HUMAN MILK OLIGOSACCHARIDES (HMOs)

2021 Compendium of Studies

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

IMPORTANT NOTICE: Breastfeeding is best for babies and is recommended for as long as possible during infancy.

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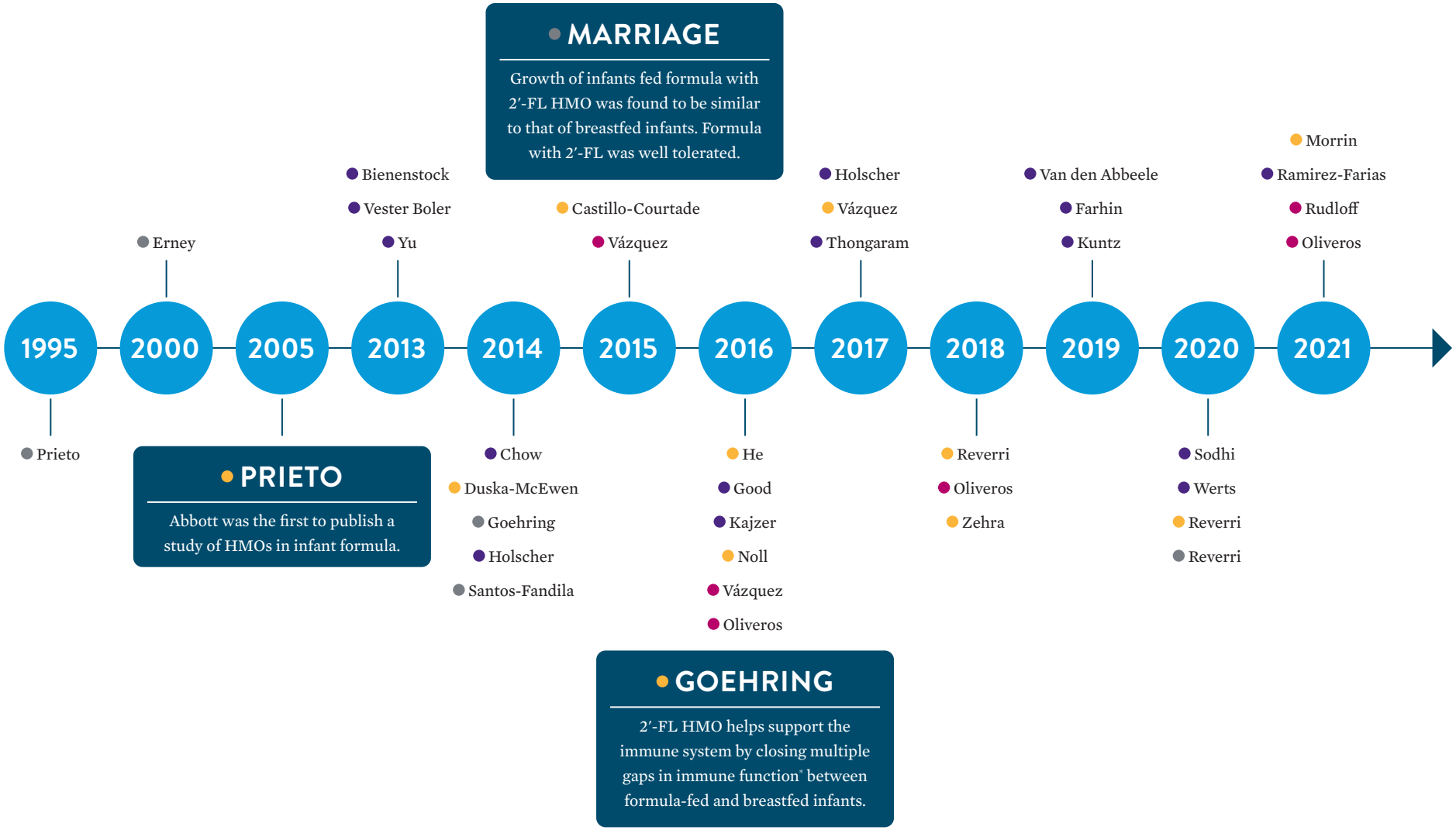
INTRODUCTION

Leading the way in HMO research

ABBOTT HAS SUPPORTED MORE THAN 35 HMO-RELATED STUDIES OVER THE PAST 25+ YEARS



* As measured by circulatory inflammatory cytokines in a clinical study of Similac Pro-Advance®.

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

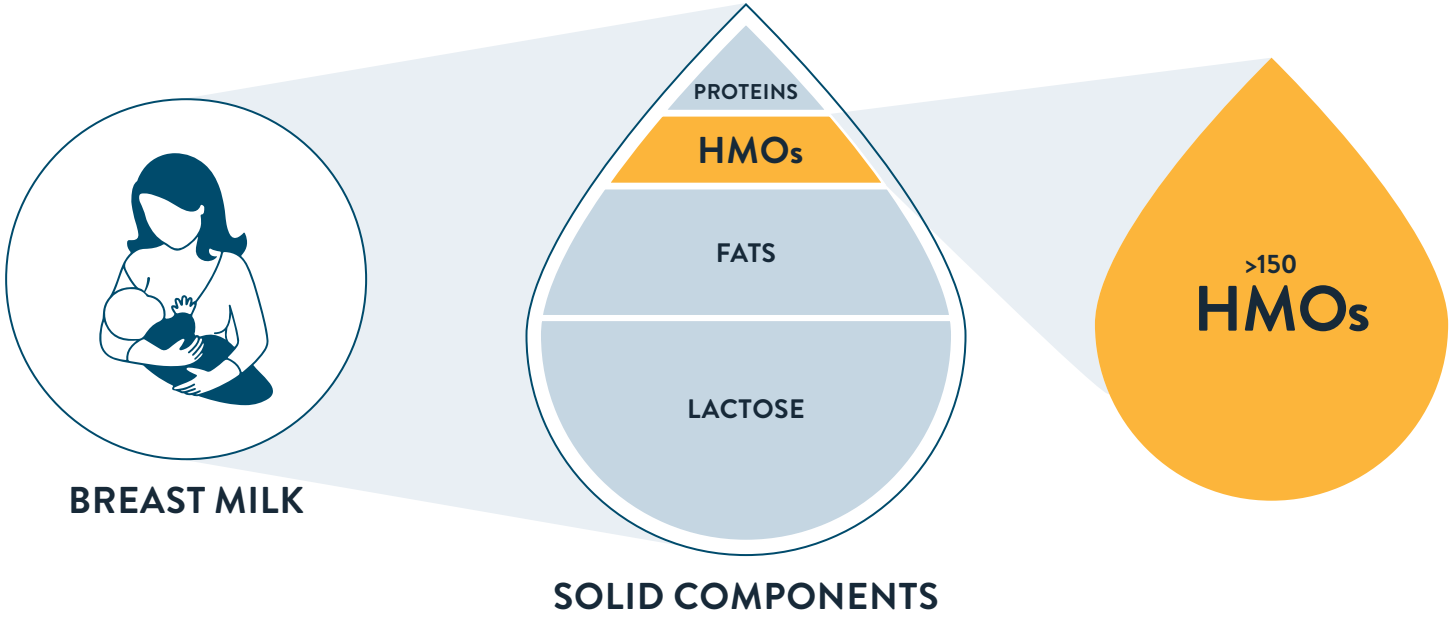
Human milk composition

HMOs: A MAJOR CLASS OF PREBIOTICS FOUND IN HUMAN MILK

Human milk remains the benchmark for infant nutrition, as it contains hundreds of different bioactive compounds. These biologically important compounds support a host of different systems and processes in the developing infant, including healthy microbial colonization in the gut (*Bode, 2012*; *Walsh, 2020*).

Analysis of human milk composition shows that water is the most abundant liquid. Lactose and fats are the most abundant solid components. Interestingly, HMOs are the third most abundant solid component of human milk, followed by proteins (*Bode, 2019*). HMOs are a unique type of prebiotic that may support gastrointestinal, neurologic, and immune system development in ways other prebiotics cannot (*Goehring, 2014*; *Ruhaak, 2014*; *Bode, 2009*; *Bode, 2012*).

This compendium summarizes selected published scientific studies around HMOs and their important role in infant nutrition.



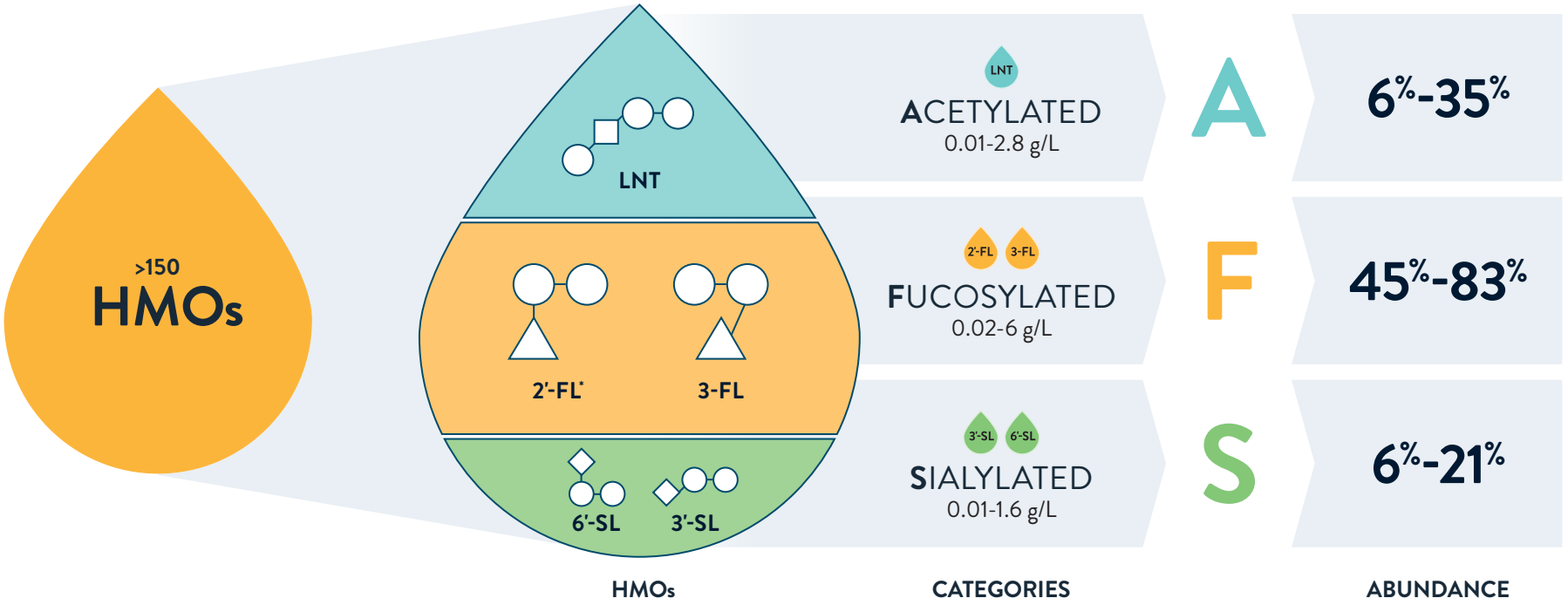
Categories of HMOs

THERE ARE 3 CATEGORIES OF HMOs—AND EACH PLAYS AN IMPORTANT ROLE

There are 3 categories of HMOs in human milk: acetylated, fucosylated, and sialylated (*Plaza-Diaz, 2018*). The categories vary structurally from one another: acetylated HMOs terminate in N-acetylglucosamine, fucosylated HMOs have a fucose at the terminal position, and sialylated HMOs have a sialic acid at the terminal position (*Walsh, 2020*).

This compendium focuses on 5 of the most abundant HMOs commonly found in human milk within the three categories (*Coppa, 1999*; *Thurl, 2010*; *Austin, 2016*; *Kunz, 2017*; *McGuire, 2017*; *Sprenger, 2017*; *Tonon, 2019*).

Because each HMO category is structurally different, emerging research suggests that they support different functions for the immune system, brain development, and digestive health (*Walsh, 2020*; *Bode, 2012*).



* Most abundant in most mothers' milk.

Support for the immune system

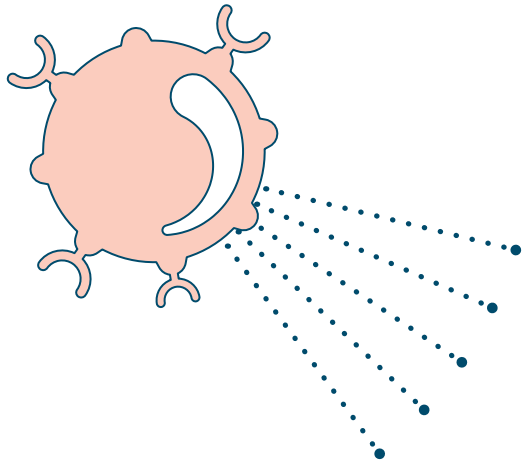
HMOs can be thought of as the "conductors of the immune orchestra" because preclinical research suggests that they act as immune cell modulators to help balance immune response—just as a conductor might set a tempo or indicate how loudly or quietly to play music.

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*).

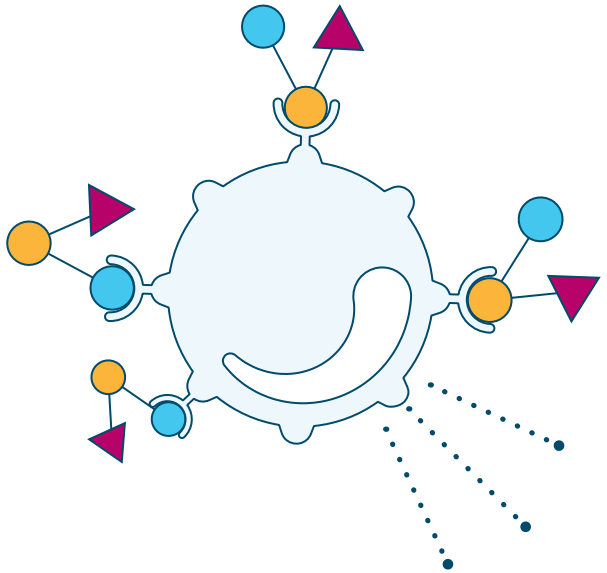
Preclinical research suggests that HMOs interact with immune cells (dendritic cells, T cells, B cells) and influence expression of pro-inflammatory and anti-inflammatory cytokines. HMOs thereby play a role in maintaining immune system homeostasis (*Donovan, 2016*; *Walsh, 2020*).

- In vitro, 2′-FL HMO has been shown to attenuate monocyte activation and modulate the release of cytokines (*Ayechu-Muruzabal, 2018*).
- In a clinical study, 2′-FL HMO was shown to lower the levels of inflammatory mediators, including TNF-α to be more like levels in breastfed infants (*Goehring, 2016*).

WHITE BLOOD CELL
WITHOUT HMOs



WHITE BLOOD CELL
WITH HMOs



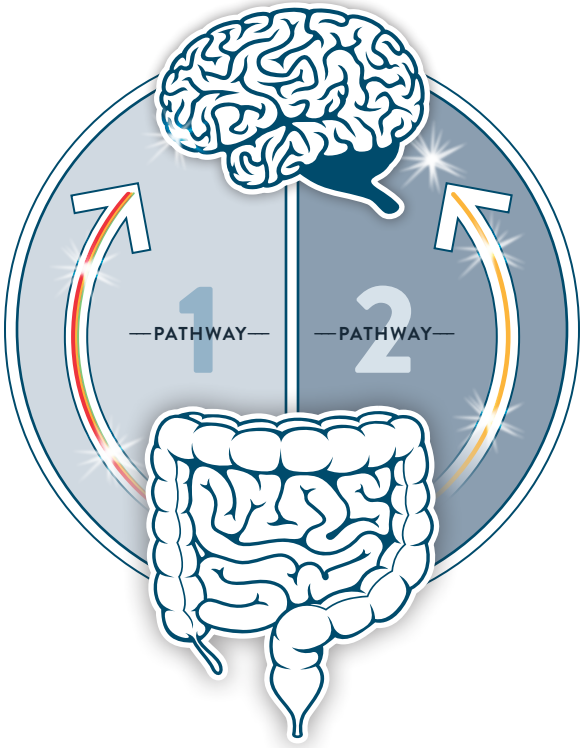
Support for brain development

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*).

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 development 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*).

SYSTEMIC BENEFITS

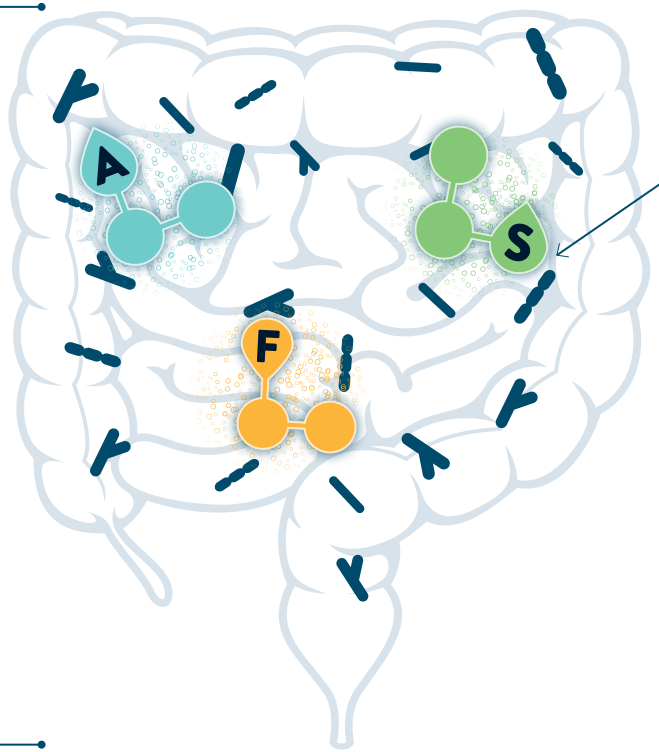
A small portion of ingested HMOs are absorbed intact into circulation and are excreted in urine (*Goehring, 2014*; *Rudloff, 2012*), which may explain some of the reported systemic benefits of HMOs (*Goehring, 2014*; *Goehring, 2016*)

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*)

SHORT-CHAIN FATTY ACIDS

SUPPORT THE DEVELOPING MUCOSAL BARRIER

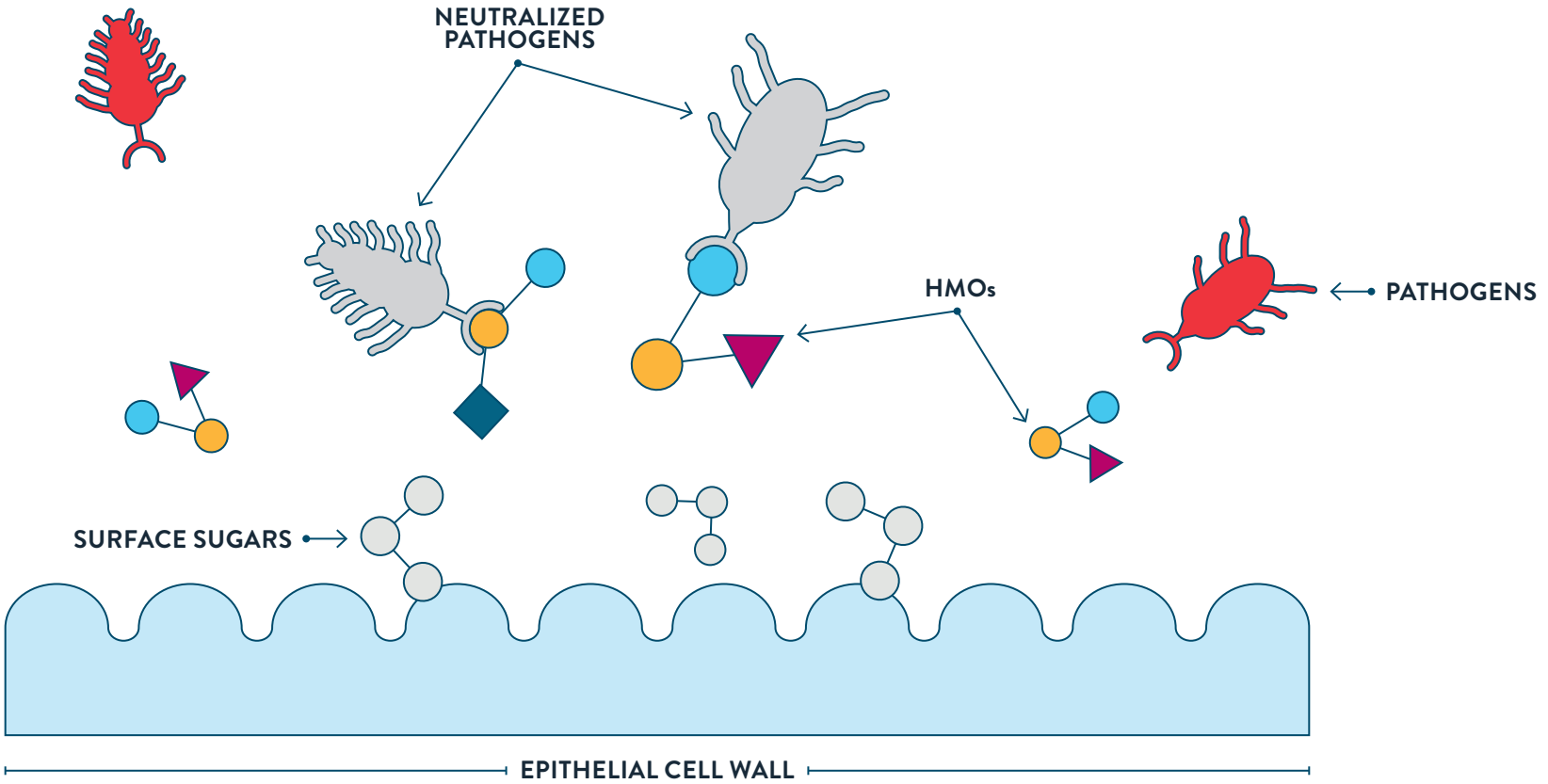
Energy for colonocytes (*Tremaroli, 2012*)

PROMOTE DIGESTIVE TRACT HEALTH

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

Support for digestive health (cont'd)

In cell culture, each of the 5 HMOs appears 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*).



HIGHLIGHTED ABBOTT STUDIES

- IMMUNE SUPPORT
- BRAIN DEVELOPMENT
- DIGESTIVE HEALTH

2018

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

Reverri E, Devitt A, Kajzer J, Baggs G, Borschel M. *Nutrients.* 2018 Sept;10, 1346: 1-11. 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 babies. 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 studies have examined HMOs' effect on preventing gastrointestinal and respiratory problems and aiding immunity for infants, and 2′-FL's role in cognitive development, as a prebiotic, preventing infections and inflammation, supporting the immune system and lessening the risk of necrotizing enterocolitis. Recently, 2′-FLs have been synthesized and added to infant formula, and have been evaluated as 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. However, the authors concluded that standard infant formula with 2′-FL fed to babies was safe, well-tolerated, absorbed and excreted similar to breast milk with 2′-FL. Parents reported less respiratory infections in infants fed formula with 2′-FL compared to the control formula. Also, infants fed with formula containing 2′-FL had immune benefits and improved formula tolerance.

CONCLUSION

The addition of 2′-FL to infant formulas brings them closer to the functionality and composition of breast milk. Several clinical studies reported that babies fed formula with 2′-FL had immune benefits, less parent-reported infections, improved formula tolerance for fussy infants, and efficient excretions.

2016

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

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

BACKGROUND

Breastfed infants have fewer infections than formula-fed babies. The human milk oligosaccharides (HMOs) in breast milk, in particular, have been shown to help with immune functions such as gut maturation, immune responses, prebiotic effects and antimicrobial activities. This study investigated how the HMO 2′-fucosyllactose (2′-FL) in feeding formulas effected immune function in healthy term infants.

METHODS

Between April 2013 and January 2014, this study was conducted as part of a larger study, as a randomized, double-blind, controlled growth and tolerance study with healthy infants of singleton birth at 28 sites in the United States. The babies were enrolled by 5 days of life and were exclusively fed formula (n=317) or breastfed (n=107) from enrollment to 4 months old. The formula-fed babies were randomly assigned one of three formulas, all of which contained 2.4 g total oligosaccharides/L [control: galacto-oligosaccharides only (GOS), GOS + 0.2 or 1.0 g 2′-FL/L]. At six weeks, 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

There were no significant differences between the feeding groups at birth. However, breastfed infants and babies fed any of the experimental formulas with 2′-FL had less concentrations of plasma inflammatory cytokines than infants fed the control formula. The ex vivo respiratory syncytial virus (RSV)-induced cultures showed that the control-fed group had significantly higher percentages of TNF-a and IFN-y than breastfed babies. Control-fed and breastfed infants had similar cytokine production in supernatants from phytohemagglutinin-stimulated PBMCs.

CONCLUSION

The study indicated that infants fed formula with 2′-FL showed lower plasma and ex vivo inflammatory cytokine profiles, similar to breastfed babies. Formulas with 2′-FL support the immune system and regulation, reducing reports of respiratory infections like RSV.

2015

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

Marriage B, Buck R, Goehring K, Oliver J, Williams J. *Journal of Pediatric Gastroenterology and Nutrition.* 2015 December; 61: 649-658. doi: 10:1097/MPG.0000000000000889

BACKGROUND

Human milk provides many benefits to breastfed infants, such as reduced risk of otitis media, gastrointestinal and respiratory tract diseases, obesity and diabetes. This study evaluated the growth and tolerance of babies fed three infant formulas with a caloric density similar to human milk. Two of the formulas were supplemented with 2′-fucosyllactose (2′-FL), a human milk oligosaccharide (HMO), to better quantify the role of HMOs and to study the uptake of HMOs.

METHODS

From April 2013 to January 2014, this prospective, randomized, controlled growth and tolerance study was conducted with healthy, full-term infants who were enrolled by 5 days of age for 119 days. A smaller group of parents agreed to biological sampling for their babies that included urine, stool and blood collection, while breastfeeding mothers provided human milk samples. Formula-fed infants were given one of the three formulas, which all contained 64.3 kcal/dL (19 kcal/fl oz) and galactooligosaccharides (GOS). Two of the experimental formulas were supplemented with different amounts of 2′-FL (at 0.2 and 1.0 g/L). The fourth group was only fed breast milk. Infants were seen at five clinic visits at 14, 28, 42, 84 and 119 days. Their growth was measured, and diet and clinical histories were taken. In the subset group of babies, 2′-FL concentration was tested in their blood and urine samples at 42 and 119 days, and in breastfed infants at 42 days.

RESULTS

At enrollment, there were no significant differences among the feeding groups for age, sex, weight, length or head circumference at birth. The groups showed no significant differences in mean weight, length or head circumference during the study, as well. In addition, the formulas were all well tolerated and led to similar average stool consistency, number of stools per day and percent of feedings associated with spitting up or vomit. Formulas with 2′-FL were well tolerated, and absorbed and excreted similar to breastfed babies.

CONCLUSION

Infants fed formula with a similar caloric density and 2′-FL amount as breast milk grew at a similar pace to breastfed babies. The 2′-FL was well tolerated and absorbed.

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 May; 1346: 1-12. 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 examines 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.

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 ability. Sham-sensitized mice were controls. To test mast cell functions response to HMOs, bone marrow-derived mast cells in vitro were induced for an immune reaction (an IgE-mediated degranulation) and measured for their response to 2′-FL and 6′-SL. 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 mice's 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 suggests that 2′-FL and 6′-SL stabilize mast cells.

CONCLUSION

Both 2′-FL and 6′-SL can reduce the risk of developing a food allergy, and lessen its 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 M, Torres-Espínola F, Segura-Moreno M, Ramírez M, Santos A, Buck R, Rueda R, Escudero M, Catena A, Azaryah H, Campoy C. *Journal of Nutrition & Food Sciences.* 2021; 4(1), 1-11.

BACKGROUND

While human milk offers multiple benefits to infants, its composition is constantly changing due to maternal conditions. In addition, little is known about how maternal factors or the environment may influence human milk oligosaccharides (HMOs) composition. To learn more, the concentrations of 2′-fucosyllactose (2′-FL) and 6′-sialyllacatose (6′-SL) in human milk and their role on infant neurodevelopment were compared with results from neurodevelopment assessments of babies born to mothers from a previous study, “Role of nutrition and maternal genetics on the programming of development of fetal adipose tissue” (PREOBE Study).

METHODS

Ultra-high performance liquid chromatography-MS/MS (UHPLC-MS/MS) was used to analyze 82 human milk samples collected 1 month postpartum from the 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 at 6 months and 18 months (through the Bayley Scales of Infant Development), Pearson correlation and multivariate linear regression analysis were conducted.

RESULTS

All of the study groups had 6′-SL and 2′-FL in their milk samples, while some mothers had low or high 2′-FL concentrations in their milk. There was a positive correlation between 6′-SL concentrations and composite cognitive scores for babies at 18 months old, and a trend was noted for 6′-SL concentrations and motor skills. For 2′-FL, a positive trend was associated with 2′-FL concentrations and motor scores at 6 months of age for infants, but no significant connections were made when low and high 2′-FL groups were assessed separately.

CONCLUSION

The amount of 6′-SL and 2′-FL in human milk was not affected by preconceptional maternal body mass index (pBMI) or gestational diabetes mellitus. 6′-SL levels in human milk were associated with infant neurodevelopment. 2′-FL concentrations were connected to positive motor scores of infants at 6 months of age, but only when evaluating low and high 2′-FL milk samples together. Both 6′-SL and 2′-FL levels in human milk correlated to infant cognitive development.

2018

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

Oliveros E, Vázquez E, Barranco A, Ramírez M, Gruart A, Delgado-García J, Buck R, Rueda R., Martín M. *Nutrients.* 2018 October; 10, 1519, 1-16. doi: 10.3390/nu10101519

BACKGROUND

The human milk oligosaccharides (HMOs) in human milk, including sialyloligosaccharides, are reported to assist with optimal development and maturation of the immune system, besides being prebiotics. Sialic acids' (Sias) connection to improved cognitive functions has been shown in studies with rodents and pigs, although the mechanism of action hasn't been determined yet. For this study, rat pups were given Sia in free form as N-acetylneuraminic acid (Neu5Ac) or conjugated as 6′-sialyllactose (6′-SL) to see if early Sia supplementation plays a role during neural development, and were evaluated later with behavioral tests and electrophysiological measurements.

METHODS

A group of rat pups were given an oral supplementation of Neu5Ac, while another group received the same molar amount of Sia as 6′-SL. Control pups were given water. After weaning, the pups' brains were examined or another set was given classical behavioral tests such as the Novel Object Recognition Test (NORT) and Y maze tests. Then, for one year the pups were given a standard chow diet. After one year, the male pups were evaluated for long-term potentiation (LTP) measurements and females were administered psychological tests to study long-term effects of learning and memory. Their brain samples were analyzed to determine Sia content as adults.

RESULTS

Adult male rats who received 6′-SL during lactation performed better on behavioral assessments and had a significant response in LTP compared to controls and the Neu5Ac group. Animals that received Sia supplementation, 6′-SL and Neu5Ac, spent longer amounts of time exploring novel objects, scored the best on the Y maze task and performed better on the IntelliCage system test.

CONCLUSION

Animals that received Sia, particularly 6′-SL, during lactation, had improved scores in behavior and electrophysiological analysis, as well as long-term cognitive development.

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, Ramírez M, Gruart A, Delgado-García J, Jimenez M, Buck R, Rueda R. *PloS ONE.* 2016 November; 11(11), 1-14 doi: 10.1371/journal.pone.0166070

BACKGROUND

Among breastfeeding's many benefits, it leads to higher performance on intelligence tests later in life for breastfed children compared to formula fed children. One of the main components of human milk is human milk oligosaccharides (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. This study measured the effects of 2′-FL or fucose on rats in vivo hippocampal long-term potentiation (LTP), and 2′-FL's effect on the cognitive skills and hippocampal LTP in rats that undergo bilateral subdiaphragmatic vagotomy.

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, and recording tetrodes. One week after surgery, the electrophysiological studies started, with different pulses applied to Schaffer collaterals. In another experiment, a high frequency protocol was employed to evoke 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 afferent vagus nerve was involved in the modulatory 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 an enhanced LTP and better results on associative learning related tests. The oral administration of 2′-FL benefited brain function and cognition in rats via the vagus nerve. Dietary 2′-FL improved cognitive abilities through the pathway of the GBA and vagus nerve connections in rodents.

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, Gruart A, Delgado-García J, Martínez-Lara E, Blanco S, Martín M, Castanys E, Buck R, Prieto P, Rueda R. *Journal of Nutritional Biochemistry.* 2015; 26, 455-465. doi: 10.1016/j.jnutbio.2014.11.016

BACKGROUND

Breastfed infants are associated with significantly higher cognitive development scores than formula-fed babies. Human milk is hard to replicate, however, with its more than 150 identified human milk oligosaccharides (HMOs) structures. 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

Mice and rats were prepared for the electrophysiological study and implanted with stimulating and recording electrodes in the hippocampus. For most of the experiments, 2′-FL was administered orally. The rodents were tested on input/output curves and long-term potentiation (LTP) after chronic administration of 2′-FL. The IntelliCage testing system evaluated spontaneous and learning behavior of radiofrequency identification (RFID)-tagged mice in a home cage environment. Rats were tested with a fixed-ratio of food pellets in the Skinner box, and were also assessed with immunohistochemistry procedures. Animals were also chronically control fed 2′-FL before their brains were assessed with biochemical techniques.

RESULTS

2′-FL has a positive effect on learning and memory in rodents. The study showed that feeding conscious mice and rats a diet with 2′-FL increased LTP at the hippocampus, enhanced memory consolidation, spatial learning and associative learning. It also increased several molecular brain markers, such as postsynaptic density protein 95.

CONCLUSION

All of the measurable results of this study can be attributed to 2′-FL as it was the only difference in the composition of the rodents' diets. Administering 2′-FL to rodents had a positive effect on their hippocampal LTP and performance in different learning tests. This is the first evidence of the active role 2′-FL plays in cognitive outcomes.

2017

Human Milk Oligosaccharides Influence Intestinal Epithelial Cell Maturation In Vitro

Holscher H, Bode L, Tappenden K. *Journal of Pediatric Gastroenterology and Nutrition*. 2017 February; 64(2), 296-301.
doi: 10.1097/MPG.0000000000001274

BACKGROUND

The human milk oligosaccharides (HMOs) in breast milk provide many benefits to infants, including prebiotic and immunologic development and may induce gut maturation. As such, they are being researched as potential ingredients in infant formulas. Previously it has been reported that HMOs affect cell dynamics and encourage epithelial cell differentiation in vitro. To our knowledge, this is the first study to assess 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 was created of the small intestinal crypt-villus axis with preconfluent HT-29, preconfluent Caco-2Bbe and postconfluent Caco-2Bbe cells. These cell cultures were seeded and allowed to adhere before incubation with HMO treatments for 72 hours. The treatment groups contained synthetic 3′-sialyllactose (3′-SL), 6′-siallylactose (6′-SL) and 2′-fucosyllactose (2′-FL) at low and high doses, combined in pairs or groups of three at both high and low doses, and controls (culture medium, 4g/L pooled HMO and lipopolysaccharide).

RESULTS

The three HMOs in the study performed as well as the more diverse, substantial pooled HMO treatment. This suggests that only a few commercially viable oligosaccharides may be needed to replicate the same results as a full set of oligosaccharides from human milk. Preconfluent HT-29 cultures were decreased by high doses of individual HMOs ($P<0.05$), combined HMOs ($P<0.05$) and pooled HMO ($P<0.001$). Preconfluent Caco-2Bbe cell proliferations were significantly reduced by pools of individual low and high treatments with 3′-SL and 6′-SL, combinations of 2 or 3 high-dose HMOs and total HMO. Postconfluent Caco-2Bbe cells treated with pooled HMO had decreased apoptosis and necrosis, amongst other results.

CONCLUSION

Specific, commercially viable 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. Decreased proliferation was also associated with enhanced differentiation. This combination suggests that HMOs support gut maturation.

2013

Fucosylated but Not Sialylated Milk Oligosaccharides Diminish Colon Motor Contractions

Bienenstock J, Buck R, Linke H, Forsythe P, Stanisz A, Kunze W. *PLoS ONE.* 2013 October; 8(10), 1-9. doi: 10.1371/journal.pone.0076236

BACKGROUND

The benefits of human milk oligosaccharides (HMOs) in breast milk to babies has been studied by many researchers, but more is left to learn about their biological effects. In particular, HMOs seem to effect colonic cell lines through promoting the growth of different types of gut bacteria, such as bifidobacteria and lactic acid bacteria. This study evaluates if HMOs like 2′-fucosyllactose (2′-FL) and 3-fucosyllactose (3-FL) can control gut motor contractions.

METHODS

The study used a standardized ex vivo colon preparation with mice; the HMOs, 2′-FL, 3-FL and 6′-sialyllactose (6′-SL), came from bacterial synthesis. Peak pressures (PPr) were applied to gut motor complexes, and the difference between baseline and maximum pressure reached were measured. HMOs were applied to each experiment only once, because their concentration did not fully wash out. To confirm the results of the PPr recordings, a video imaging system recorded peristalsis of colonic motor contractions.

RESULTS

Only 2′-FL and 3-FL effected PPr, decreasing the frequency of gut contractions and velocity. No significant effects were observed with the other HMOs: 6′-SL, 3′-sialyllactose, lacto-N-neotetraose and galactooligosaccharides. Lactose was the negative control in this experiment.

CONCLUSION

HMOs with fucosylated molecules, such as 2′-FL and 3-FL, calmed colon contractions within 5 to 15 minutes. Fucose seems to play a significant role in reducing gut motility. It is worth exploring if fucosylated HMOs can reduce the pain associated with disordered motility, such as infantile colic. The results also showed potential for fucosylated oligosaccharides to positively effect cognition and memory.

2013

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

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

BACKGROUND

Breast milk is full of human milk oligosaccharides (HMOs), which are considered prebiotics because they stimulate the growth of several bifidobacteria of the infant gut microbiota. HMOs cannot be digested by infants' intestines. But this actually allows them to greater nourish infants, as the majority of ingested oligosaccharides are able to be used as a source of energy and enrich gut bacteria. However, most gut bacteria need more than just HMOs to grow. This study measured the degree to which bacteria are able to use fucosylated HMOs and sialylated HMOs for their growth.

METHODS

The human intestinal microbiota has 25 major isolates. In this study, each of the 25 major isolates was fed individual major fucosylated and sialylated HMOs in an anaerobic culture. Then, specific HMOs were assessed for their effect on the growth and metabolic products of individual microbiota bacteria.

RESULTS

The major fucosylated HMOs, 2′-fucosyllactose (2′-FL), 3-fucosyllactose (3-FL) and lactodifucotetraose (LDFT), affected the growth of gut microorganisms. The induction of 2′-FL and acid production led to significant changes: 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 human milk trisaccharides, 3′-sialyllactose (3′-SL) and 6′-siallactose (6′-SL), also affected the growth of gut-related microorganisms, but not as much as 2′-FL and 3-FL.

CONCLUSION

Major fucosylated HMOs can strongly stimulate gut bacteria (bifidobacteria and bacteroides), reduce pH and create an acidic environment in their role as prebiotics.

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IMPORTANT NOTICE: Breastfeeding is best for babies and is recommended for as long as possible during infancy.

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