our understanding of structure-function relationships, optimal dosage strategies, interindividual differences, and in vivo metabolic mechanisms. Well-designed randomized controlled trials are required to identify the optimal HMO combination, dosage range, and intervention window, especially by comparing single HMO supplements with multiple HMO mixtures. Additionally, stratifying populations based on maternal secretory status, gestational age, and disease risk characteristics may help explain interindividual differences in HMO efficacy. From a mechanistic perspective, integrating multi-omics technologies, including gut microbiome analysis, metabolomics, transcriptomics, and stable isotope tracing, is crucial for elucidating host-microbiota co-metabolism and establishing robust structure-function relationships. Addressing these unresolved issues is crucial for translating HMO research into more precise and evidence-based nutritional and therapeutic applications. In summary, HMOs are the optimal nutritional source for infants, and breastfeeding remains the most effective strategy for providing nutrition and protection.

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