Abbott

HUMAN MILK OLIGOSACCHARIDES (HMOs)

2022 Compendium of Publications

With a focus on brain development, immune support, and digestive health.

IMPORTANT NOTICE: Breastfeeding is recommended for as long as possible, as mutually desired by mother and infant.

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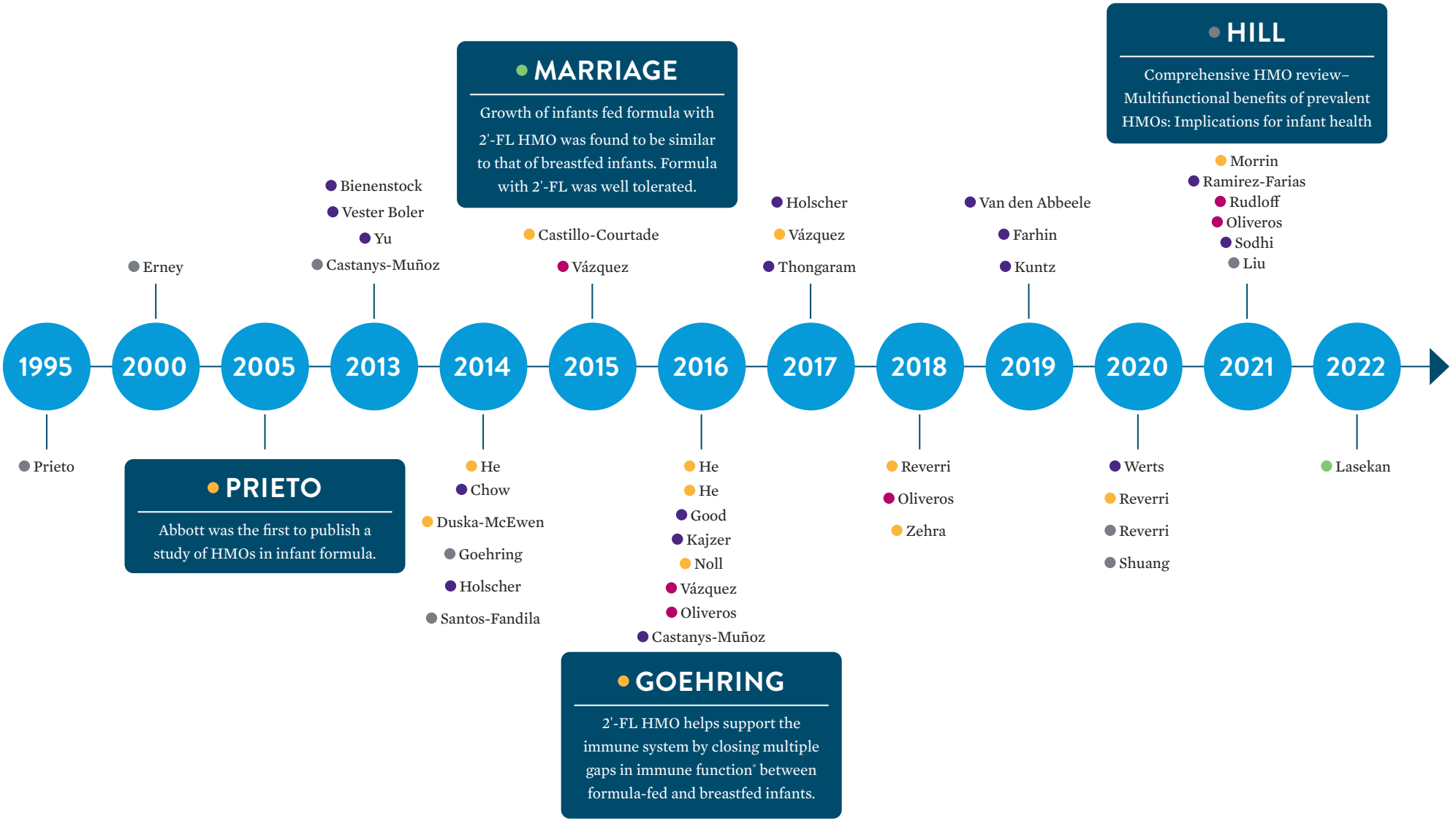
INTRODUCTION

Leading the way in HMO research

ABBOTT HAS SUPPORTED MORE THAN 40 HMO-RELATED PUBLICATIONS OVER THE PAST 25+ YEARS



* As measured by circulatory inflammatory cytokines in a clinical study of Similac with HMO.

HMO research focus: Growth and Tolerance · Immune Support · Brain Development · Digestive Health · Other HMO Research

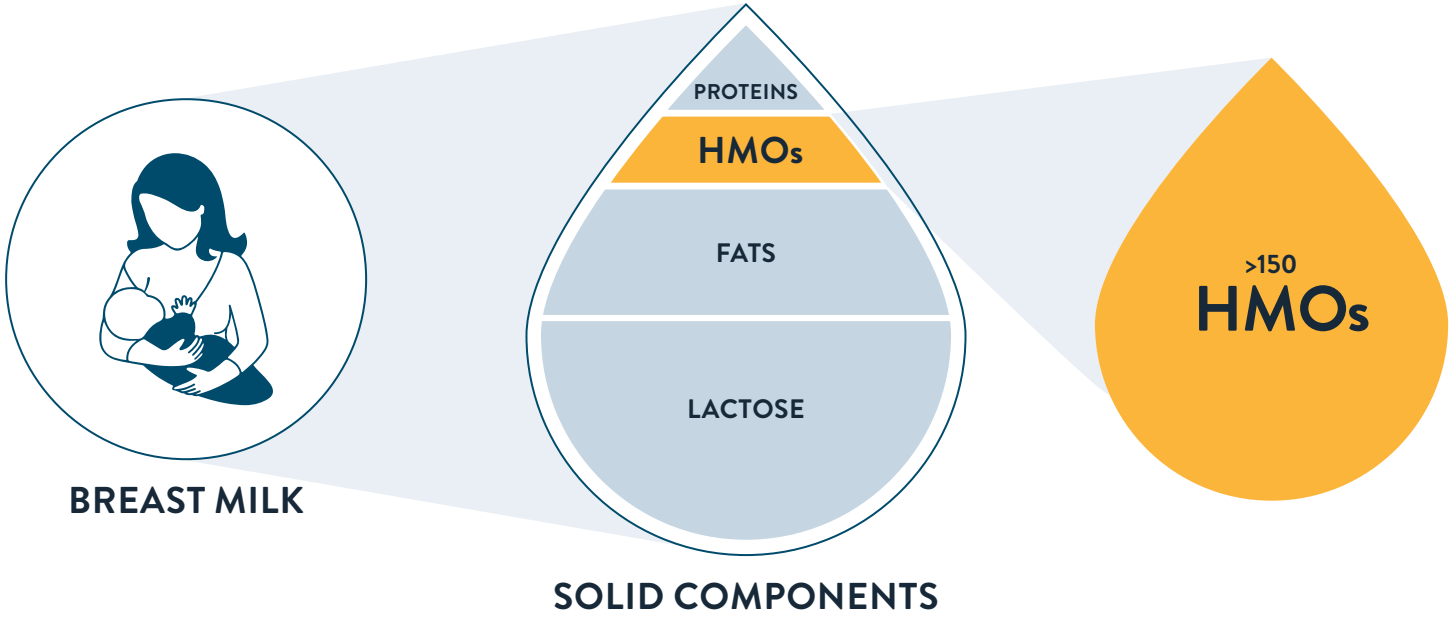
Human milk composition

HMOs: A MAJOR CLASS OF PREBIOTICS FOUND IN HUMAN MILK

Human milk is the "gold standard" for infant nutrition (*Walker, 2010*). Not only does human milk provide essential nutrients required for infant growth and development, it also contains hundreds of different bioactive compounds that may have health benefits (*Bode, 2009*).

After lactose and fat, HMOs are the third most abundant solid component (*Bode, 2019*). HMOs are a unique type of bioactive carbohydrate that act as a prebiotic and may have roles in supporting the immune system, brain development, and digestive health (*Goehring, 2014*; *Bode, 2009*; *Bode, 2012*; *Hill, 2021*).

This compendium summarizes selected scientific publications around HMOs and their potential role in infant health.



Categories of HMOs

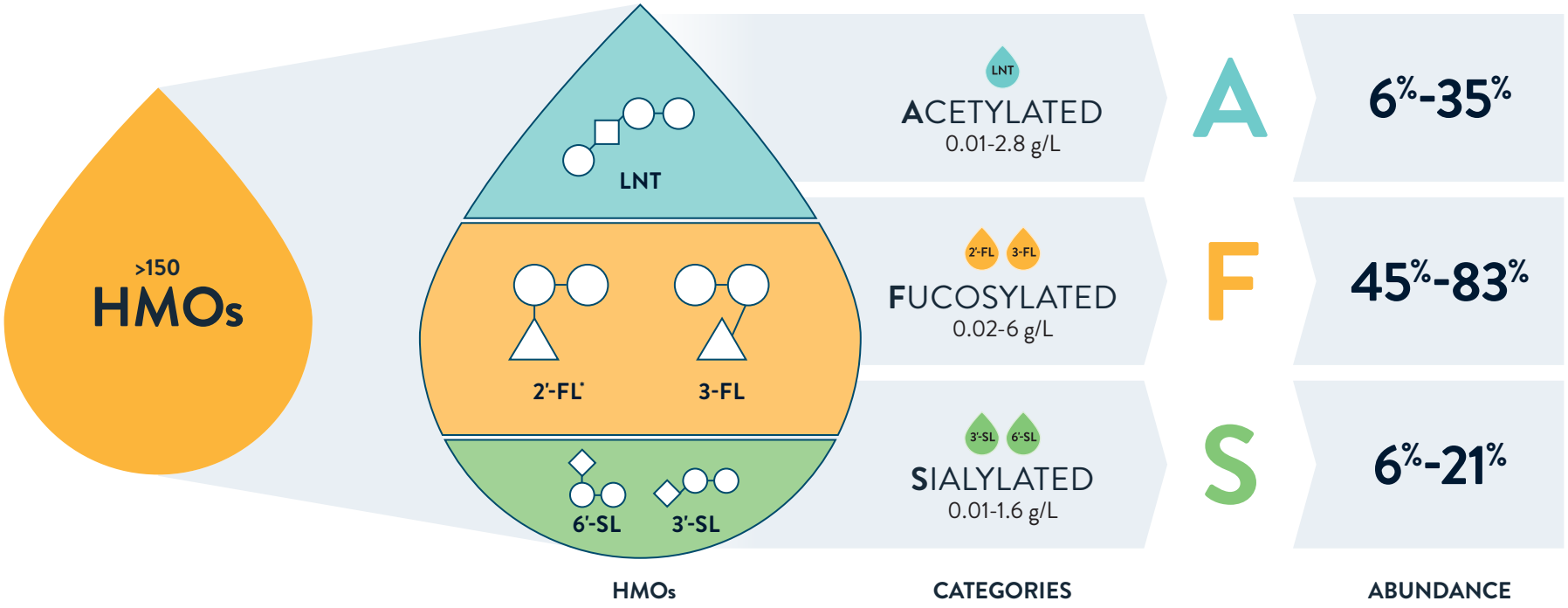
HMOs FUNCTION IN UNIQUE WAYS DUE TO THEIR STRUCTURES—AND EACH MAY PLAY AN IMPORTANT ROLE

There are 3 categories of HMOs in human milk: acetylated (nonfucosylated), fucosylated, and sialylated (*Plaza-Diaz, 2018*). Each HMO category varies structurally from one another: acetylated HMOs contains N-acetylglucosamine at the terminal position, whereas fucosylated HMOs contains fucose and sialylated HMOs contains sialic acid at the terminal positions (*Plaza-Diaz, 2018*).

Within these 3 categories, there are 5 HMOs that are the most abundant in human milk (*Coppa, 1999*; *Thurl, 2010*; *Austin, 2016*; *Kunz, 2017*; *McGuire, 2017*; *Sprenger, 2017*; *Tonon, 2019*).

Emerging research suggests that different HMOs support different functions for the immune system, brain development, and digestive health (*Walsh, 2020*; *Bode, 2012*; *Hill, 2021*).

This compendium focuses on these 5 HMOs.



* Most abundant in most mothers' milk.

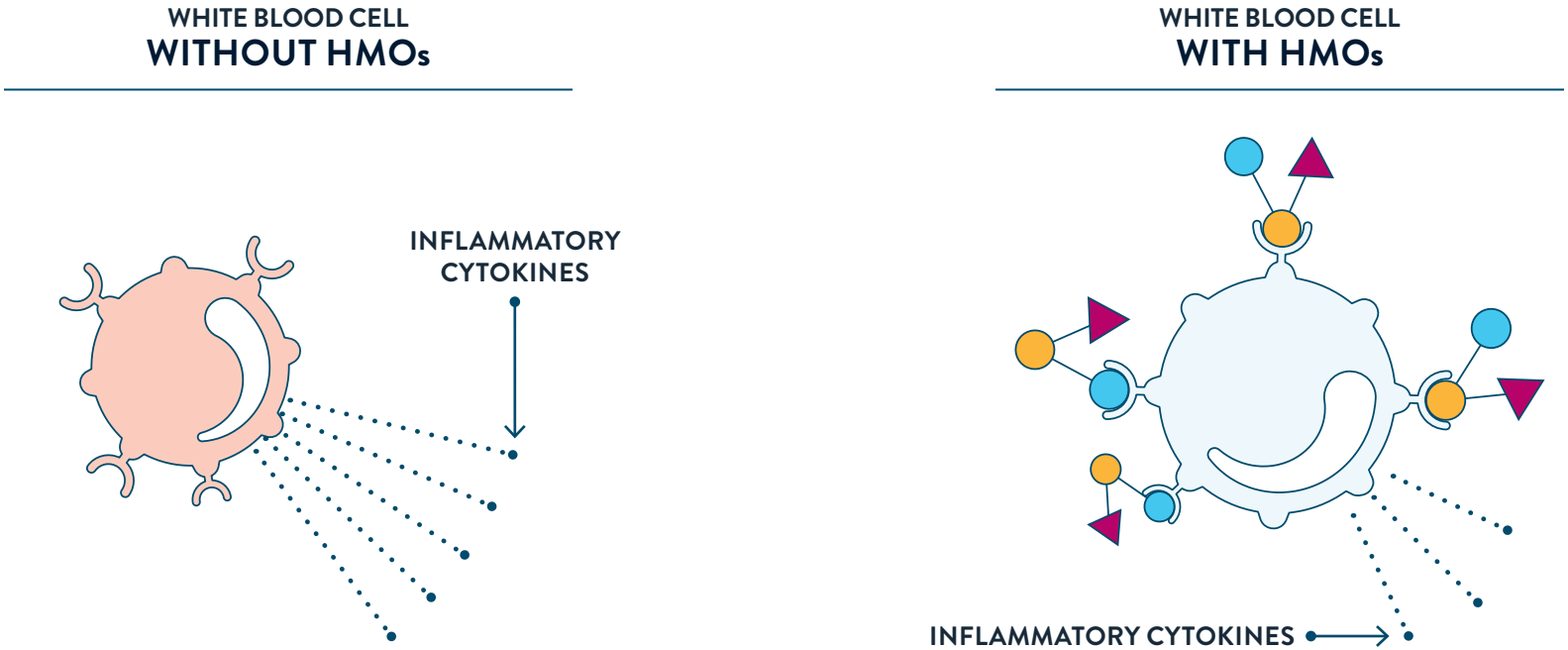
Support for the immune system

HMOs can be thought of as the "conductors of the immune orchestra" because preclinical research suggests that they act as immune cell modulators to help balance immune response—just as a conductor might set a tempo or indicate how loudly or quietly to play music.

If a cytokine balance cannot be established, an excessive pro-inflammatory reaction or an anti-inflammatory reaction may ensue. A range of clinical sequelae may then follow (*Oberholzer, 2000*).

Preclinical research suggests that HMOs interact with immune cells (dendritic cells, T cells, B cells) and influence expression of pro-inflammatory and anti-inflammatory cytokines. HMOs thereby play a role in maintaining immune system homeostasis (*Donovan, 2016*; *Walsh, 2020*; *Hill, 2021*).

- In vitro, 2'-FL HMO has been shown to attenuate monocyte activation and modulate the release of cytokines *(Sotgiu, 2006)*.
- In a clinical study, infants fed human milk or formulas with 2'-FL HMO had no significant differences in inflammatory mediators, including TNF-α. When compared to infants fed formula without 2'-FL, those fed formulas with 2'-FL HMO had significantly lower inflammatory mediators ($p < 0.05$) (*Goehring, 2016*).



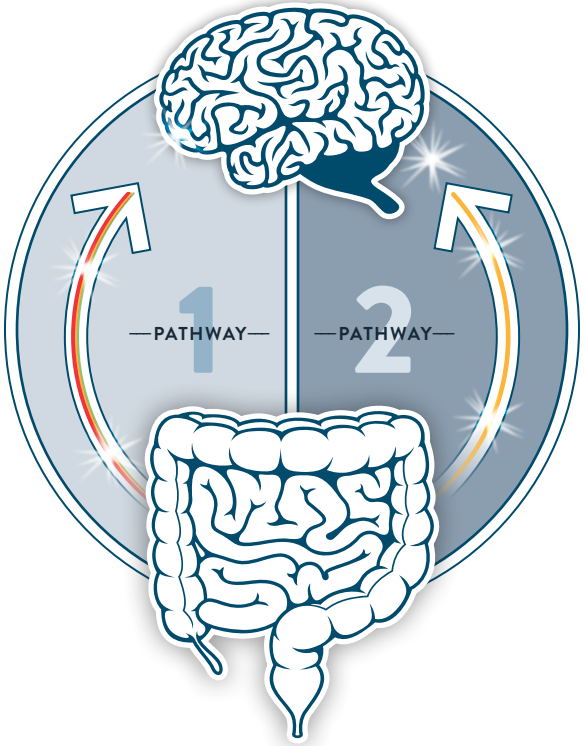
Support for brain development

Preclinical research suggests that HMOs may play a beneficial role in the brain through communication via **1) circulation** and **2) the vagus nerve**. (*Oliveros, 2018*; *Jacobi, 2016*; *Lis-Kuberka, 2019*; *Mudd, 2017*; *Tarr, 2015*; *Vázquez, 2016*; *Vázquez, 2015*; *Wang, 2007*; *Wang, 2009*; *Wang, 2012*; *Krug, 1994*; *Matthies, 1996*; *Al-Khafaji, 2020*; *Kuntz, 2019*; *Hill, 2021*).

PATHWAY 1
CIRCULATION

Fucose metabolites, sialic acid, and microbiota-derived metabolites may be absorbed into the bloodstream, where they can travel to the brain to support cognitive development.

2′-FL 3-FL 3′-SL 6′-SL



PATHWAY 2
VAGUS NERVE

2′-FL and microbiota-derived metabolites may activate the vagus nerve and thus stimulate the developing brain.

2′-FL

CLINICAL OUTCOMES

Human milk concentrations of 2′-FL HMO and 6′-SL HMO have been associated with measures of improved cognitive developmental outcomes through 24 months of age (Oliveros, 2021; Berger, 2020; Jorgensen, 2021).

Support for digestive health

HMOs act as selective prebiotics that resist digestion by human intestinal enzymes and promote growth of healthy microbiota in the gut to support baby's developing immune system (*Bode, 2012*). HMOs are also thought to play an important role in development of the immune system through several mechanisms in the gut and beyond via systemic absorption (*Bode, 2012*; *Castanys-Muñoz, 2016*; *Stepans, 2006*; *Triantis, 2018*; *Hill, 2021*).

SYSTEMIC BENEFITS

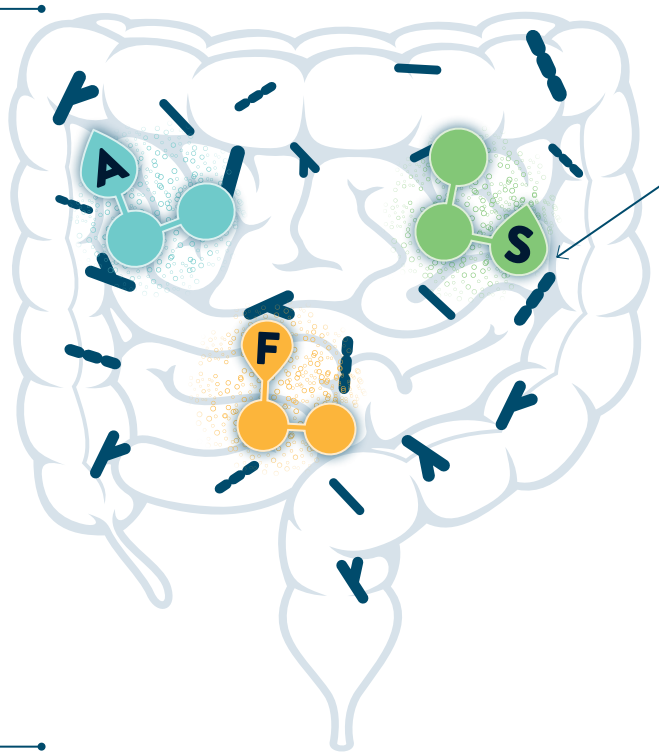
A small portion of ingested HMOs are absorbed intact into circulation and are excreted in urine (*Goehring, 2014*; *Rudloff, 2012*), which may explain some of the reported systemic benefits of HMOs (*Goehring, 2014*; *Goehring, 2016*)

SUPPORT METABOLISM

Feed beneficial bacteria which help synthesize vitamins (*LeBlanc, 2017*)

SUPPORT IMMUNE RESPONSES

Stimulate lymphocytes to produce IgA antibodies (*Kleinman, 2014*)



SUPPORT GUT HEALTH

Selectively support the growth of beneficial gut bacteria (*Walsh, 2020*; *Hill, 2021*)

SHORT-CHAIN FATTY ACIDS

SUPPORT THE DEVELOPING MUCOSAL BARRIER

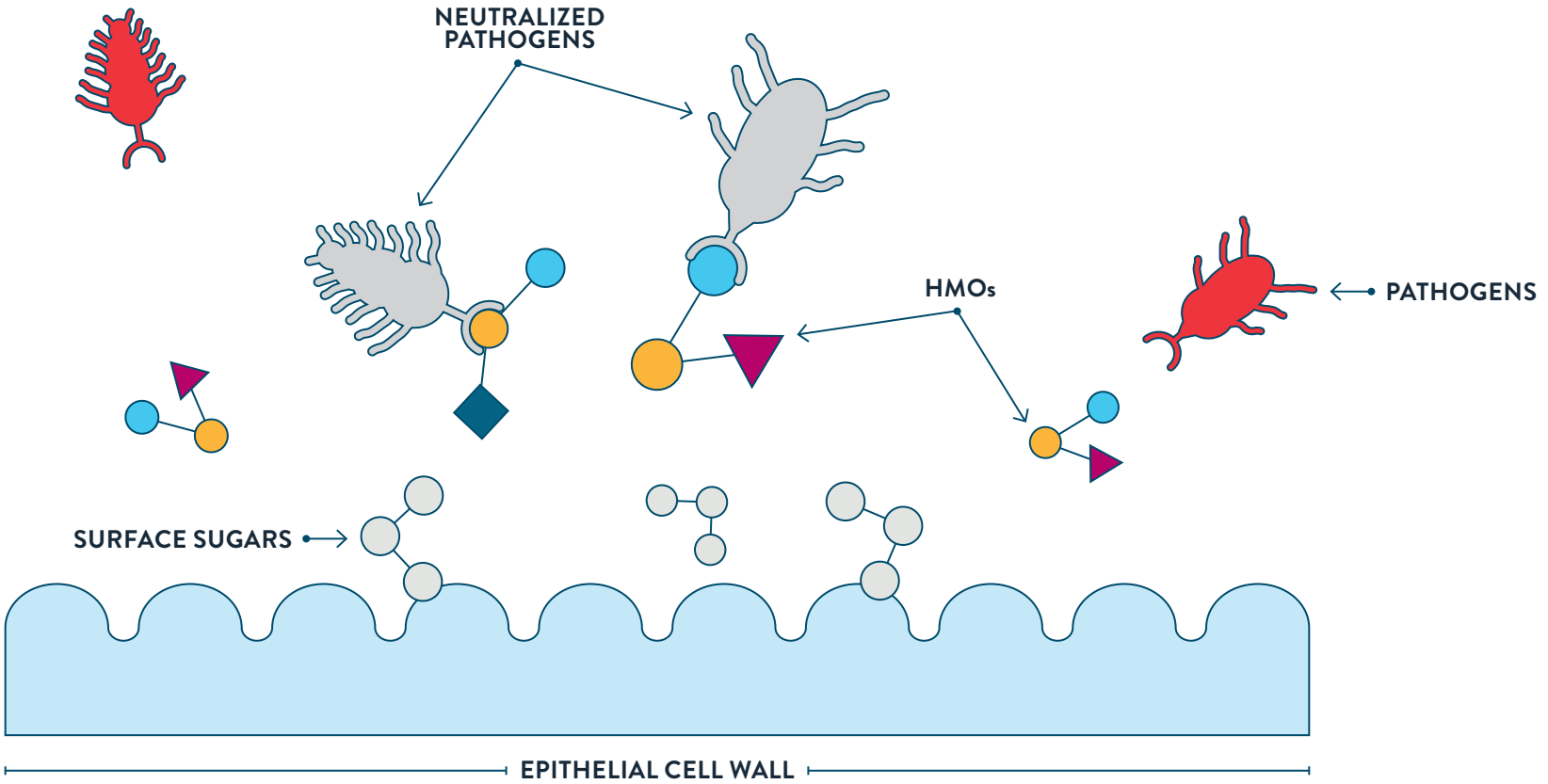
Energy for colonocytes (*Tremaroli, 2012*)

PROMOTE DIGESTIVE TRACT HEALTH

Decreased pH and unfavorable bacteria (*Yu, 2013*)

Support for digestive health (cont'd)

In cell culture, certain HMOs appear to act as a receptor decoy for specific pathogens, which may help block pathogen adhesion to epithelial cell walls (*Bode, 2012*; *Walsh, 2020*; *Kunz, 2000*; *Newburg, 2005*; *Hill, 2021*).



HIGHLIGHTED ABBOTT PUBLICATIONS

- GROWTH AND TOLERANCE
- IMMUNE SUPPORT
- BRAIN DEVELOPMENT
- DIGESTIVE HEALTH
- COMPREHENSIVE HMO REVIEW

2022

Growth and Gastrointestinal Tolerance in Healthy Term Infants Fed Milk-Based Infant Formula Supplemented with Five Human Milk Oligosaccharides (HMOs): A Randomized Multicenter Trial

Lasekan J, Choe Y, Dvoretskiy S, et al. *Nutrients.* 2022;14(13):2625. Published 2022 Jun 24. doi:10.3390/nu14132625

BACKGROUND

The three categories of human milk oligosaccharides (HMOs) include fucosylated HMOs such as 2'-fucosyllactose (2'-FL) and 3-fucosyllactose (3-FL), acetylated HMOs such as lacto-*N*-tetraose (LNT), and sialylated HMOs such as 3'-sialyllactose (3'-SL) and 6'-sialyllactose (6'-SL). After lactose and lipids, HMOs are the third most prevalent solid component in breast milk, which highlight the importance of HMOs for infant nutrition. Five of the most abundant HMOs (out of >150 identified) are 2'-FL, 3-FL, LNT, 3'-SL and 6'-SL. The objective of this clinical study was to evaluate the growth, gastrointestinal tolerance and health outcomes in healthy term-born infants fed an experimental cow's milk protein-based infant formula containing the 5 aforementioned HMOs, each at concentrations in the range found in human milk, representing each category of HMOs and the most abundant HMOs usually found in most women's breast milk.

METHODS

This randomized, controlled, multicenter, double-blind, parallel study was conducted between September 2019 through December 2020. Formula-fed infants were enrolled between 0 and 14 days of life and randomized to receive either the control formula (CF) without HMOs or the experimental formula (EF) containing 5 HMOs at 5.75 g/L through approximately 4 months of age. Human milk-fed infants were enrolled and served as a human milk-fed reference group (HM).

RESULTS

Baseline characteristics of study groups (N=363) were similar at enrollment except that a greater percentage of infants in the EF group included mothers who smoked compared to the CF or HM groups. The study primary variable, weight gain per day from day 14 to day 119 of life among the protocol-evaluable (PE) participants, was not significantly different between CF and EF groups, or when comparing formula-fed groups to the HM group. Gains in weight, length and head circumferences from day 14 to day 119 between the 3 study groups did not differ. Compared to the CF group, the EF group had stools that were closer to the stools of HM reference group; they were more frequent, softer and yellow. Study groups did not differ in rates of adverse events; however, more infants in the CF group compared to the EF group were seen by a health care professional for illness during study entry to day 56 and to day 84 ($p<0.05$ for each time period).

CONCLUSION

This study demonstrated that a cow's milk protein-based infant formula containing 5 of the most abundant HMOs at 5.75 g/L, representing the 3 categories of HMOs, supported normal growth, tolerance and health outcomes in healthy term infants. Some parameters of stool output favored the study group fed the 5 HMO-containing formula compared to infants fed control formula and were closer to those of the human milk-fed reference group. The safety of the 5 HMO-containing formula was supported by no differences seen in adverse events among study groups.

2015

Infants Fed a Lower Calorie Formula With 2'-FL Show Growth and 2'-FL Uptake Like Breastfed Infants

Marriage BJ, Buck RH, Goehring KC, Oliver JS, Williams JA. *J Pediatr Gastroenterol Nutr.* 2015;61(6):649-658. doi:10.1097/MPG.0000000000000889

BACKGROUND

Human milk provides many benefits to infants, such as reduced risk of ear infections, gastrointestinal and respiratory tract diseases, obesity, and diabetes. Ongoing research is starting to reveal the role of HMOs in health protection and development in infants. This study evaluated the growth and tolerance of infants fed formula with the HMO 2'-fucosyllactose (2'-FL).

METHODS

This prospective, randomized, controlled growth and tolerance study was conducted from April 2013 to January 2014. Healthy, full-term infants were enrolled by day of life (DOL) 5 days. Formula-fed infants were randomized to receive one of three formulas. Each formula had a caloric density of 64.3 kcal/dL. The formula in the control group contained the prebiotic, galactooligosaccharides (GOS), while the other two experimental formulas contained GOS and varying amounts of 2'-FL (0.2 vs 1.0 g/L). The human milk-fed infants served as the reference group.

After study enrollment, on DOL 14, 28, 42, 84, and 119, infants had five additional clinic visits. At these clinic visits, growth measurements and diet and clinical histories were collected. Among the participants, a subset group of parents consented to the biological sampling, including urine, stool, and blood, of their infants. Biological samples were collected at DOL 42 and 119. Mothers of human milk-fed babies who consented for biological sampling provided milk samples on DOL 42.

RESULTS

At birth and during the study, there were no significant differences in weight, length, or head circumference among groups. All of the formulas were well tolerated and had comparable mean rank stool consistency, number of stools per day, and percent of feedings with spitting up or vomiting. Plasma and urine samples detected the presence of 2'-FL in infants fed formulas containing 2'-FL. Despite different levels of HMO, the uptake of 2'-FL between the two experimental formulas containing 2'-FL were not significantly different.

CONCLUSION

In this study, the formula-fed infants had no significant differences in growth when compared to human milk fed infants. The formulas that contain 2'-FL at 0.2 g/L and 0.1 g/L were well tolerated, and 2'-FL uptake of these formulas were similar to those of breastfed infants.

2018

Review of the Clinical Experiences of Feeding Infants Formula Containing the Human Milk Oligosaccharide 2′-Fucosyllactose

Reverri EJ, Devitt AA, Kajzer JA, Baggs GE, Borschel MW. *Nutrients.* 2018;10(10):1346. Published 2018 Sep 21. doi:10.3390/nu10101346

BACKGROUND

This review summarized the clinical experiences of feeding infants formula containing the human milk oligosaccharide (HMO) 2′-fucosyllactose (2′-FL) in healthy infants. HMOs are of interest because they are the third most abundant solid component in breast milk besides lactose and lipids. In particular, 2′-FL is the most prevalent HMO in most mothers′ milk. Previous preclinical and human milk association studies have demonstrated HMOs′ effects on immune, gastrointestinal, and brain health. 2′-FL can be synthesized and added to infant formula, and has been determined to be structurally identical to the 2′-FL found in human milk.

METHODS

This narrative review summarized the clinical experiences of feeding healthy infants formula that was supplemented with 2′-FL. The authors searched databases for studies on infant formulas with 2′-FL being fed to infants. Some of the search terms were 2′-FL HMO, 2′-FL, human milk oligosaccharide, HMO, infant formula, and formula.

RESULTS

A systematic review was unable to be conducted due to the limited number of studies with variation in results. Formulas supplemented with 2′-FL were well tolerated, and 2′-FL absorption profiles were similar to those of breastfed infants. Parents reported fewer respiratory infections in infants fed formula with 2′-FL compared to control formula. Formula with 2′-FL was shown in a clinical study to lower levels of multiple inflammatory cytokines to be more like levels in breastfed infants, suggesting 2′-FL had an immune benefit.

CONCLUSION

Several clinical studies have investigated infants fed formulas supplemented with 2′-FL HMO. The addition of 2′-FL to infant formulas brings them closer to the functionality and composition of breast milk compared to similar formulas without 2′-FL.

2016

Similar to Those Who Are Breastfed, Infants Fed a Formula Containing 2'-Fucosyllactose Have Lower Inflammatory Cytokines in a Randomized Controlled Trial

Goehring KC, Marriage BJ, Oliver JS, Wilder JA, Barrett EG, Buck RH. *J Nutr.* 2016;146(12):2559-2566. doi:10.3945/jn.116.236919

BACKGROUND

Human milk-fed infants tend to have fewer infections than formula-fed babies. In preclinical studies, human milk oligosaccharides (HMOs) have been shown to support gut maturation; have prebiotic, antiadhesive and antimicrobial effects; and affect immune responses. In this study, investigators evaluated how formula supplemented with the HMO 2'-fucosyllactose (2'-FL) affects biomarkers of immune function in infants.

METHODS

This study was conducted between April 2013 and January 2014 as part of a larger study, a randomized, double-blind, controlled growth and tolerance study. Healthy term infants were enrolled by day of life 5 and were either exclusively formula-fed or breastfed from enrollment to 4 months of age. The formula-fed babies were randomly assigned 1 of 3 formulas, all of which contained 2.4 g total oligosaccharides/L [control: galacto-oligosaccharides only (GOS), (experimental: GOS + 0.2 or 1.0 g) GOS + 0.2 or 1.0 g 2'-FL/L]. At 6 weeks of age, those for whom consent was given, blood samples were drawn from the infants, and peripheral blood mononuclear cells (PBMCs) were isolated for examination and cytokine concentrations were measured in plasma and in ex vivo-stimulated culture supernatants.

RESULTS

At enrollment, groups were similar with the exception of more babies characterized as black randomized to the experimental formula with 1.0 g 2'-FL/L compared to exclusively breastfed babies that were characterized as white. Compared to control, breastfed infants and infants fed either experimental formula had lower concentrations of 5 plasma inflammatory cytokines ($P<=0.05$). The ex vivo respiratory syncytial virus (RSV)-induced cultures showed that the control-fed group had significantly higher percentages of TNF-α and IFN-y than breastfed infants. Cultures of infants fed either experimental formula did not differ from cultures of breastfed infants. Cytokine production and cellular proliferation in phytohemagglutinin-stimulated PBMCs were not different between study groups.

CONCLUSION

The study indicated that infants fed formula with 2'-FL showed plasma and ex vivo inflammatory cytokine profiles similar to breastfed babies. Similar to breastfed infants, formulas with 2'-FL at the levels studied supported development and regulation of immunity.

2015

Attenuation of Food Allergy Symptoms Following Treatment with Human Milk Oligosaccharides in a Mouse Model

Castillo-Courtade L, Han S, Lee S, Mian FM, Buck R, Forsythe P. *Allergy.* 2015;70(9):1091-1102. doi:10.1111/all.12650

BACKGROUND

Allergic diseases, such as asthma, eczema and food allergies, have significantly increased in the past 30 years, with the World Health Organization even calling them an epidemic. Patients clearly need more effective therapeutic approaches. This study examined the role of two human milk oligosaccharides (HMOs), the neutral 2'-fucosyllactose (2'-FL) and acidic 6'-sialyllactose (6'-SL), and lactose on the symptoms and immune responses to food allergies in mice.

METHODS

Mice were sensitized with oral ovalbumin (OVA) to induce anaphylactic symptoms. Then, 2'-FL, 6'-SL or lactose was administered to OVA-sensitized mice to gauge their treatment response. Sham-sensitized mice were used as controls. To test mast cell response to HMOs, bone marrow-derived mast cells were stimulated in vitro for an immune reaction (an IgE-mediated degranulation) in the presence of 2'-FL, 6'-SL, or control treatment. In another experiment, mice were given 2'-FL, 6'-SL or lactose for either 5 days, 3 days or an hour before an antigen challenge to measure the mast-cell-dependent passive cutaneous anaphylaxis (PCA) reaction.

RESULTS

Oral treatment with 2'-FL or 6'-SL significantly reduced an OVA-induced intestinal allergy, decreasing diarrhea and hypothermia symptoms. The HMOs also both lessened the amount of mast cell protease-1 (mMCP-1) released from mucosal mast cells in serum, while lactose had no significant effect on the food allergy symptoms or mMCP-1 release. 2'-FL and 6'-SL treatments also reduced the number of mast cells in the colon. However, the HMOs had no effect on the levels of antigens in OVA-sensitized mice. The 2'-FL and 6'-SL treatments were associated with increases in the CD4+ CD25+ IL-10+ cell populations in the mice's Peyer's patches and mesenteric lymph nodes. In addition, the HMOs also reduced the PCA response, which suggested that 2'-FL and 6'-SL stabilized mast cells.

CONCLUSION

In a mouse model, both 2'-FL and 6'-SL reduced the risk of developing food allergy and lessened allergic symptoms.

2021

Human Milk Levels of 2'-Fucosyllactose and 6'-Sialyllactose Are Positively Associated With Infant Neurodevelopment and Are Not Impacted by Maternal BMI or Diabetic Status

Oliveros E, Martín MJ, Torres-Espínola FJ, et al. *J Nutr Food Sci.* 2021;4:024.

BACKGROUND

Human milk offers multiple benefits to infants and its composition depends on various factors. Little is known about how maternal factors may influence human milk oligosaccharides (HMOs) composition. This pilot study aimed to determine if maternal BMI or diabetic status during pregnancy are linked to HMO profiles, specifically 2'-fucosyllactose (2'-FL) and 6'-sialyllactose (6'-SL) concentrations, and whether those HMOs impact neurodevelopment in infants.

METHODS

In this secondary analysis of a cohort study, UHPLC-MS/MS was used to analyze 82 human milk samples collected 1 month postpartum from the "Role of Nutrition and Maternal Genetics on the Programming of Development of Fetal Adipose Tissue" (PREOBE Study), and determine 2'-FL and 6'-SL concentrations. The samples came from overweight, obese, normal weight, and gestational diabetic mothers. To evaluate the relationship between 2'-FL and 6'-SL concentrations and infants' neurodevelopment assessed by the Bayley Scales of Infant Development at 6 months and 18 months, Pearson correlation and multivariate linear regression analyses were conducted.

RESULTS

All human milk samples had 6'-SL and 2'-FL. There was a positive association (p=0.023) found between 6'-SL HMO concentrations and composite motor scores at 18 months of age. 2'-FL HMO concentrations were associated with composite scores for motor skills at 6 months of age.

CONCLUSION

6'-SL and 2'-FL levels in human milk were not affected by maternal pre-gestational BMI or diabetic status. 6'-SL was associated with infant neurodevelopment at 18 months of age and 2'-FL was associated with motor scores at 6 months of age.

2018

Sialic Acid and Sialylated Oligosaccharide Supplementation During Lactation Improves Learning and Memory in Rats

Oliveros E, Vázquez E, Barranco A, et al. *Nutrients*. 2018;10(10):1519. Published 2018 Oct 16. doi:10.3390/nu10101519

BACKGROUND

The human milk oligosaccharides (HMOs) in human milk, including sialylated oligosaccharides, are reported to assist with optimal development and maturation of the immune system, in addition to being prebiotics. Sialic acids' (Sia) connection to improved cognitive function has been shown in studies with rodents and pigs, although the mechanism of action hasn't been determined yet. The purpose of this study was to determine if early Sia supplementation plays a role in neural development in rats.

METHODS

A group of rat pups received daily oral supplementation of less Sia than they would get from normal lactation, and other groups received the same levels as they would from normal lactation in the free form of Sia as 6'-sialyllactose (6'-SL). The control group received daily oral supplementation of water alone. Long-term potentiation (LTP) as a test of behavior was conducted, along with IntelliCage system behavior testing. Classic behavioral tests were also conducted, specifically the novel object recognition test and the Y Maze with blocked arm test. Brain samples were collected and analyzed using western blotting and HPLC techniques.

RESULTS

The content of Sia in the brain at weaning and at 1 year did not show significant differences among groups regardless of age. Rats that were fed 6'-SL during the lactation period had significantly more polysialylated neural cell adhesion molecule expression, a marker of memory formation and synaptic connections, compared to rats supplemented with Neu5Ac or control animals. Both Neu5Ac- and 6'-SL-supplemented groups showed improved cognitive development as measured by LTP, but the 6'- SL group was significantly higher relative to the Neu5Ac group.

CONCLUSION

While the mechanisms of action underlying cognitive benefits of Neu5Ac and 6'-SL are not clear, this study demonstrates that the supplementation of either during lactation improves cognitive development in rats. The results show enhanced scores in behavior and electrophysiological analysis in the 6'-SL supplemented group relative to the Neu5Ac group.

2016

Dietary 2'-Fucosyllactose Enhances Operant Conditioning and Long-Term Potentiation via Gut-Brain Communication Through the Vagus Nerve in Rodents

Vázquez E, Barranco A, Ramirez M, et al. *PLoS One*. 2016;11(11):e0166070. Published 2016 Nov 16. doi:10.1371/journal.pone.0166070

BACKGROUND

Breastfeeding is associated with numerous benefits including improved cognitive performance later in life. One of the main components of human milk is human milk oligosaccharide (HMOs), of which 2'-fucosyllactose (2'-FL) is thought to positively influence the central nervous system (CNS) through the gut-brain axis (GBA), specifically the vagus nerve. The purpose of this work is to test the hypothesis that dietary 2'-FL modulates the CNS through the vagus nerve by measuring the effects of dietary 2'-FL or fucose on cognitive skills in rats.

METHODS

The rats were prepared for the experiments and divided into 3 groups: 2'-FL, fucose, and control. 2'-FL and fucose were orally administered to the rats. Two weeks before the feeding treatments, vagotomy was performed. The animals in the experiments were implanted with stimulating and recording electrodes in the hippocampus. In another experiment, a high frequency protocol was employed to evoke long term potentiation (LTP). Animals were also trained and tested in basic Skinner box modules.

RESULTS

Chronic delivery of 2'-FL, but not fucose, enhanced LTP, while vagotomy inhibited the effect of 2'-FL on LTP. 2'-FL also improved cognitive performance in the Skinner box, but a bilateral vagotomy eliminated those conditioning results.

CONCLUSION

The vagus nerve mediated the beneficial effect of ingested 2'-FL on brain function. Furthermore, bilateral vagotomy prevented the beneficial effects of 2'-FL on LTP. Animals fed a diet supplemented with 2'-FL showed enhanced LTP and better results on associative learning related tests. The oral administration of 2'-FL improved brain function and cognition in rats via the vagus nerve.

2015

Effects of a Human Milk Oligosaccharide, 2'-Fucosyllactose, on Hippocampal Long-Term Potentiation and Learning Capabilities in Rodents

Vázquez E, Barranco A, Ramírez M, et al. *J Nutr Biochem.* 2015;26(5):455-465. doi:10.1016/j.jnutbio.2014.11.016

BACKGROUND

Breastfed infants have better cognitive development than formula-fed infants. Human milk is composed of more than 150 individual human milk oligosaccharides (HMOs). Several studies with rats, mice, and chicks have evaluated the role of fucose on brain networks. This study further examined how the HMO 2'-fucosyllactose (2'-FL) effects synaptic plasticity and learning capabilities in rodents.

METHODS

For most of the experiments, 2'-FL was administered orally. Mice were tested for spatial learning, working memory, and operant conditioning using the IntelliCage system, while rats were submitted to long term potentiation (LTP) testing and Skinner box tests. At the end of the experiments, animals were sacrificed and the brains were analyzed using immunohistochemistry, western blotting, and biochemical testing.

RESULTS

Rodents fed 2'-FL demonstrated significantly better performance on tests for spatial learning and working memory relative to the controls. Chronic administration of 2'-FL in the diet increased expression of postsynapstic density protein 95 in the cerebral cortex and hippocampus, increased protein levels of calcium/calmodulin-dependent kinase II in the hipocampus, and increased brain-derived neurotrophic factor expression in the hippocampus.

CONCLUSION

The results of this study demonstrate an enhancement of synaptic plasticity in rats fed a diet with 2'-FL HMO. This was the first evidence for effects of a dietary HMO on brain function and cognition using rodent models.

2021

The Human Milk Oligosaccharides 2'-Fucosyllactose and 6'-Sialyllactose Protect Against the Development of Necrotizing Enterocolitis by Inhibiting Toll-Like Receptor 4 Signaling

Sodhi CP, Wipf P, Yamaguchi Y, et al. *Pediatr Res.* 2021;89(1):91-101. doi:10.1038/s41390-020-0852-3

BACKGROUND

Necrotizing enterocolitis (NEC) is the sudden necrosis of the small intestine, leading to sepsis and potential death in premature infants. NEC develops in part through an overactive signaling pathway in the intestinal epithelium, toll-like receptor 4 (TLR4). The purpose of this study is to determine if human milk oligosaccharides (HMOs) 2'-fucosyllactose (2'-FL) and 6'-sialyllactose (6'-SL) can inhibit the TLR4 signalling pathway and thereby reduce NEC in animal models.

METHODS

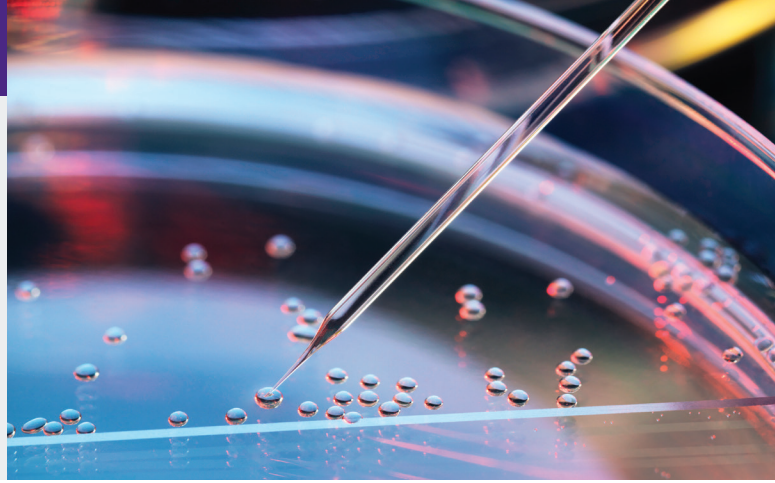
NEC was induced in premature piglets and newborn mice. Animals were fed infant formula supplemented with either lactose, 2'-FL HMO alone, 6'-SL HMO alone, or the combination of 2'-FL and 6'-SL HMOs. Intestinal tissue from the animals was assessed for TLR4 inhibition, in a cell culture model of IEC-6 enterocytes, and in human intestinal explants.

RESULTS

Infant formula supplemented with either 2'-FL and/or 6'-SL was shown to inhibit TLR4 signaling in cultured enterocytes, mouse intestinal tissue and in human intestinal explants, while the infant formula with lactose did not. Furthermore, the mechanism of action may be due to infant formula with 2'-FL and 6'-SL directly binding to TLR4.

CONCLUSION

This study showed that 2'-FL and 6'-SL HMOs protected against the development of NEC in mice and piglet models. These findings suggest that HMOs can be part of a preventative strategy against NEC for infants.



2017

Human Milk Oligosaccharides Influence Intestinal Epithelial Cell Maturation In Vitro

Holscher HD, Bode L, Tappenden KA. *J Pediatr Gastroenterol Nutr.* 2017;64(2):296-301. doi:10.1097/MPG.0000000000001274

BACKGROUND

Human milk oligosaccharides (HMOs) provide prebiotic and immunologic benefits to infants fed human milk and may contribute to gut maturation. HMOs have been added to infant formulas. Previously it was reported that HMOs affect cell dynamics and encourage epithelial cell differentiation in vitro. This study assessed the impact of specific combinations of HMOs of commercial interest on intestinal epithelial cell function, which is critical to gut maturation.

METHODS

An in vitro epithelial model consisting of preconfluent HT-29 cells, preconfluent Caco-2Bbe cells, and postconfluent Caco-2Bbe cells was used to mimic the small intestinal crypt-villus axis. There were 17 treatments of 72-hour incubations including 3 HMOs at low and high doses, individually and in combinations.

RESULTS

Proliferation in HT-29 cultures was decreased by high doses of individual HMOs ($P<0.05$), combined HMOs ($P<0.05$), and pooled HMOs ($P<0.001$). Preconfluent Caco-2Bbe cell proliferations were significantly reduced by pools of individual low and high dose treatments with 3'-SL and 6'-SL, combinations of 2 or 3 high-dose HMOs and pooled HMO. Postconfluent Caco-2Bbe cells treated with pooled HMO had decreased apoptosis and necrosis.

CONCLUSION

Commercially available HMOs in infant formula have the potential to support gastrointestinal tract development. Individual and combined treatments with 2'-FL, 3'-SL and 6'-SL reduced proliferation in preconfluent transformed small intestinal cell lines HT-29 and Caco-2Bbe. This combination suggests that HMOs support gut maturation.

2013

Fucosylated but Not Sialylated Milk Oligosaccharides Diminish Colon Motor Contractions

Bienenstock J, Buck RH, Linke H, Forsythe P, Stanisz AM, Kunze WA. *PLoS One*. 2013;8(10):e76236. Published 2013 Oct 2. doi:10.1371/journal.pone.0076236

BACKGROUND

The benefits of human milk oligosaccharides (HMOs) to babies have been studied by many. Emerging research suggests that HMOs may influence the gut epithelium and have some immune benefits beyond the gut. The purpose of this study is to utilize an ex vivo murine colon model to determine the effects of multiple types of HMOs on colonic motor contractions.

METHODS

The study used a standardized ex vivo colon preparation from mice. The HMOs, 2'-fucosyllactose (2'-FL), 3-fucosyllactose (3-FL), 3'-siallylactose (3'-SL) were synthesized using a bacterial fermentation method. Peak pressures (PPr) were applied to gut motor complexes and the difference between baseline and maximum pressure was measured. HMOs were applied to each experiment only once. Lactose was the negative control in this experiment. To confirm the results of the PPr recordings, a video imaging system recorded colonic motor contractions.

RESULTS

Lactose served as a negative control and did not change the PPr at 1 mg/mL. Of all the HMOs tested, only the fucosylated (2'-FL and 3-FL) HMOs demonstrated effects on PPr by decreasing contractility in a concentration-dependent manner. None of the sialylated (3'-SL and 6'-SL) HMOs decreased contractility, nor did the acetylated HMO (Lacto-N-neotetraose), nor galactooligosaccharides.

CONCLUSION

Using an ex vivo murine colon preparation model, only the fucosylated HMOs (2'-FL and 3-FL) were able to diminish gut motor contractility. A potential mechanism of action is that the fucose or fucose residues may interact with gut tissue receptors to regulate gut motility.

2013

Utilization of Major Fucosylated and Sialylated Human Milk Oligosaccharides by Isolated Human Gut Microbes

Yu ZT, Chen C, Newburg DS. *Glycobiology.* 2013;23(11):1281-1292. doi:10.1093/glycob/cwt065

BACKGROUND

Breast milk contains non-digestable human milk oligosaccharides (HMOs), which are considered prebiotics due to their ability to stimulate the growth of bifidobacteria in the infant gut. The purpose of this study is to measure the degree of utilization of multiple fucosylated and sialylated HMOs by isolated mutualist bacteria.

METHODS

Major fucosylated and sialylated HMOs were added to cell culture media as the sole source of sugar. Physiological digestion of HMOs was measured in vitro in 25 individual microbial strains using liquid chromotography-mass spectrometry.

RESULTS

The major fucosylated HMOs, 2'-fucosyllactose (2'-FL), 3-fucosyllactose (3-FL) and lactodifucotetraose (LDFT), affected the growth of gut microorganisms. The introduction of 2'-FL led to significant changes: in growth (r=0.442, P=0.027) and pH (r=0.514, P=0.009), as well as changes in organic acid production correlated with changes in pH (r=0.739, P=0.001). The major sialylated HMOs, 2'-sialyllactose (3'-SL) and 6'-siallactose (6'-SL) also promoted the growth of gut-related microorganisms, but not as much as 2'-FL and 3-FL.

CONCLUSION

Specific bacteria digest HMOs differently, with the fucosylated HMOs stimulating key species of mutualist symbionts more than other HMO categories. HMOs may be useful in development of novel therapeutic agents to address dysbiosis of the microbiota.

2021

Multifunctional Benefits of Prevalent HMOs: Implications for Infant Health

Hill DR, Chow JM, Buck RH. *Nutrients.* 2021;13(10):3364. Published 2021 Sep 25. doi:10.3390/nu13103364

BACKGROUND

Breast milk provides the best source of nutrition for infants and supports digestion, brain development, the immune system, and a multitude of other functions necessary for optimal growth and development. The addition of HMOs to infant formula represents a significant innovation in infant formula technology. The authors proposed a set of core HMOs that mimic a wide range of the benefits that are provided naturally in breast milk. This review summarizes how HMOs across three categories (acetylated, fucosylated, and sialylated) may function in supporting the developing immune system, promoting digestive health, and supporting cognitive function. It describes HMO structures and composition of HMOs found in breast milk and evaluates functions HMOs may have in supporting digestive health, the immune system, and cognitive development.

SUMMARY

As HMOs pass through the gastrointestinal tract, they exert a multitude of direct and indirect digestive health benefits. Preclinical research suggests that HMOs function as prebiotics, supporting the growth of beneficial microbes. The metabolic byproducts of HMO fermentation, such as short-chain fatty acids, can act as anti-inflammatory agents and support gut motility and intestinal barrier function. HMOs may support the immune system through direct anti-infective activity and immune modulation. Cell culture studies suggest that individual HMO structures in all 3 categories of HMOs inhibit specific pathogen adhesion to host cells, suggesting that multiple diverse HMO structures expand the cumulative profile of anti-adhesive activity in human milk. Furthermore, HMOs can act as acute modulators of immune signaling with potential to shape immediate and long-term health outcomes. A new area of research evaluates HMOs and their role in cognitive development, as well as their relationship to brain health via the gut-brain axis. Breastfeeding association studies demonstrated that exposure to 2'-FL HMO in infancy was correlated with improved performance on tests of cognitive development and motor function. Sialylated HMOs were associated with a multitude of molecular and cellular changes in the developing brain and appeared to play an important role in cognitive development.

CONCLUSION

HMOs may play essential roles in promotion of digestive health, specifically the development of the microbiome. HMOs may also provide direct and indirect immune support, as well as supporting cognitive function in the developing brain, as evidenced by breastfeeding association studies. The authors proposed that a set of 5 core HMOs (2'-FL, 3-FL, 6'-SL, 3'-SL, and LNT) could encompass many key benefits currently attributed to HMOs in breast milk.

REFERENCES

ABBOTT-SPONSORED PUBLICATIONS: ALPHABETICAL BY AUTHOR

B

Bienenstock J, Buck RH, Linke H, Forsythe P, Stanisz AM, Kunze WA. **Fucosylated but not sialylated milk oligosaccharides diminish colon motor contractions.** *PLoS One.* Published 2013 Oct 2. 2013;8(10):e76236. doi:10.1371/journal.pone.0076236

C

Castillo-Courtade L, Han S, Lee S, Mian FM, Buck R, Forsythe P. **Attenuation of food allergy symptoms following treatment with human milk oligosaccharides in a mouse model.** *Allergy*. 2015;70(9):1091-1102. doi:10.1111/all.12650

Chow J, Panasevich MR, Alexander D, et al. **Fecal metabolomics of healthy breastfed versus formula-fed infants before and during in vitro batch culture fermentation.** *J Proteome Res*. 2014;13(5):2534-2542. doi:10.1021/pr500011w

D

Duska-McEwen G, Senft A, Ruetschilling T, Barrett EG, Buck R. **Human milk oligosaccharides enhance innate immunity to respiratory syncytial virus and influenza in vitro.** *Food Nutr Sci*. 2014;5:1387-1398. doi:10.4236/fns.2014.514151

E

Erney RM, Malone WT, Skelding MB, et al. **Variability of human milk neutral oligosaccharides in a diverse population.** *J Pediatr Gastroenterol Nutr*. 2000;30(2):181-192. doi:10.1097/00005176-200002000-00016

F

Farhin S, Wong A, Delungahawatta T, et al. **Restraint stress induced gut dysmotility is diminished by a milk oligosaccharide (2'-fucosyllactose) in vitro.** *PLoS One*. 2019;14(4):e0215151. Published 2019 Apr 24. doi:10.1371/journal.pone.0215151

G

Goehring KC, Kennedy AD, Prieto PA, Buck RH. **Direct evidence for the presence of human milk oligosaccharides in the circulation of breastfed infants.** *PLoS One*. 2014;9(7):e101692. Published 2014 Jul 7. doi:10.1371/journal.pone.0101692

Goehring KC, Marriage BJ, Oliver JS, Wilder JA, Barrett EG, Buck RH. **Similar to those that are breastfed, infants fed a formula containing 2'-fucosyllactose have lower inflammatory cytokines in a randomized controlled trial.** *J Nutr*. 2016;146(12):2559-2566. doi:10.3945/jn.116.236919

Good M, Sodhi CP, Yamaguchi Y, et al. **The human milk oligosaccharide 2'-fucosyllactose attenuates the severity of experimental necrotising enterocolitis by enhancing mesenteric perfusion in the neonatal intestine.** *Br J Nutr*. 2016;116(7):1175-1187. doi:10.1017/S0007114516002944

H

He Y, Lawlor NT, Newburg DS. **Human milk components modulate toll-like receptor–mediated inflammation.** *Adv Nutr*. 2016;7(1): 102-111. Published 2016 Jan 15. doi:10.3945/an.115.010090

Hill DR, Chow JM, Buck RH. **Multifunctional benefits of prevalent HMOs: Implications for infant health.** *Nutrients*. 2021;13(10), 3364. Published 2021 Sep 25. doi:10.3390/nul3103364

Holscher HD, Davis SR, Tappenden KA. **Human milk oligosaccharides influence maturation of human intestinal Caco-2Bbe and HT-29 cell lines.** *J Nutr*. 2014;144(5):586-591. doi:10.3945/jn.113.189704

Holscher HD, Bode L, Tappenden KA. **Human milk oligosaccharides influence intestinal epithelial cell maturation in vitro.** *J Pediatr Gastroenterol Nutr*. 2017;64(2):296-301. doi:10.1097/MPG.0000000000001274

ABBOTT-SPONSORED PUBLICATIONS: ALPHABETICAL BY AUTHOR (CONT'D)

K

Kajzer J, Oliver J, Marriage B. **Gastrointestinal tolerance of formula supplemented with oligosaccharides.** *FASEB J*. 2016;30(suppl 1):671.4. doi:10.1096/fasebj.30.1_supplement.671.4

Kuntz S, Kunz C, Borsch C, et al. **Metabolic fate and distribution of 2′-fucosyllactose: direct influence on gut microbial activity but not on brain.** *Mol Nutr Food Res*. 2019;63(13):e1900035. doi:10.1002/mnfr.201900035

L

Lasekan J, Choe Y, Dvoretskiy S, Borsch C, et al. **Growth and gastrointestinal tolerance in healthy term infants fed milk-based infant formula supplemented with five human milk oligosaccharides (HMOs): A randomized multicenter trial.** *Nutrients*. 2022;14(13):2625. Published 2022 Jun 24. doi:10.3390/nu14132625

M

Marriage BJ, Buck RH, Goehring KC, Oliver JS, Williams JA. **Infants fed a lower calorie formula with 2′-FL show growth and 2′-FL uptake like breastfed infants.** *J Pediatr Gastroentrol Nutr*. 2015;61(6):649-658. doi:10.1097/MPG.0000000000000889

Morrin S, Buck R, Farrow M, Hickey R. **Milk-derived anti-infectives and their potential to combat bacterial and viral infection.** *J Funct Foods*. 2021;81:104442. doi:10.1016/j.jff.2021.104442

N

Noll AJ, Yu Y, Lasanajak Y, et al. **Human DC-SIGN binds specific human milk glycans.** *Biochem J*. 2016;473(10):1343-1353. doi:10.1042/BCJ20160046

O

Oliveros E, Ramirez M, Vázquez E, et al. **Oral supplementation of 2′-fucosyllactose during lactation improves memory and learning in rats.** *J Nutr Biochem*. 2016;31:20-27. doi:10.1016/j.jnutbio.2015.12.014

Oliveros E, Vázquez E, Barranco A, et al. **Sialic acid and sialylated oligosaccharide supplementation during lactation improves learning and memory in rats.** *Nutrients*. 2018;10(10):1519. Published 2018 Oct 16. doi:10.3390/nu10101519

Oliveros E, Martín MJ, Torres-Espínola FJ, et al. **Human milk levels of 2′-fucosyllactose and 6-sialyllactose are positively associated with infant neurodevelopment and are not impacted by maternal BMI or diabetic status.** *J Nutr Food Sci*. 2021;4:024.

P

Prieto PA, Mukerji P, Kelder B, et al. **Remodeling of mouse milk glycoconjugates by transgenic expression of a human glycosyltransferase.** *J Biol Chem*. 1995;270(49):29515-29519. doi:10.1074/jbc.270.49.29515

Prieto PA. **In vitro and clinical experiences with a human milk oligosaccharide, Lacto-N-neoTetraose, and fructooligosaccharides.** *Foods Food Ingredients J Jpn*. 2005;210(11):1018-1030.

R

Ramirez-Farias C, Baggs GE, Marriage BJ. **Growth, tolerance, and compliance of infants fed an extensively hydrolyzed infant formula with added 2′-fucosyllactose (2′-FL) human milk oligosaccharide.** *Nutrients*. 2021;13(1):186. doi:10.3390/nu13010186

ABBOTT-SPONSORED PUBLICATIONS: ALPHABETICAL BY AUTHOR (CONT'D)

Reverri EJ, Devitt AA, Kajzer JA, Baggs GE, Borschel MW. **Review of the clinical experiences of feeding infants formula containing the human milk oligosaccharide 2′-fucosyllactose.** *Nutrients*. 2018;10(10):1346. Published 2018 Sep 21. doi:10.3390/nu10101346

Reverri EJ, Baggs GE, Xie W, et al. **Parent reported health parameters of infants fed HMO containing infant formula, infant formula without HMO, or human milk.** Abstract presented at: North American Society for Pediatric Gastroenterology, Hepatology and Nutrition Online Conference. November 1-7, 2020.

Reverri EJ, Xie W, DeWitt T, et al. **Parent reported health of young children fed HMO containing growing up milk vs no growing up milk.** Abstract presented at: North American Society for Pediatric Gastroenterology, Hepatology and Nutrition Online Conference. November 1-7, 2020.

Rudloff S, Kuntz S, Borsch C, et al. **Fucose as a cleavage product of 2′-fucosyllactose does not cross the blood-brain barrier in mice.** *Mol Nutr Food Res*. 2021;65(16):e2100045. doi:10.1002/mnfr.202100045

S

Santos-Fandila A, Zafra-Gómez A, Vázquez E, Navalón A, Rueda R, Ramírez M. **Ultra high performance liquid chromatography–tandem mass spectrometry method for the determination of soluble milk glycans in rat serum.** *Talanta*. 2014;118:137-146. doi:10.1016/j.talanta.2013.10.013

Sodhi CP, Wipf P, Yamaguchi Y, et al. **The human milk oligosaccharides 2′-fucosyllactose and 6′-sialyllactose protect against the development of necrotizing enterocolitis by inhibiting toll-like receptor 4 signaling.** *Pediatr Res*. 2021;89(1):91-101. doi:10.1038/s41390-020-0852-3

T

Thongaram T, Hoeflinger JL, Chow J, Miller MJ. **Human milk oligosaccharide consumption by probiotic and human-associated bifidobacteria and lactobacilli.** *J Dairy Sci*. 2017;100(10):7825-7833. doi:10.3168/jds.2017-12753

V

Van den Abbeele P, Duysburgh C, Vázquez E, Chow J, Buck R, Marzorati M. **2′-fucosyllactose alters the composition and activity of gut microbiota from formula-fed infants receiving complementary feeding in a validated intestinal model.** *J Funct Foods*. 2019;61:103484. doi:10.1016/j.jff.2019.103484

Vázquez E, Barranco A, Ramírez M, et al. **Effects of a human milk oligosaccharide, 2′-fucosyllactose, on hippocampal long-term potentiation and learning capabilities in rodents.** *J Nutr Biochem*. 2015;26(5):455-465.

Vázquez E, Barranco A, Ramirez M, et al. **Dietary 2′-fucosyllactose enhances operant conditioning and long-term potentiation via gut-brain communication through the vagus nerve in rodents.** *PLoS ONE*. 2016;11(11):e0166070. Published 2016 Nov 16. doi:10.1371/journal.pone.0166070

Vázquez E, Santos-Fandila A, Buck R, Rueda R, Ramirez M. **Major human milk oligosaccharides are absorbed into the systemic circulation after oral administration in rats.** *Br J Nutr*. 2017;117(2):237-247. doi:10.1017/S0007114516004554

Vester Boler BM, Rossoni Serao MC, Faber TA, et al. **In vitro fermentation characteristics of select nondigestible oligosaccharides by infant fecal inocula.** *J Agric Food Chem*. 2013;61(9):2109-2119. doi:10.1021/jf305056f

ABBOTT-SPONSORED PUBLICATIONS: ALPHABETICAL BY AUTHOR (CONT'D)

W

Werts AD, Fulton WB, Ladd MR, et al. **A novel role for necroptosis in the pathogenesis of necrotizing enterocolitis.** *Cell Mol Gastroenterol Hepatol*. 2020;9(3):403-423. doi:10.1016/j.jcmgh.2019.11.002

Y

Yu Z-T, Chen C, Newburg DS. **Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes.** *Glycobiology*. 2013;23(11):1281-1292. doi:10.1093/glycob/cwt065

Z

Zehra S, Khambati I, Vierhout M, Firoz Mian M, Buck R, Forsythe P. **Human milk oligosaccharides attenuate antigen–antibody complex induced chemokine release from human intestinal epithelial cell lines.** *J Food Sci*. 2018;83(2):499-508. doi:10.1111/1750-3841.14039

REFERENCED PUBLICATIONS: ALPHABETICAL BY AUTHOR

Al-Khafaji A, Jepsen S, Christensen K, Vigsnæs L. The potential of human milk oligosaccharides to impact the microbiota-gut-brain axis through modulation of the gut microbiota. *J Funct Foods*. 2020;74:104176. doi:10.1016/j.jff.2020.104176

Austin S, De Castro CA, Bénet T, et al. Temporal change of the content of 10 oligosaccharides in the milk of Chinese urban mothers. *Nutrients*. 2016;8(6):346. Published 2016 Jun 8. doi:10.3390/nu8060346

Berger PK, Plows JF, Jones RB, et al. Human milk oligosaccharide 2′-fucosyllactose links feedings at 1 month to cognitive development at 24 months in infants of normal and overweight mothers. *PLoS One*. 2020;15(2):e0228323. Published 2020 Feb 12. doi:10.1371/journal.pone.0228323

Bode L. Human milk oligosaccharides: prebiotics and beyond. *Nutr Rev*. 2009;67 Suppl 2:S183-S191. doi:10.1111/j.1753-4887.2009.00239.x

Bode L. Human milk oligosaccharides: every baby needs a sugar mama. *Glycobiology*. 2012;22(9):1147-62. doi:10.1093/glycob/cws074

Bode L. Human milk oligosaccharides: Next-generation functions and questions. *Nestle Nutr Inst Workshop Ser*. 2019;90:191-201. doi:10.1159/000490306

Castanys-Muñoz E, Martin MJ, Vazquez E. Building a beneficial microbiome from birth. *Adv Nutr*. 2016;7(2):323-330. Published 2016 Mar 15. doi:10.3945/an.115.010694

Coppa GV, Pierani P, Zampini L, et al. Oligosaccharides in human milk during different phases of lactation. *Acta Paediatrica*. Suppl. 1999;88(430):89-94. doi:10.1111/j.1651-2227.1999.tb01307.x

Donovan SM, Comstock, SS. Human milk oligosaccharides influence neonatal mucosal and systemic immunity. *Ann Nutr Metab*. 2016;69 Suppl 2(Suppl 2):42-51. doi:10.1159/000452818

Jacobi SK, Yatsunenko T, Li D, et al. Dietary isomers of sialyllactose increase ganglioside sialic acid concentrations in the corpus callosum and cerebellum and modulate the colonic microbiota of formula-fed piglets. *J Nutr*. 2016;146(2):200-208. doi:10.3945/jn.115.220152

Jorgensen JM, Young R, Ashorn P, et al. Associations of human milk oligosaccharides and bioactive proteins with infant growth and development among Malawian mother-infant dyads. [published online ahead of print, 2020 Oct 23]. *Am J Clin Nutr*. 2020;113(1):209-220. doi:10.1093/ajcn/nqaa272

Kleinman RE, Greer FR, eds. *Pediatric Nutrition: Policy of the American Academy of Pediatrics*. 7th ed. American Academy of Pediatrics, 2014.

Krug M, Wagner M, Staak S, Smalla KH. Fucose and fucose-containing sugar epitopes enhance hippocampal long-term potentiation in the freely moving rat. *Brain Res*. 1994;643(1-2):130-135. doi:10.1016/0006-8993(94)90018-3

Kunz C, Rudloff S, Baier W, Klein N, Strobel S. Influence of gestational age, secretor, and lewis blood group status on the oligosaccharide content of human milk. *J Pediatr Gastroenterol Nutr*. 2017;64(5):789-798. doi:10.1097/MPG.0000000000001402

Kunz C, Rudloff S, Baier W, et al. Oligosaccharides in human milk: structural, functional, and metabolic aspects. *Annu Rev Nutr*. 2000;20:699-722. doi:10.1146/annurev.nutr.20.1.699

REFERENCED PUBLICATIONS: ALPHABETICAL BY AUTHOR (CONT'D)

LeBlanc JG, Chain F, Martín R, Bermúdez-Humarán LG, Courau S, Langella P. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb Cell Fact*. 2017;16(1):79. Published 2017 May 8. doi:10.1186/s12934-017-0691-z

Lis-Kuberka J, Orczyk-Pawiłowicz M. Sialylated Oligosaccharides and glycoconjugates of human milk. The impact on infant and newborn protection, development and well-being. *Nutrients*. 2019;11(2):306. Published 2019 Feb 1. doi:10.3390/nu11020306

Matthies H, Staak S, Krug M. Fucose and fucosyllactose enhance in-vitro hippocampal long-term potentiation. *Brain Res*. 1996;725(2):276-280. doi:10.1016/0006-8993(96)00406-4

McGuire MK, Meehan CL, McGuire MA, et al. What's normal? Oligosaccharide concentrations and profiles in milk produced by healthy women vary geographically. *Am J Clin Nutr*. 2017;105(5):1086-1100. doi:10.3945/ajcn.116.139980

Mudd AT, Fleming SA, Labhart B, et al. Dietary sialyllactose influences sialic acid concentrations in the prefrontal cortex and magnetic resonance imaging measures in corpus callosum of young pigs. *Nutrients*. 2017;9(12):1297. Published 2017 Nov 28. doi:10.3390/nu9121297

Newburg DS, Ruiz-Palacios GM, Morrow AL. Human milk glycans protect infants against enteric pathogens. *Annu Rev Nutr*. 2005;25:37-58. doi:10.1146/annurev.nutr.25.050304.092553

Oberholzer A, Oberholzer C, Moldawer LL. Cytokine signaling regulation of the immune response in normal and critically ill states. *Crit Care Med*. 2000;28(4 Suppl):N3-N12. doi:10.1097/00003246-200004001-00002

Plaza-Díaz J, Fontana L, Gil A. Human milk oligosaccharides and immune system development. *Nutrients*. 2018;10(8):1038. Published 2018 Aug 8. doi:10.3390/nu10081038

Rudloff S, Kunz C. Milk oligosaccharides and metabolism in infants. *Adv Nutr*. 2012;3(3):398S-405S. Published 2012 May 1. doi:10.3945/an.111.001594

Ruhaak LR, Stroble C, Underwood MA, Lebrilla CB. Detection of milk oligosaccharides in plasma of infants. *Anal Bioanal Chem*. 2014;406(24):5775-5784. doi:10.1007/s00216-014-8025-z

Sotgiu S, et al. Immunomodulation of fucosyl-lactose and lacto-N-fucopentaose on mononuclear cells from multiple sclerosis and healthy subjects. *Int J Biomed Sci*. 2006;2(2):114-120.

Sprenger N, Lee LY, De Castro CA, Steenhout P, Thakkar SK. Longitudinal change of selected human milk oligosaccharides and association to infants' growth, an observatory, single center, longitudinal cohort study. *PLoS One*. 2017;12(2):e0171814. Published 2017 Feb 9. doi:10.1371/journal.pone.0171814

Stepans MB, Wilhelm SL, Hertzog M, et al. Early consumption of human milk oligosaccharides is inversely related to subsequent risk of respiratory and enteric disease in infants. *Breastfeed Med*. 2006;1(4):207-215. doi:10.1089/bfm.2006.1.207

Tarr AJ, Galley JD, Fisher SE, Chichlowski M, Berg BM, Bailey MT. The prebiotics 3'Sialyllactose and 6'Sialyllactose diminish stressor-induced anxiety-like behavior and colonic microbiota alterations: Evidence for effects on the gut-brain axis. *Brain Behav Immun*. 2015;50:166-177. doi:10.1016/j.bbi.2015.06.025

REFERENCED PUBLICATIONS: ALPHABETICAL BY AUTHOR (CONT'D)

Thurl S, Munzert M, Boehm G, Matthews C, Stahl B. Systematic review of the concentrations of oligosaccharides in human milk. *Nutr Rev.* 2017;75(11):920-933. doi:10.1093/nutrit/nux044

Thurl S, Munzert M, Henker J, et al. Variation of human milk oligosaccharides in relation to milk groups and lactational periods. *Br J Nutr.* 2010;104(9):1261-1271. doi:10.1017/S0007114510002072

Tonon KM, Miranda A, Abrão ACFV, de Morais MB, Morais TB. Validation and application of a method for the simultaneous absolute quantification of 16 neutral and acidic human milk oligosaccharides by graphitized carbon liquid chromatography - electrospray ionization - mass spectrometry. *Food Chem*. 2019;274:691-697. doi:10.1016/j.foodchem.2018.09.036

Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. *Nature*. 2012;489:242-249. doi:10.1038/nature11552

Triantis V, Bode L, van Neerven RJJ. Immunological Effects of Human Milk Oligosaccharides. *Front Pediatr.* 2018;6:190. Published 2018 Jul 2. doi:10.3389/fped.2018.00190

Walker A. Breast milk as the gold standard for protective nutrients. *J Pediatr.* 2010;156(2 Suppl):S3-7. doi: 10.1016/j.jpeds.2009.11.021.

Walsh C, Lane JA, van Sinderen D, Hickey RM. Human milk oligosaccharides: Shaping the infant gut microbiota and supporting health. *J Funct Foods.* 2020;72:104074. doi:10.1016/j.jff.2020.104074

Wang B. Molecular mechanism underlying sialic acid as an essential nutrient for brain development and cognition. *Advances in Nutrition.* 2012;3(3):465S–472S. Published 2012 May 1. doi:10.3945/an.112.001875

Wang B. Sialic acid is an essential nutrient for brain development and cognition. *Annu Rev Nutr.* 2009;29:177-222. doi:10.1146/annurev.nutr.28.061807.155515

Wang B, Yu B, Karim M, et al. Dietary sialic acid supplementation improves learning and memory in piglets. *Am J Clin Nutr.* 2007;85(2):561-569. doi:10.1093/ajcn/85.2.561

202210246/September 2022 LITHO IN USA

IMPORTANT NOTICE: Breastfeeding is recommended for as long as possible, as mutually desired by mother and infant.