Bioactive Components of Human Milk

Including HMOs and Other Prebiotics

Lars Bode, PhD

Pediatric Nutrition
CONTINUING EDUCATION FOR CLINICIANS
pnce.org

Annenberg Center For Health Sciences
at Eisenhower
Imparting knowledge. Improving patient care.

This activity is supported by an educational grant from
Mead Johnson Nutrition.

Faculty Presenter

Lars Bode, PhD
Professor of Pediatrics
Endowed Chair of Collaborative Human Milk Research
Director, Larsson-Rosenquist Foundation
Mother-Milk-Infant Center of Research Excellence
University of California, San Diego
La Jolla, California

Faculty Disclosures

Lars Bode, PhD

Speaker	Abbott Nutrition, Nestlé Nutrition, Nutricia/Danone, Medela
Research Grants	NIH, Bill & Melinda Gates Foundation, Larsson-Rosenquist Foundation
Endowment	Larsson-Rosenquist Foundation

Learning Objectives

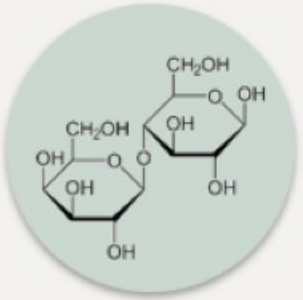
- Summarize ongoing research into the bioactive components of human milk
- Describe the evidence for use of prebiotics in infant feeding

Human Breast Milk Composition

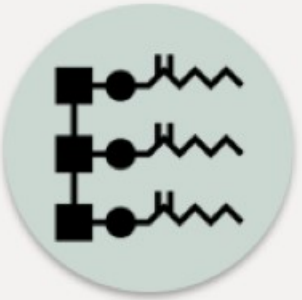
Introduction to Human Milk Oligosaccharides

The Composition of Human Breast Milk

Water



Carbohydrates
Lactose, HMOs



Lipids
Phospholipids, sphingolipids

Proteins

Immune Cells and Factors
Macrophages, cytokines, chemokines, immunoglobulins

Growth Factors and Hormones

HMO, human milk oligosaccharide.

1. Ballard O, Morrow AL. *Pediatr Clin North Am*. 2013;60(1):49-74.
2. Sánchez C, et al. *Nutrients*. 2021;13(3):1026.

Overview of Human Milk Oligosaccharides

- Nonnutritive carbohydrates found in breast milk [1]
  - Indigestible and reach the small intestine and colon virtually intact
- Third most abundant bioactive component in human breast milk (after lipids and lactose) [1]
- Unique to human breast milk [1],[2]
  - Synthesis is highly energy intensive [1]

Macronutrient Concentrations in Human and Cow's Milk [2]

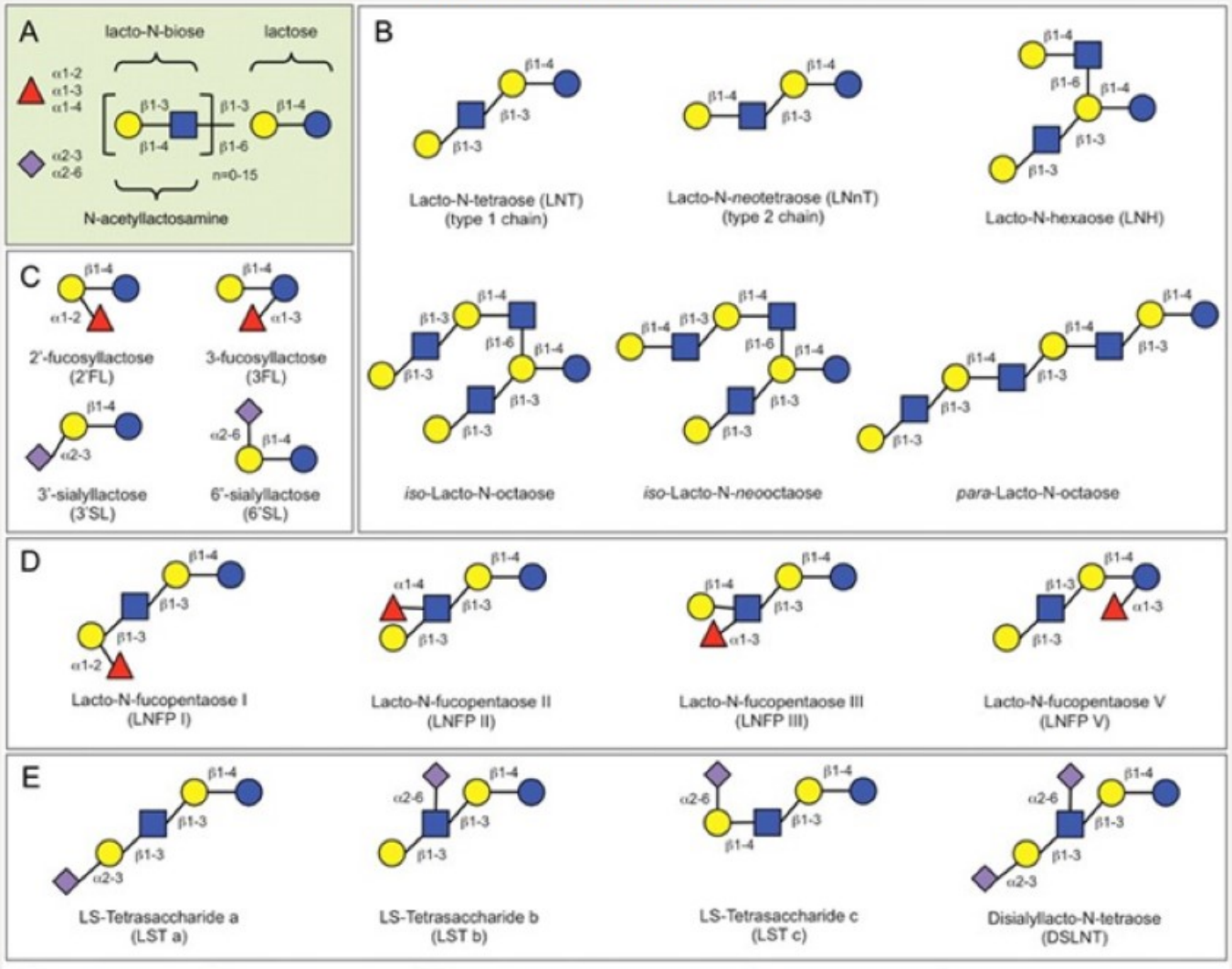
	Human	Cow
Protein (g/L)	8	32
Fat (g/L)	41	37
Lactose (g/L)	70	48
Oligosaccharides (g/L)	**5-15**	**0.05**

1. Sánchez C, et al. *Nutrients*. 2021;13(3):1026.
2. Bode L. *Glycobiology*. 2012;22(9):1147–1162.

HMO Structural Blueprint

- **5 basic building blocks** (monosaccharides) of HMOs [1],[2]:
  - glucose
  - galactose
  - N-acetylglucosamine
  - fucose
  - sialic acid
- **Over 150** HMOs have been identified [1]

HMO Structures [2]

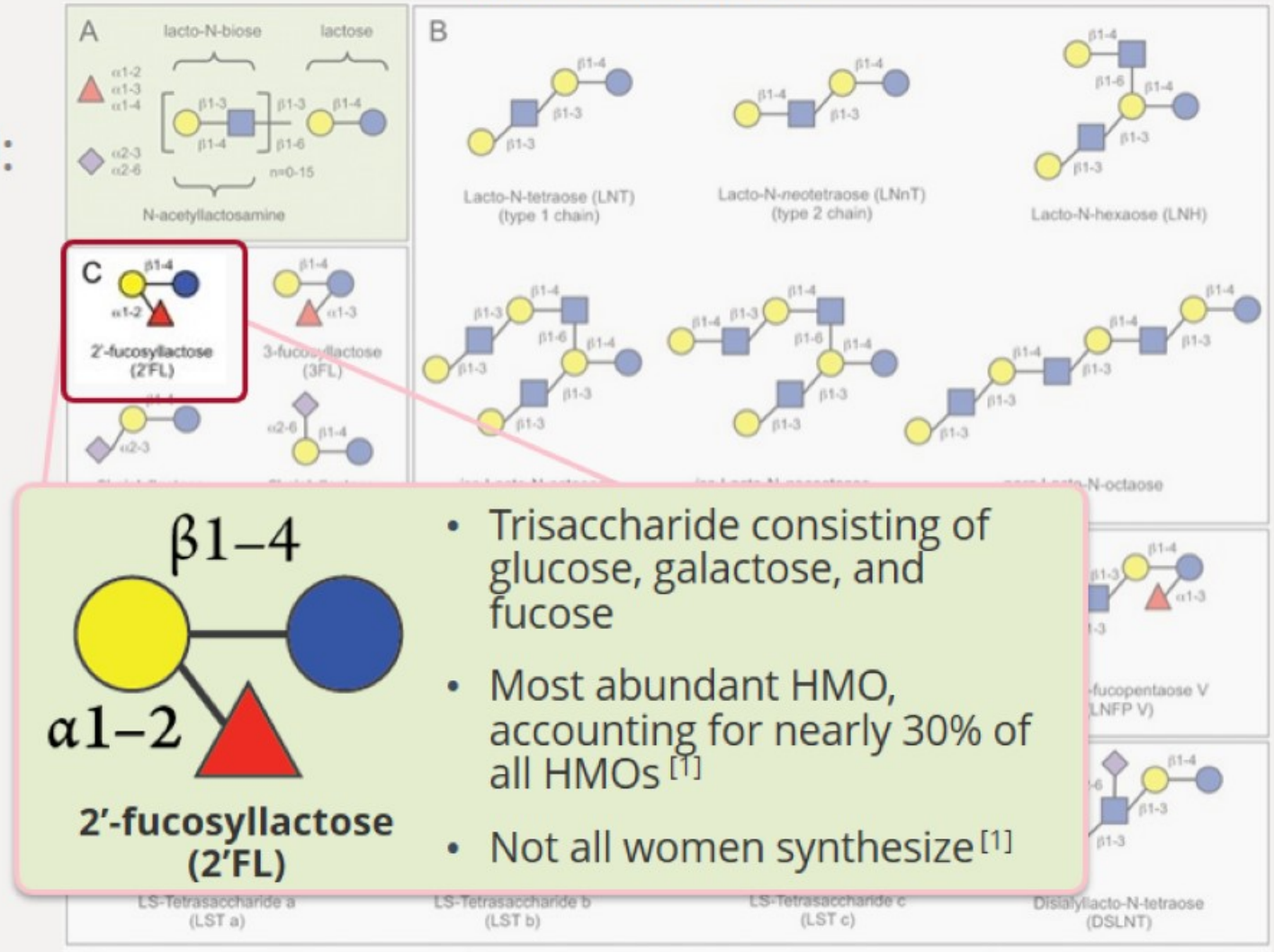


1. Sánchez C, et al. *Nutrients*. 2021;13(3):1026.
2. Bode L. *Glycobiology*. 2012;22(9):1147–1162. Used by permission of *Oxford University Press* on behalf of the American Society for Nutrition.

HMO Structural Blueprint

- **5 basic building blocks** (monosaccharides) of HMOs [1],[2]:
  - glucose
  - galactose
  - N-acetylglucosamine
  - fucose
  - sialic acid
- **Over 150** HMOs have been identified [1]

HMO Structures [2]

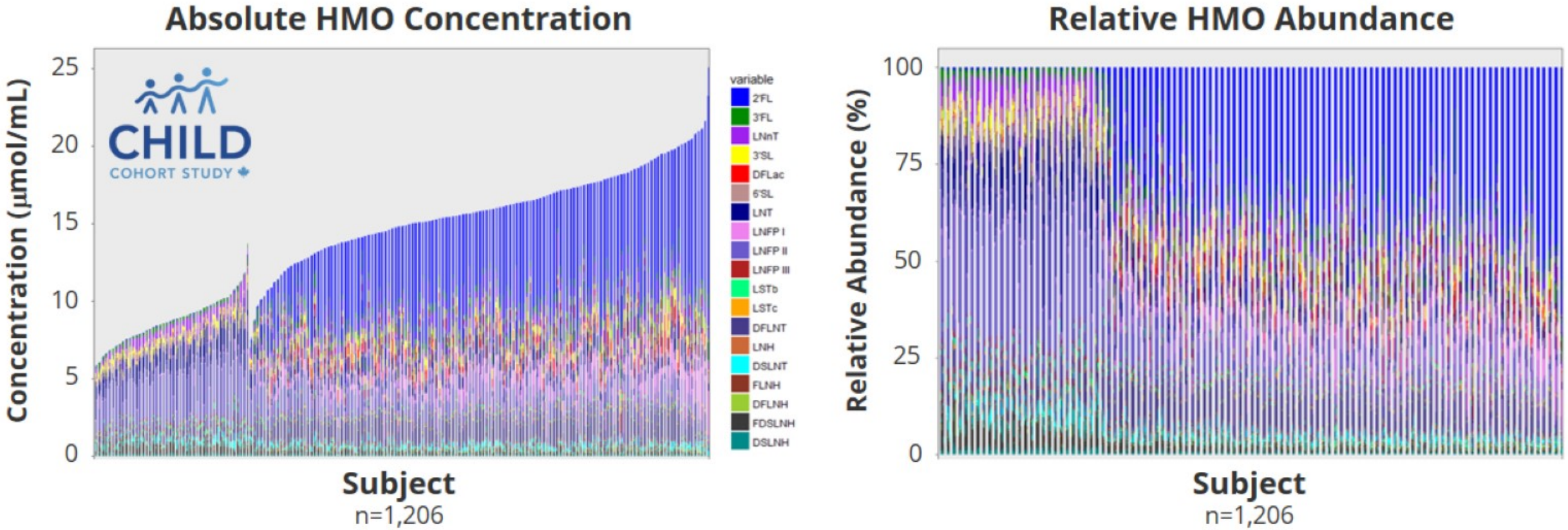


2'-fucosyllactose (2'FL)

- Trisaccharide consisting of glucose, galactose, and fucose
- Most abundant HMO, accounting for nearly 30% of all HMOs [1]
- Not all women synthesize [1]

1. Cheng YJ, Yeung CY. *Pediatr Neonatol*. 2021;62(4):347-353.
2. Bode L. *Glycobiology*. 2012;22(9):1147–1162. Used by permission of *Oxford University Press* on behalf of the American Society for Nutrition.

Maternal Variation in HMO Concentration



CHILD, Canadian Healthy Infant Longitudinal Development study.

1. Azad MB, et al. *J Nutr*. 2018;148(11):1733-1742. Used by permission of *Oxford University Press* on behalf of the American Society for Nutrition.

Genetic Drivers of Maternal Variation in HMO Production

- HMO composition in breast milk is determined in part by maternal genetics and the activity of genes in the Lewis antigen system
  - ***Se* (secretor) gene:** α1-2-fucoslyltransferase (FUT2)
  - ***Le* (Lewis) gene:** α1-3/4-fucosyltransferase (FUT3)
- FUT2 is responsible for synthesis of 2′-FL and other α1-2-fucosylated HMOs
  - Women who do not encode a functional FUT2 enzyme cannot synthesize α1-2-fucosylated HMOs

Maternal Genetic Background and Associated HMO Composition

Lewis Gene (FUT3)	Secretor Gene (FUT2)	Phenotype	Associated HMOs
+	+	Lewis positive secretor	All HMOs
+	-	Lewis positive non-secretor	LNT, LNFP II, LNFP III, LNDFH II
-	+	Lewis negative secretor	2′-FL, 3FL, LNFP I, LNFP II
-	-	Lewis negative non-secretor	3FL, LNT, LNFP III, LNFP V

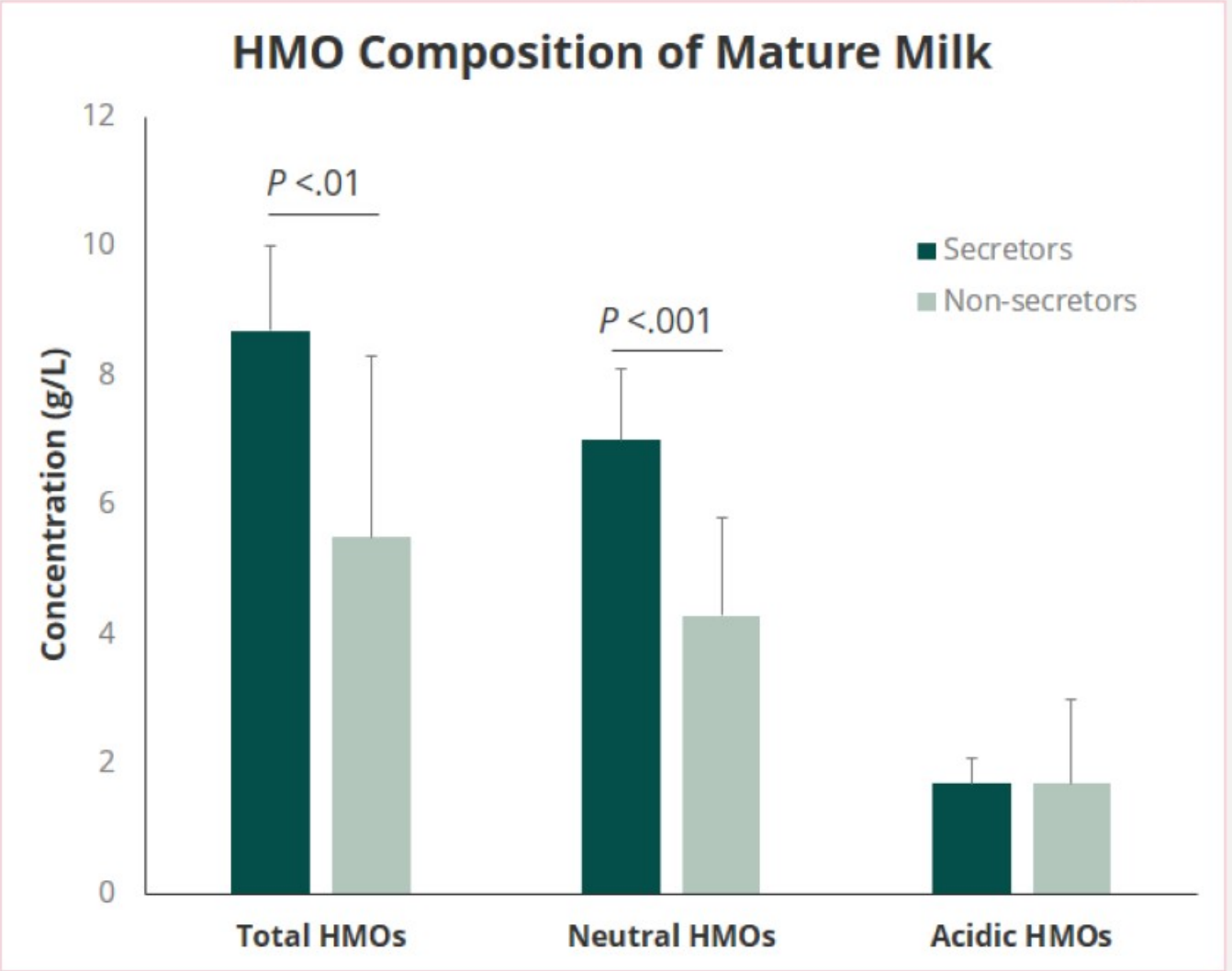
LNT, lacto-N-tetraose; LNFP, lacto-N-fucopentaose; LNDFH, lacto-N-difucohexaose; FL, fucosyllactose.

1. Cheng YJ, Yeung CY. *Pediatr Neonatol*. 2021;62(4):347-353.

HMO Composition of Mature Breast Milk Varies by Secretor Status

- Study of 96 milk samples from 32 mothers with preterm and term infants found significant differences in HMO composition based on genotype

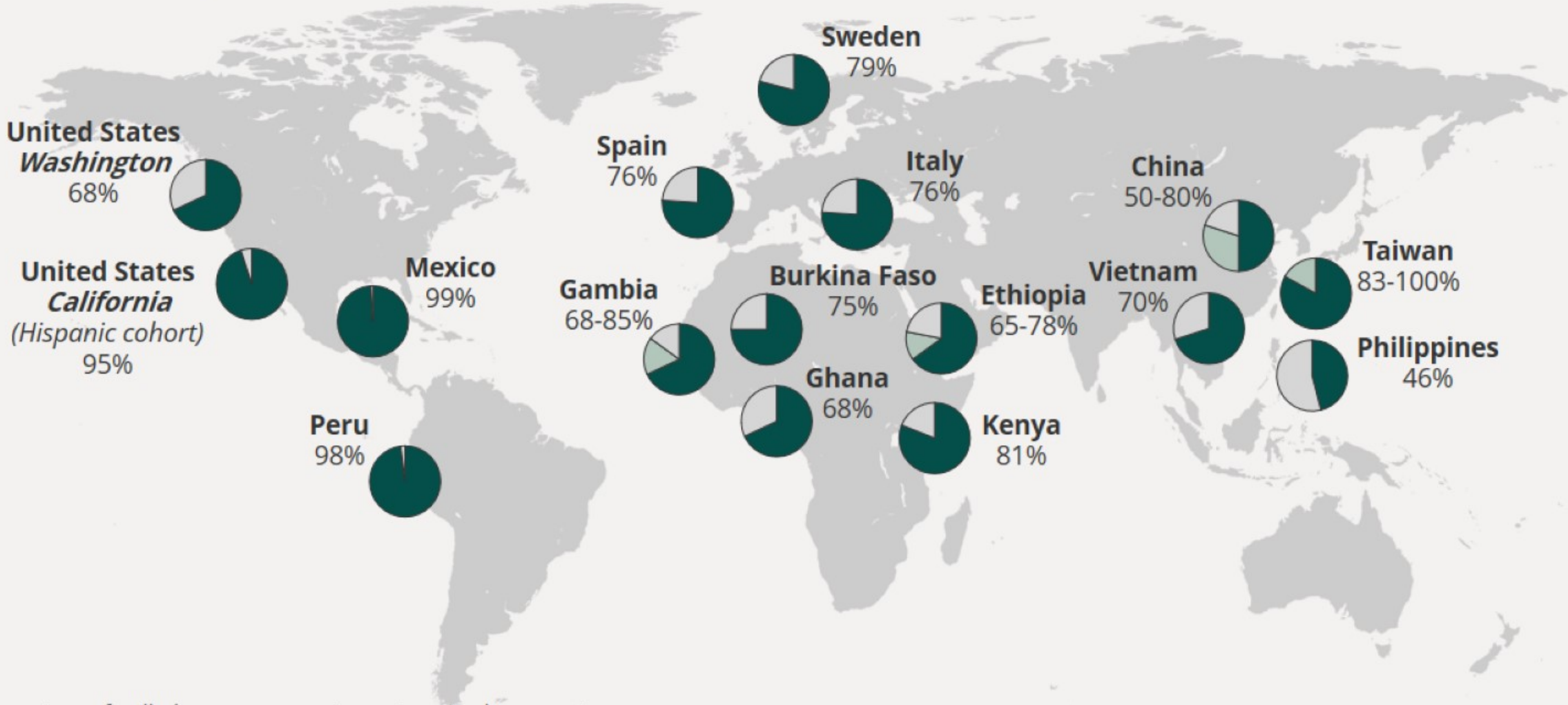
- Secretors had higher concentrations of **2'-FL** and **LNFP I**, and lower concentrations of **LNT**, **LNFP II**, and **LNDFH II**



LNFP, lacto-N-fucopentaose; LNT, lacto-N-tetraose; LNFDH, lacto-N-difucohexaose.

1. Kunz C, et al. *J Pediatr Gastroenterol Nutr*. 2017;64(5):789-798.

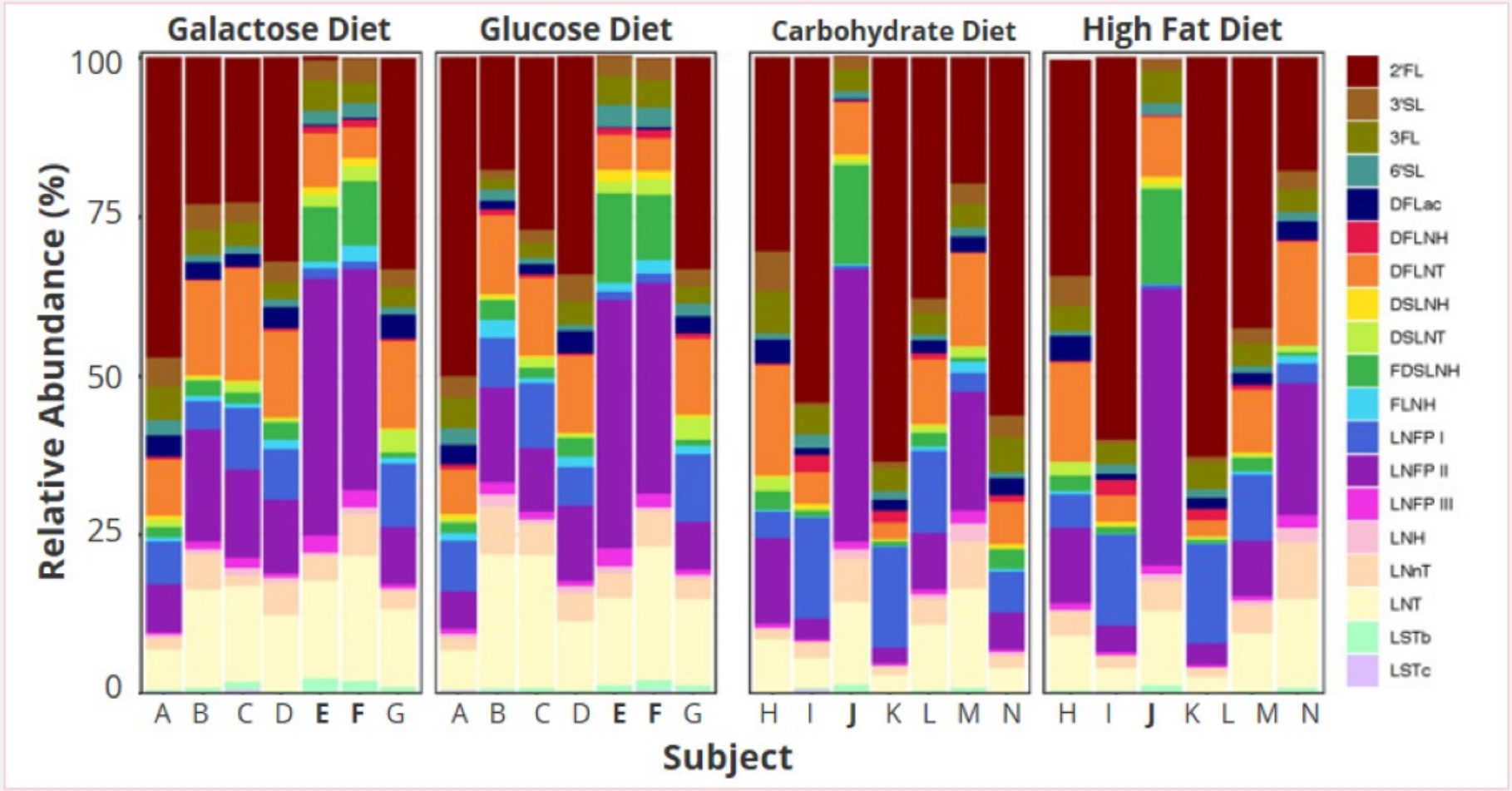
Geographic Variation in Secretor Status



Percentage of milk donors per country categorized as secretors

1. McGuire MK, et al. *Am J Clin Nutr*. 2017;105(5):1086-1100.
2. Cheng YJ, Yeung CY. *Pediatr Neonatol*. 2021;62(4):347-353.

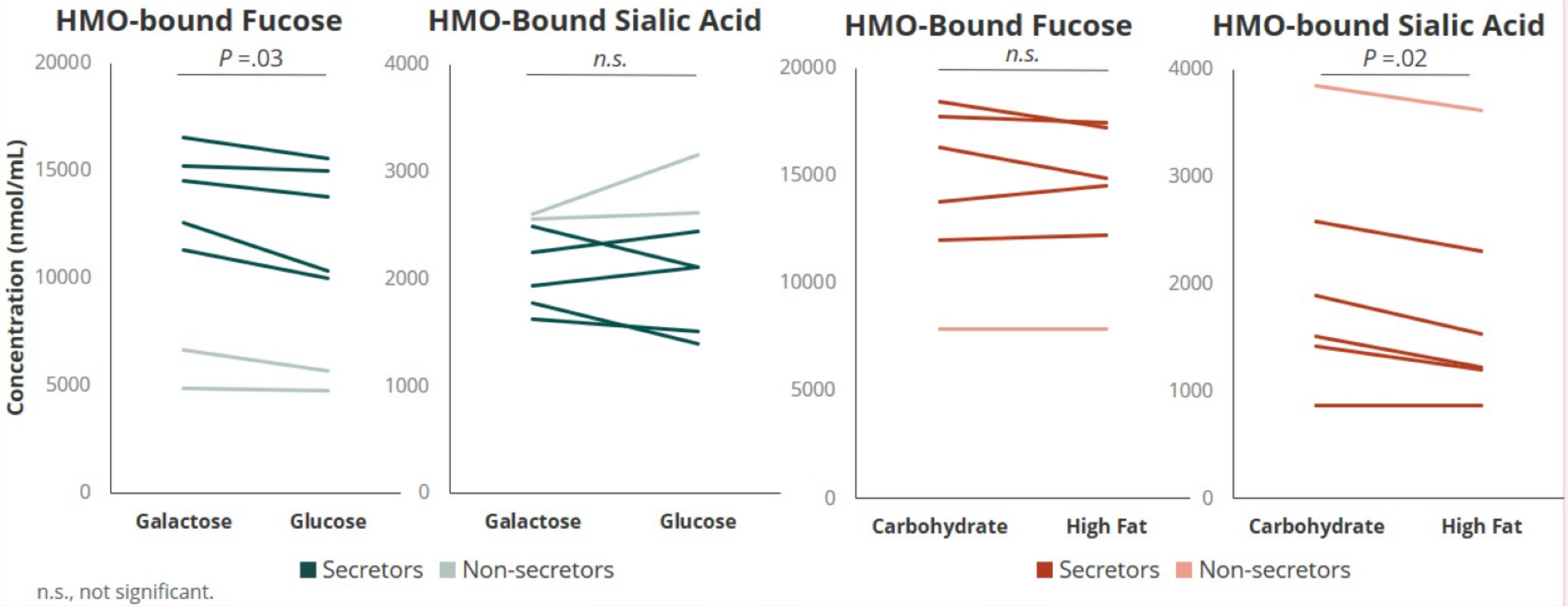
Maternal Diet Affects the HMO Composition of Breast Milk [a]



a. According to a human crossover study of 14 lactating women (7 8–11 weeks postpartum and 7 9–12 weeks postpartum); bolded letters indicate non-secretors.

1. Seferovic MD, et al. *Sci Rep*. 2020;10(1):22092. Use under terms of a Creative Commons license (CC BY 4.0).

Maternal Diet Alters the Abundance of HMO-Bound Fucose and Sialic Acid in Breast Milk

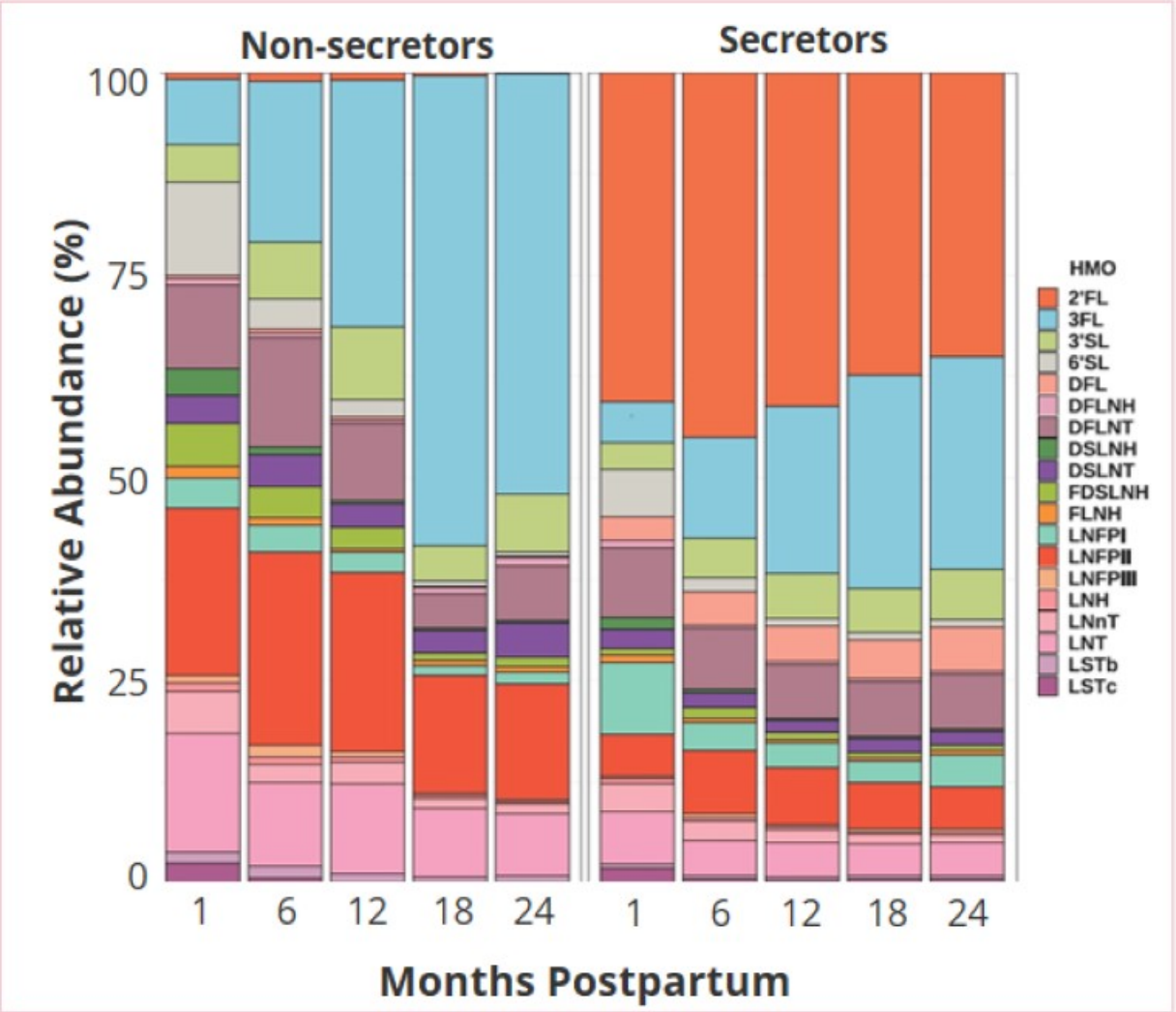


n.s., not significant.

1. Seferovic MD, et al. *Sci Rep*. 2020;10(1):22092.

HMO Composition of Breast Milk Changes Over Time

- The HMO composition of breast milk changes over the postpartum period.
- The concentration of most HMOs decreases whereas **2'-FL remains stable** and **3FL increases**.
- Changing composition of breast milk may reflect the changing needs of a growing infant.



1. Plows JF, et al. *J Nutr*. 2021;151(4):876-882. Used by permission of *Oxford University Press* on behalf of the American Society for Nutrition.

Key Takeaways

- Human milk oligosaccharides represent a major bioactive component of human breast milk
- Maternal variation in HMO composition is driven by a variety of factors, including genetics, geography, diet, and time

Effects of HMOs

HMOs Promote Gut and Immune Health in Infants

A variety of HMO-dependent effects promote gut and immune health:

Prebiotic Effects

HMOs serve as metabolic substrates for beneficial bacteria within the gut

Antimicrobial Effects

Attachment of HMOs to epithelial receptors in the gut prevents microbial attachment

Transcriptional Effects

Signaling via HMOs alters the gene expression of intestinal epithelial cells

Immunomodulatory Effects

Cytokine production and inflammatory cell infiltration are modulated by HMOs

1. Bode L. *Glycobiology*. 2012;22(9):1147–1162.

HMOs Stimulate the Growth of Beneficial Bacteria in the Infant Gut

- Undigested HMOs in the gut serve as **metabolic substrates** for beneficial bacteria, which form the basis of the infant microbiome [1]

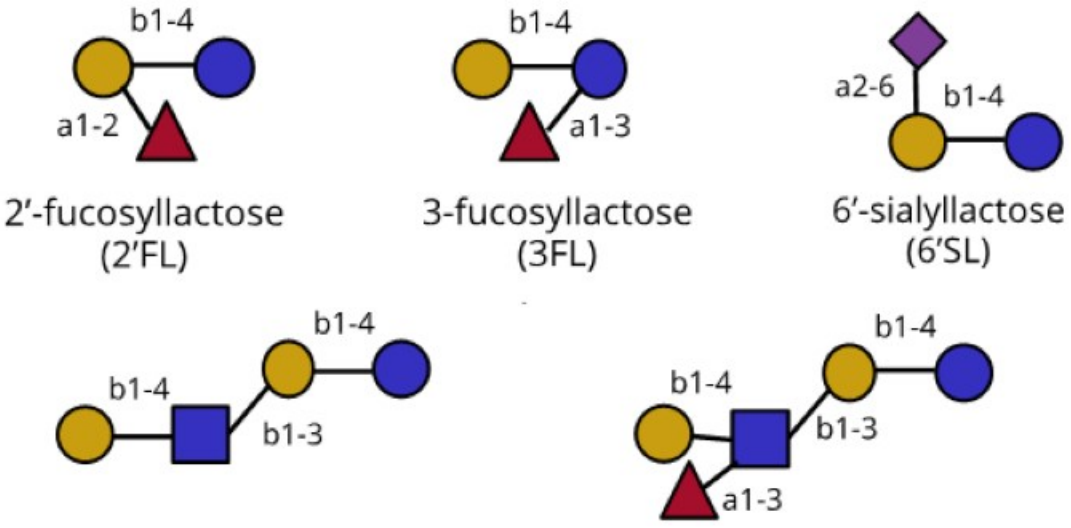
- HMOs promote the growth of bacteria that express sialidases and fucosidases, which can utilize HMOs, over other bacteria that cannot utilize HMOs as an energy source [1],[2]
  - The ***Bifidobacterium* genus** is the dominant HMO-utilizing species in the gut of breastfed infants [1]
  - HMOs can also be utilized by some strains of ***Bacteroides*** and ***Lactobacillus*** [1]

1. Sánchez C, et al. *Foods*. 2021;10(6):1429.
2. Garrido D, et al. *Sci Rep*. 2016;6:35045.

Microbial Diversity in the Gut Is Influenced by the HMO Composition of Breast Milk

- In an analysis of 412 milk and 406 infant fecal samples, the concentrations of several HMOs in maternal breast milk were significantly correlated with microbial community structures [1]
- In breast milk, most bacterial variants were negatively associated with sialylated HMOs and positively associated with fucosylated HMOs [2],[a]
  - Reversed for *Staphylococcus*

Selected HMOs Associated with Infant Fecal Microbial Community Structural [1]

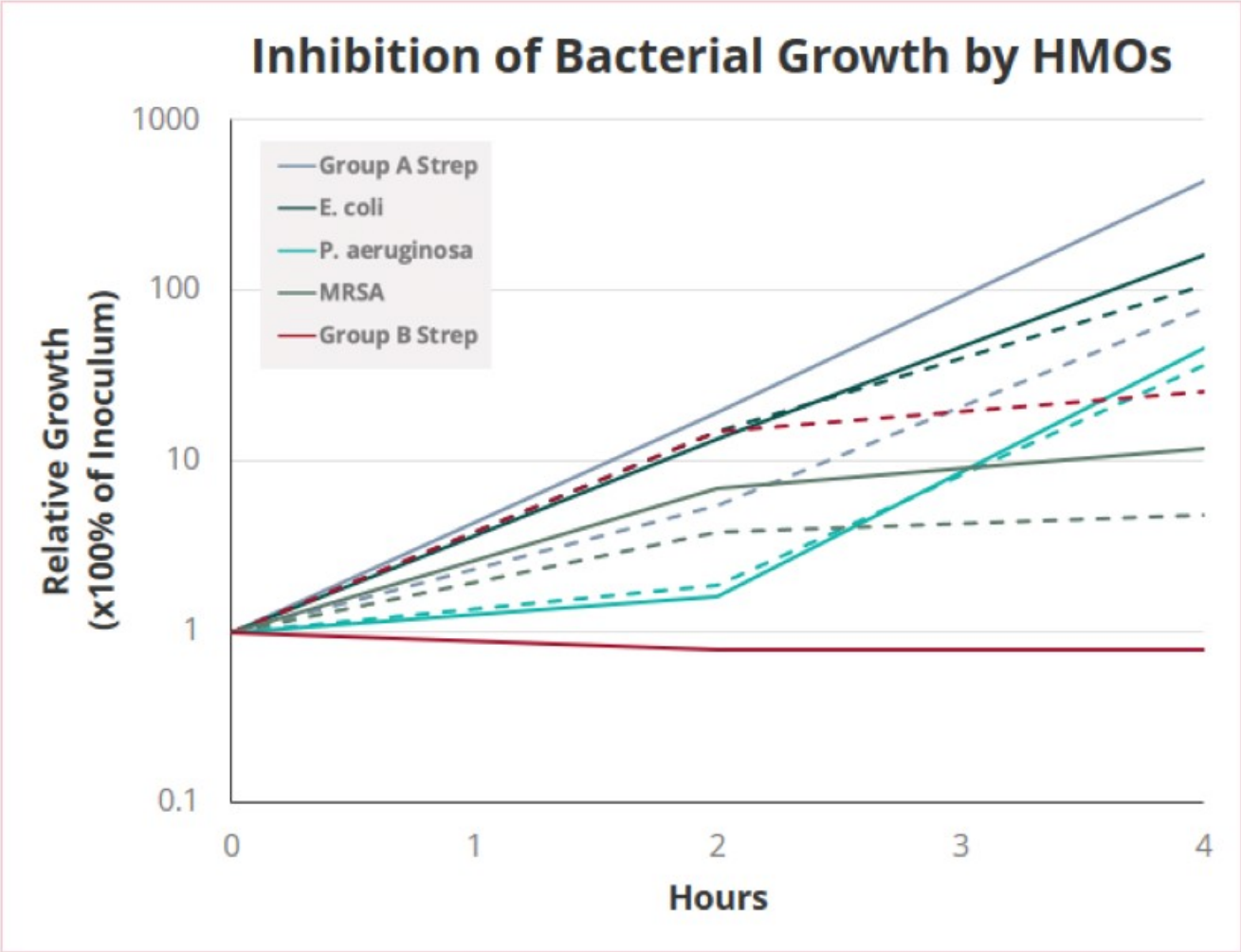


a. According to results from the CHILD study.

1. Pace RM, et al. *Microorganisms*. 2021;9(6):1153..
2. Moossavi S, et al. *Front Nutr*. 2019;6:58.

HMOs Exhibit Unique Antimicrobial Properties Against Group B *Streptococcus*

- Approximately 10^5 cfu *Streptococcus* were resuspended in tissue culture media with (solid) or without (dashed) HMOs (2 mg/mL)
  - Isolated from pooled human milk
- Group B *Streptococcus* growth was inhibited **10-fold** (*P* <0.05)
- **Antimicrobial HMO:** LNT
- **Bacterial target:** Glycosyltransferase



cfu, colony-forming units; LNT, lacto-N-tetraose; *Strep*, *Streptococcus*; MRSA, methicillin-resistant *S. aureus*.

1. Lin AE, et al. *J Biol Chem*. 2017;292(27):11243-11249.

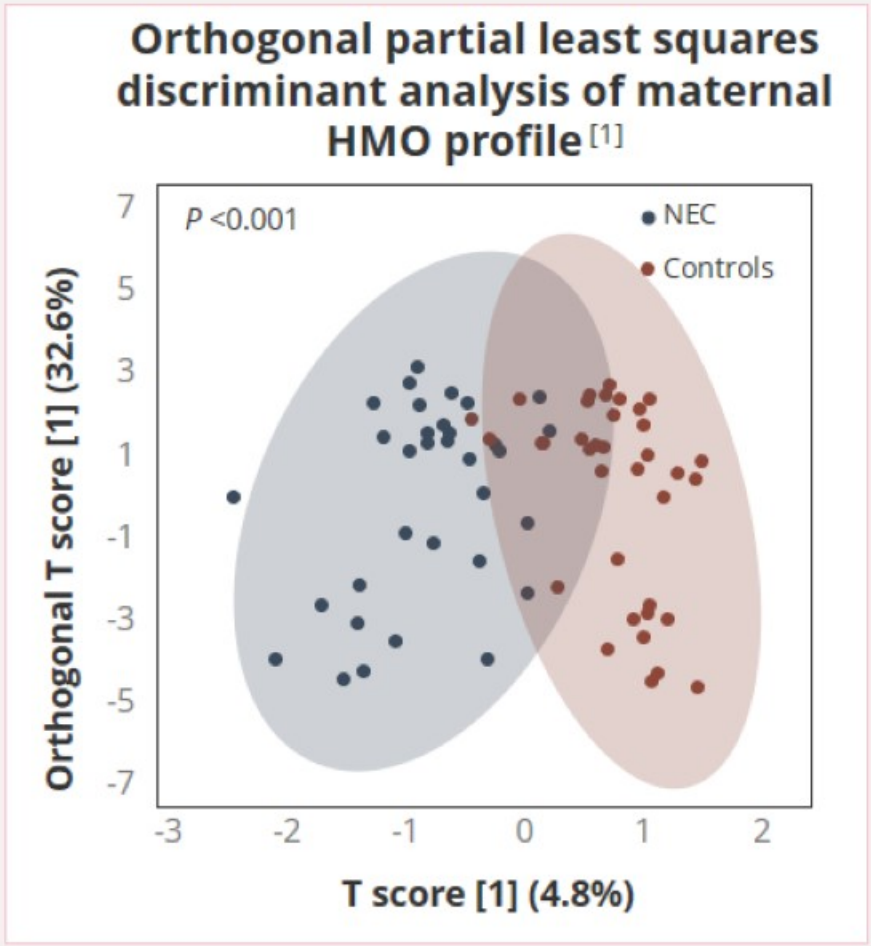
Signaling by HMOs Regulates Immune Activity

- **~70%** of immune cells are located in the gut [1]
- HMO binding to cellular receptors can modulate immune-cell signaling and activity [2]
  - **Galectins:** regulation of T-cell function
  - **Selectins:** leukocyte trafficking
  - **Integrins:** regulation of leukocyte interactions
- Acidic HMOs play a variety of immunomodulatory roles: [2]
  - Downregulation of type 2 immune responses
  - Inhibition of T-cell proliferation

1. Furness JB, et al. *Am J Physiol*. 1999;277(5):G922-8.
2. Triantis V, et al. *Front Pediatr*. 2018;6:190.

Health Implications: Necrotizing Enterocolitis

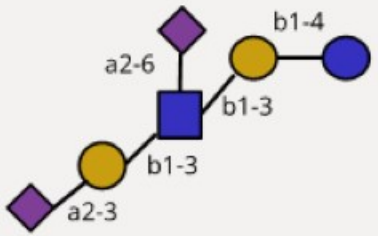
- Necrotizing enterocolitis (NEC) is a deadly intestinal disorder that occurs in upwards of 7% of preterm infants with very low birth weight (500–1500 g).[1]
  - The mortality rate of NEC ranges from 10% to 50%, up to 100% for the most severe forms.
- The etiology of NEC is still unknown but is related to inflammation in the gut and failure of the intestinal epithelial barrier.[1]
- Preterm infants who are breastfed are 6- to 10-times less likely to develop NEC compared with formula-fed infants.[1]



1. Bode L. *Front Pediatr*. 2018;6:385.
2. Masi AC, et al. *Gut*. 2020:gutjnl-2020-322771.

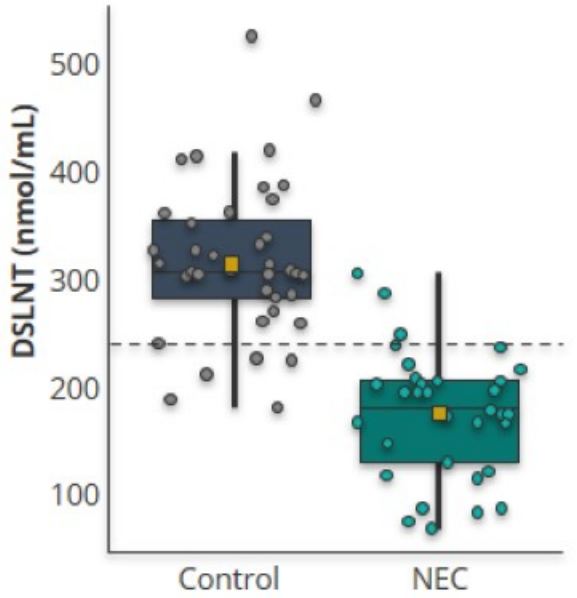
Protection Against NEC May Be Associated With a Specific HMO

- In neonatal rats, HMOs (specifically, DSLNT) with 2 sialic acids were found to play a protective role against NEC [1]
- In human infants, maternal breast milk DSLNT concentration was predictive of NEC development [2]
  - DSLNT threshold level of 241 nmol/mL had a sensitivity and specificity of 0.9 for NEC
- Development of NEC may be related to microbiome composition [2]
  - Infants with NEC had lower relative abundance of *Bifidobacterium longum* and higher relative abundance of *Enterobacter cloacae*



Disialyllacto-N-tetraose (DSLNT)

Concentration of DSLNT by NEC Status[1]



1. Jantscher-Krenn E, et al. *Gut*. 2012;61(10):1417-1425.
2. Masi AC, et al. *Gut*. 2020:gutjnl-2020-322771.

HMOs Protect Against Some Infections[a]...

Diarrhea

Higher relative abundance of fucosylated HMOs was associated with reduced incidence of diarrhea by 2 years.

Respiratory Tract Infections & Gastroenteritis

Higher LNFP II concentrations were associated with fewer cases of respiratory tract infections and gastroenteritis at 6 and 12 weeks.

HIV

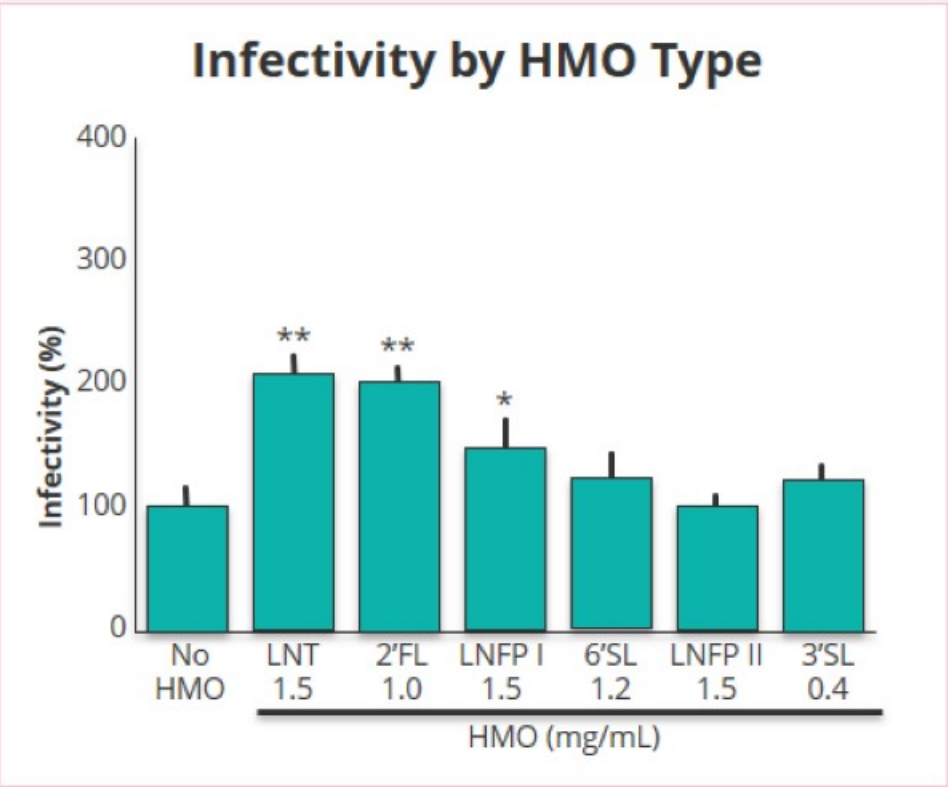
Higher HMO concentration was associated with reduced risk for HIV transmission and mortality risk among HIV-exposed infants.

[a]According to a systematic review of 6 original studies.[1]
LNFP, lacto-N-fucopentaose; HIV, human immunodeficiency virus.

1. Doherty AM, et al. *Front Pediatr*. 2018;6:91.

…But May Enhance Infectivity of Other Pathogens

- Maternal breast milk HMO profile of infants with symptomatic rotavirus infection was distinct from that of rotavirus-negative and asymptomatic rotavirus-positive infants [a]
- Concentrations of LNT were most highly predictive of symptomatic rotavirus infection
  - Infectivity was also enhanced with the addition of 2′-FL and LNFP I *in vitro*
- LNT concentrations were positively correlated with *Enterobacter/Klebsiella* abundance and symptomatic infection

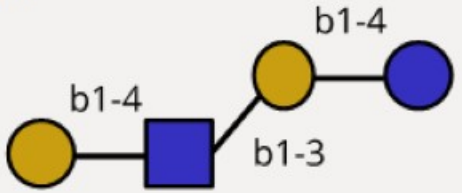


a. According to an analysis of 181 mother-infant pairs from Vellore, India.[1]
LNFP, lacto-N-fucopentaose.

1. Ramani S, et al. *Nat Commun*. 2018;9(1):5010.

Is HMO Supplementation a Substitute for Natural HMO Composition?

- In breast milk, HMOs exist as a complex mixture that changes with infant growth and development
  - Adding single oligosaccharides to infant formula is **not** equivalent to the mixture observed in breast milk
- Individual HMOs may have variable effects on infant health.
  - **Lacto-n-neotetraose (LNnT):** higher concentrations in breast milk are negatively associated with child height and weight between 3 months and 12 years of age [1]
  - **Disialyllacto-N-tetraose (DSLNT):** lower concentrations in milk samples in NEC relative to controls [2]
- Overall HMO composition and the ratios between them are likely to be more important than single oligosaccharides [1]

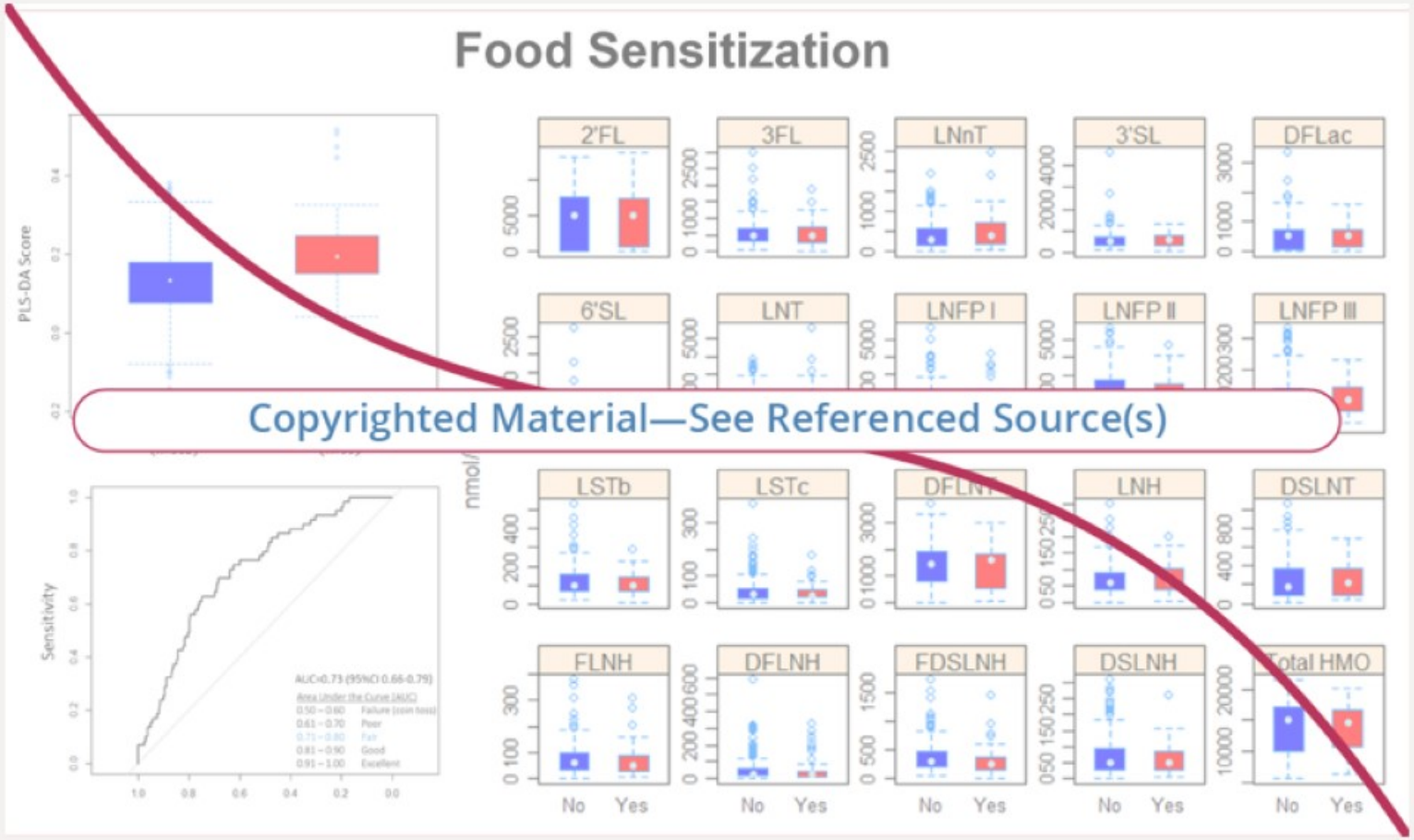


Lacto-N-*neo*tetraose (LNnT)

1. Autran CA, et al. *Gut*. 2018 Jun;67(6):1064-1070.
2. Lagström H, et al. *Am J Clin Nutr*. 2020;111(4):769-778.

Emerging Research: HMO Composition Is Associated With Food Sensitivity Risk

- Overall HMO composition, but **not individual HMOs**, is associated with food sensitivity [1]
- **LNFP III** may be protective against cow's milk allergy [2]
  - Infants with LNFP III concentrations <60 µM were **6.7-times more likely** to develop cow's milk allergy



1. Miliku K, et al. *Allergy*. 2018;73(10):2070-2073.
2. Seppo AE, et al. *J Allergy Clin Immunol*. 2017;139(2):708-711.e5.

Emerging Research: HMO Composition and Weight

- In a small cohort study (n=30 infants), HMO composition in breast milk was linked to excessive weight gain (weight-for-age z-score >2) over 6 months of age [1]

- Individual HMOs may be associated with weight changes, but more research is needed
  - May have important implications for **obesity risk** [1] and **malnutrition**
  - Considerations for formula supplementation

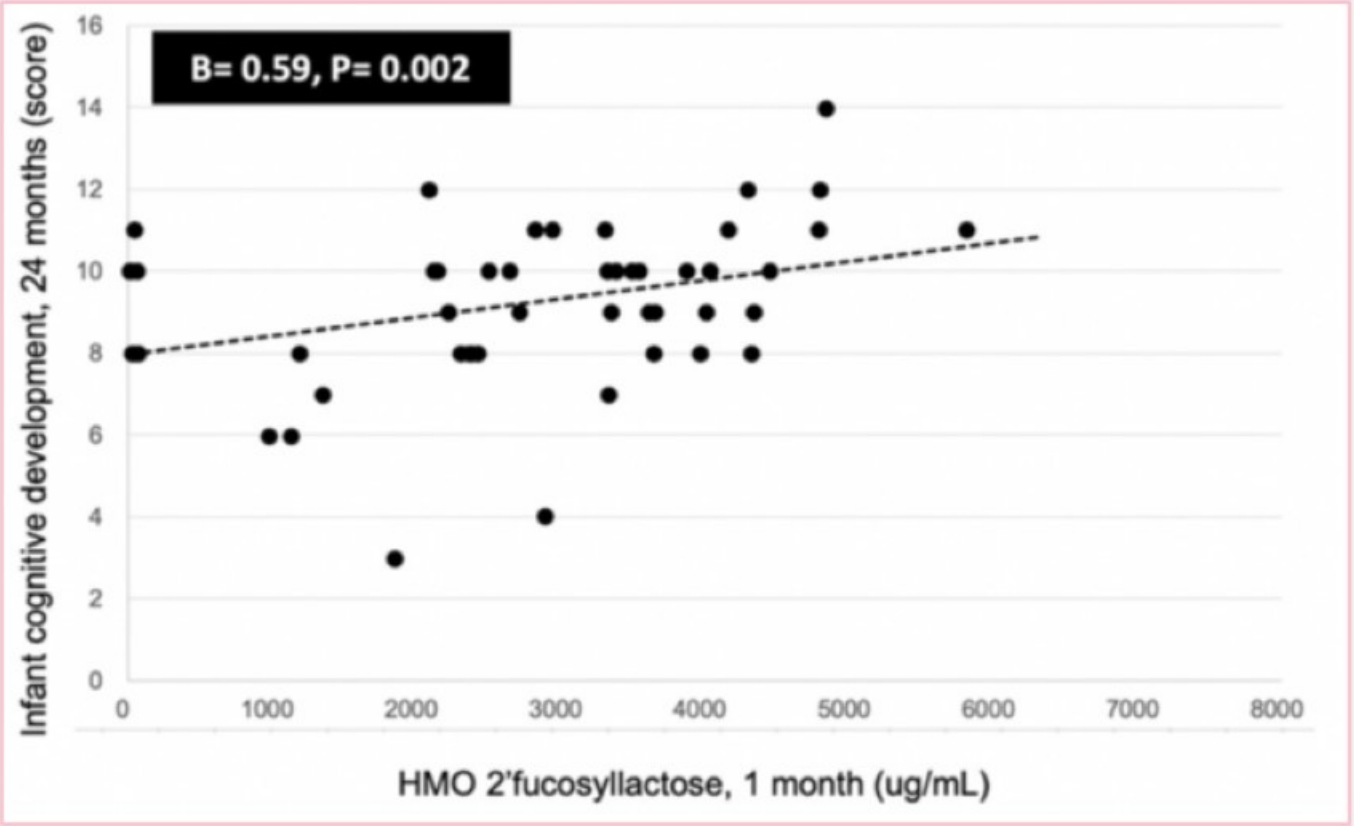
HMO Composition and Weight Gain		
Positive Associations Reported		
DSLNT [1],[3]	3FL [3]	6′-SL [3]
2′-FL [2]	3′-SL [3]	DSLNH [3]
Negative Associations Reported		
LNFP II [1]		
Conflicting Associations Reported		
LNnT [1],[2]		

DSLNT, disialyllacto-N-tetraose; 3FL, 3-fucosyllactose; 3′-SL, 3-sialyllactose, 6′-SL, 6-sialyllactose; DSLNH, disialyllacto-N-hexaose.

1. Berger PK, et al. *Obesity (Silver Spring)*. 2020;28(8):1519-1525.
2. Larsson MW, et al. *Front Pediatr*. 2019;7:297.
3. Saben JL, et al. *Nutrients*. 2021;13(2):446.

Emerging Research: Cognitive Development

Breastfeeding frequency and breast milk concentrations of **2'-FL** at 1 month also correlated with **infant cognitive development scores** at 24 months[1]



1. Berger PK, et al. *PLoS One*. 2020;15(2):e0228323. Use under terms of a Creative Commons license (CC BY 4.0).

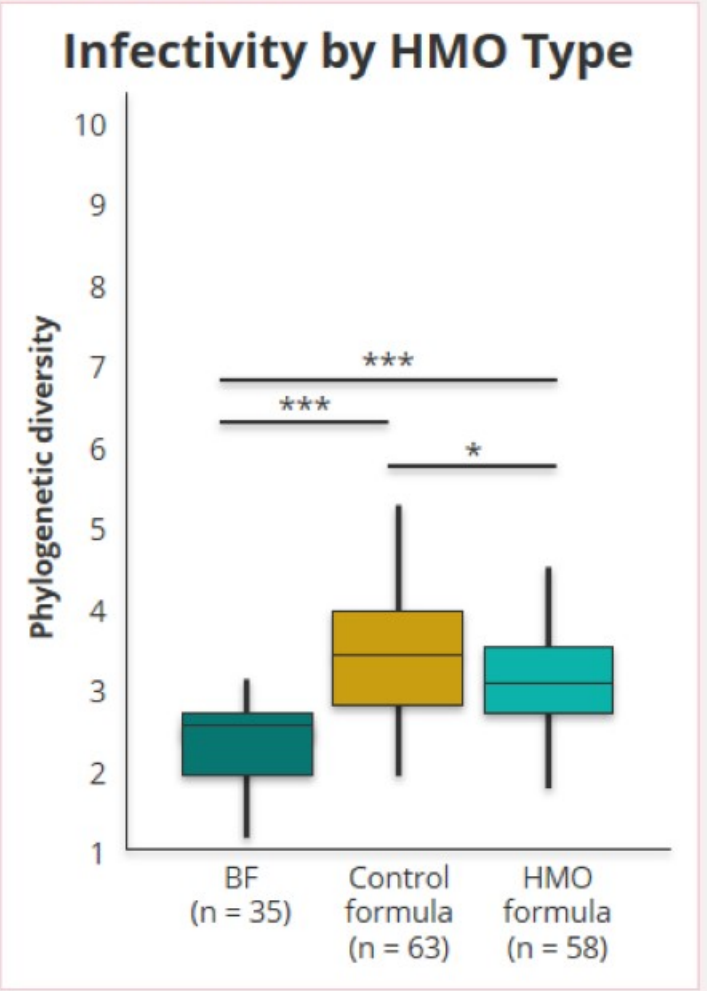
Key Takeaways

- HMOs support the development of the gut microbiome and immune system by serving as prebiotics, antimicrobials, and regulators of immune and epithelial cells
- HMOs play a protective role against NEC and some intestinal infections
- Emerging research suggests a role for HMOs in infant growth and development, including food sensitivities and cognitive function
- Overall HMO composition may be more important for associated health benefits than individual HMOs

Oligosaccharides in Infant Formula

HMO Supplementation in Infant Formula

- HMOs currently used to supplement infant formula: **2'-FL** and **LNnT**
- In a multicenter RCT, infants fed formula supplemented with 2'-FL (1 g/L) and LNnT (0.5 g/L) experienced a shift in their fecal **microbiome signature** closer to that of EBF infants compared with those fed control formula [1]
- Compared with control formula, HMO supplementation was associated with less frequent **respiratory infections**, including bronchitis, and less **antibiotic use** [2]



RCT, randomized, controlled trial; EBF, exclusively breastfed.

1. Berger B, et al. *mBio*. 2020;11(2):e03196-19.
2. Puccio G, et al. *J Pediatr Gastroenterol Nutr*. 2017;64(4):624-631.

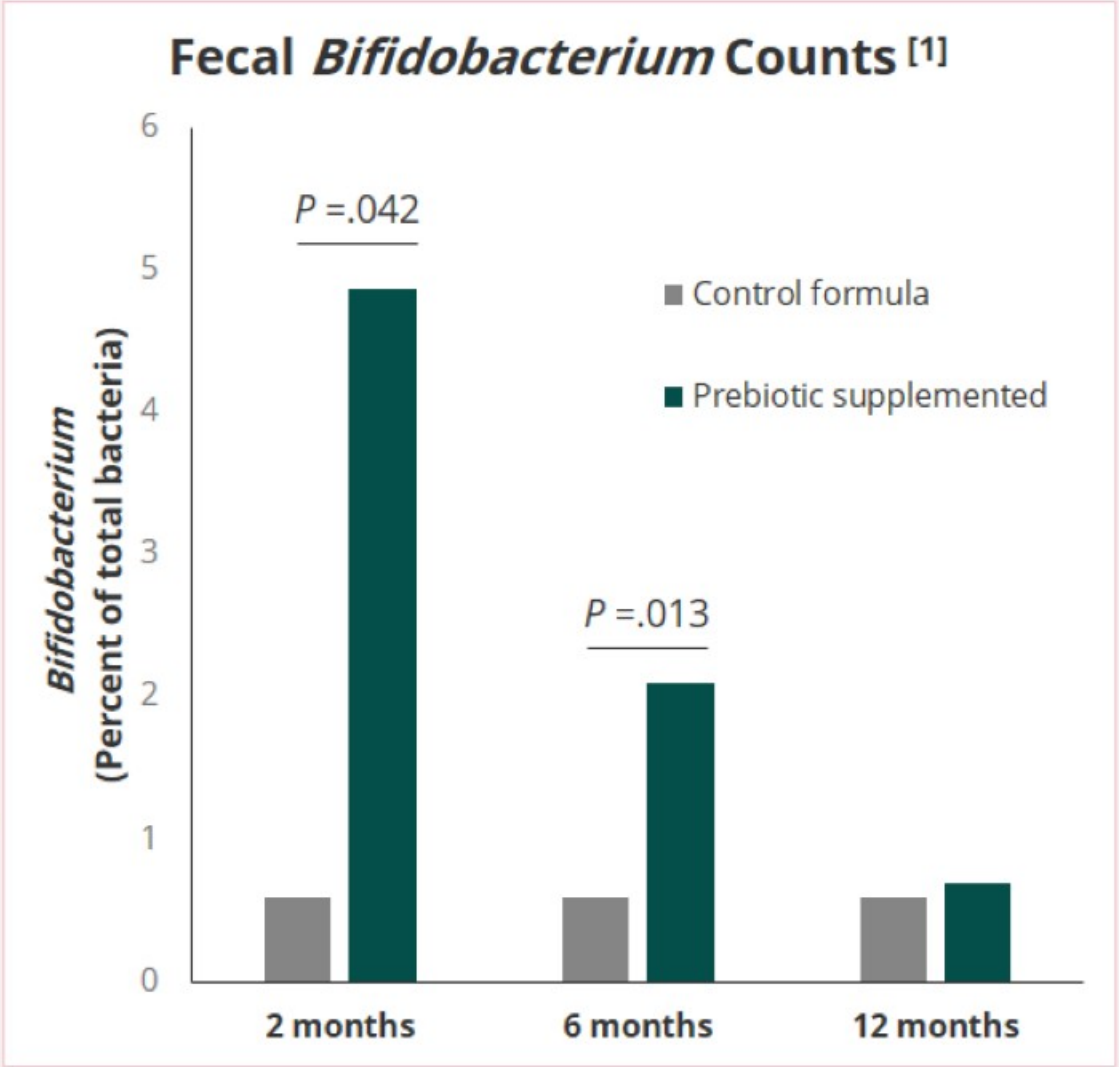
Non-HMO Carbohydrate Prebiotics in Infant Formula

- Several non-HMO prebiotics are used to supplement commercial and clinical infant formula as well, including:
  - Galactooligosaccharides (GOS)
  - Fructooligosaccharides (FOS)
  - Inulin
  - Lactulose
  - Polydextrose
- The goal of addition of these prebiotics to infant formula is to produce a product that results in a **microbiome composition** closer to that of breastfed infants
  - May also serve as anti-adhesive microbials and possess anti-inflammatory properties

1. Ackerman DL, et al. *Carbohydr Res*. 2017;437:16-27.

Non-HMO Prebiotics Support *Bifidobacterium* Growth

- In clinical trials, infant formula supplemented with inulin [1] or GOS [2] supported the growth of *Bifidobacterium* within the infant gut
  - High-fat infant formula supplemented with GOS increased the proportion of fecal *Bifidobacterium* to a level that was not statistically different from that of **breastfed infants** [2]
- No difference in the incidence of gastrointestinal infections was observed, [1] but prebiotic supplementation reduced the duration of infections [2]

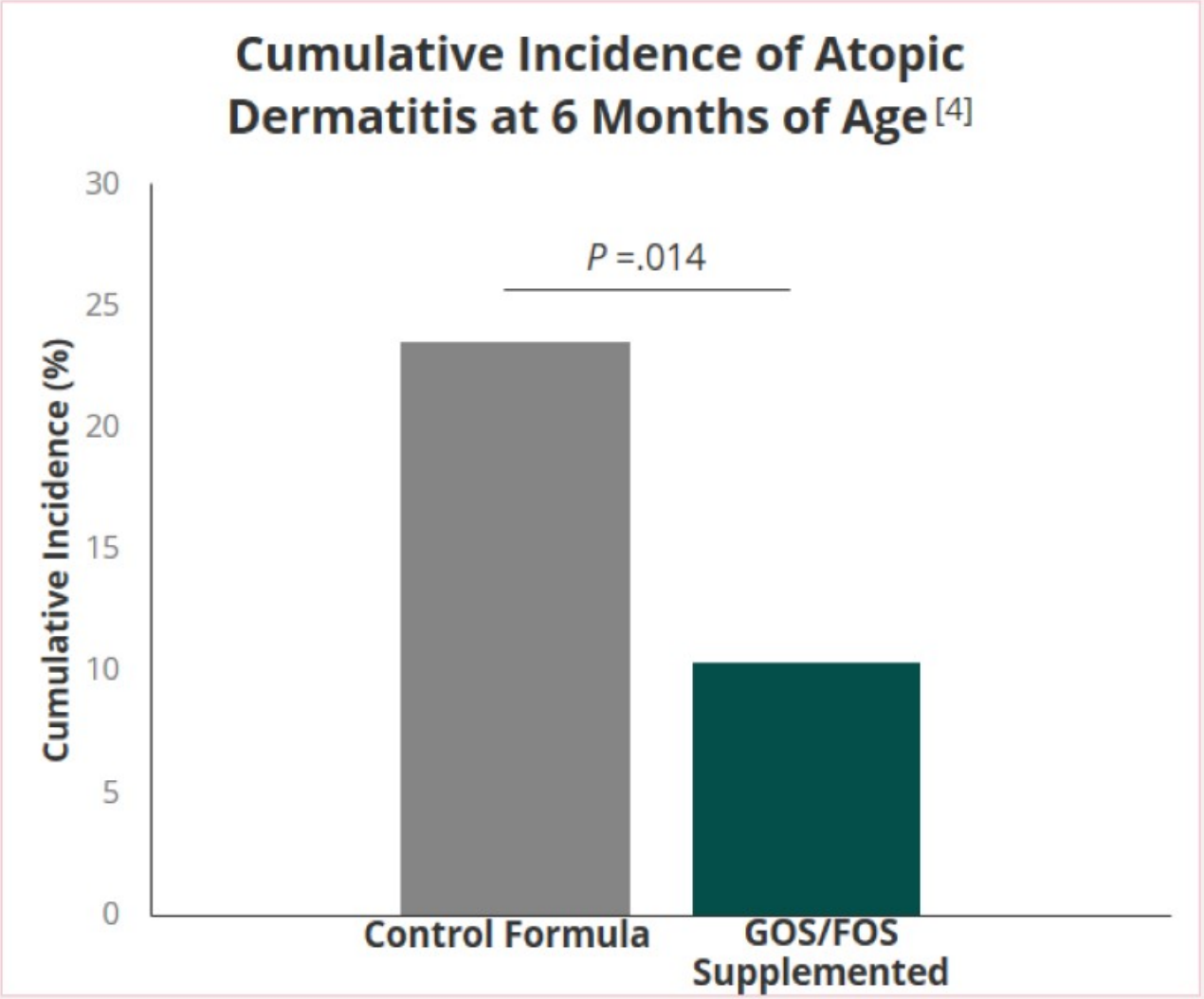


1. Neumer F, et al. *Nutrients*. 2021;13(4):1276.
2. Nomayo A, et al. *Mol Cell Pediatr*. 2020;7(1):6.

Prebiotic Supplementation Provides Additional Health Benefits

Supplementation of infant formula with non-HMO prebiotics provides a variety of additional health benefits, including:

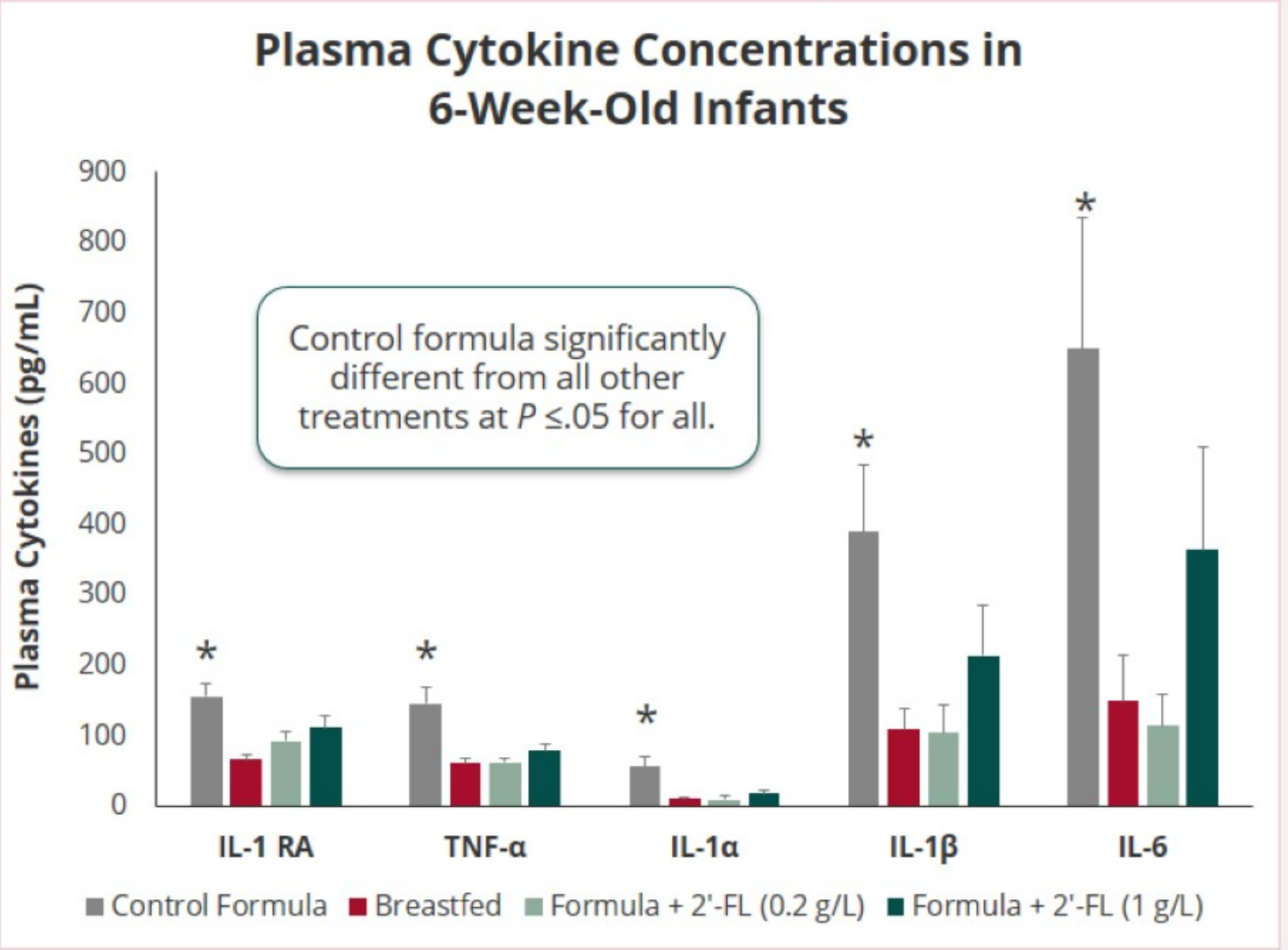
- Immune development and infection control [1],[2]
- Healthy infant growth [3]
- Prevention of allergy and atopic dermatitis [4]



1. Scholtens PA, et al. *J Nutr*. 2008;138(6):1141-1147.
2. Bruzzese E, et al. *J Pediatr Gastroenterol Nutr*. 2006;42(5):E95.
3. Vandenplas Y, et al. *Nutrients*. 2020;12(11):3560.
4. Moro G, et al. *Arch Dis Child*. 2006;91(10):814-819.

Non-HMO and HMO Prebiotics Are Not Equal

- In an RCT, infants who were exclusively formula-fed were randomized to receive control formula containing GOS or formula supplemented with 2′-FL (0.2 or 1.0 g/mL)
- Infants fed 2′-FL-supplemented formula had lower inflammatory cytokine profiles compared with those fed control formula
  - Similar to exclusively breastfed infants



1. Goehring KC, et al. *J Nutr*. 2016;146(12):2559-2566.

Discussion: Implications for Counseling New Parents

- Research on the effects of HMOs and other human milk bioactive components is still in the early stages
  - More research is needed to fully understand potential impact
- A detailed mechanistic understanding of HMO effects is required to guide formula product development
- There are potential opportunities for personalized early life nutrition
  - Not every HMO (either alone or in a blend) may be valuable or effective for every baby and situation

Key Takeaways

- HMO (2’-FL and LNnT) and non-HMO prebiotics are added to infant formulas to support healthy gut development.
- While non-HMO prebiotics provide a variety of health benefits, they are not equivalent to HMO prebiotics.