Human Milk Oligosaccharides (HMOs) and Brain Development

Dr. Garba D. GWARZO MBBS, FMCPaed

Associate Professor/ Consultant Paediatrician,

Bayero University/ Aminu Kano Teaching Hospital, Kano, Nigeria.

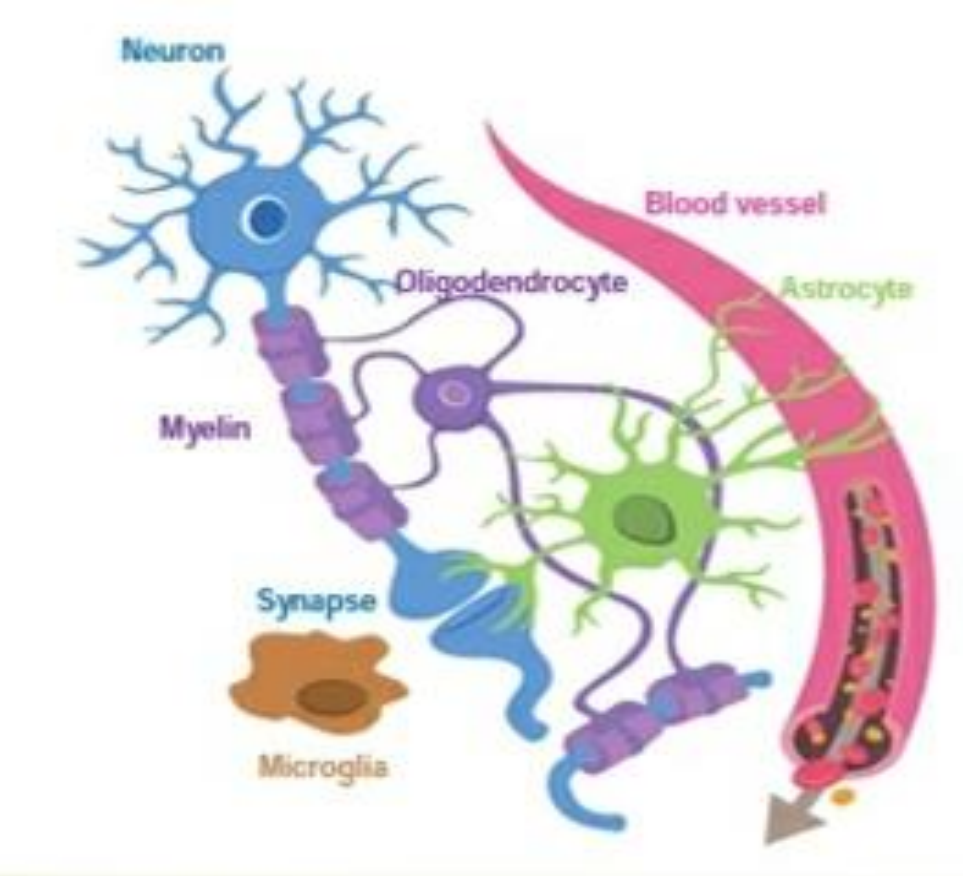
Outline

- Introduction
- Brain development
- What are HMOs?
- Functions of HMOs
- Importance of HMOs in cognition
- Key messages
- References

Introduction

- The brain weighs 350-400 grams and contains 100 billion neurons at birth
- After birth, billions of neurons get connected by synaptogenesis: 700,000 synapses/second are formed
- 90% brain development by age 5 years and 90% of brain volume by 6 years
- Rapid growth and development in infants and children need adequate amounts of nutrients

The Brain Is More Than Neurons

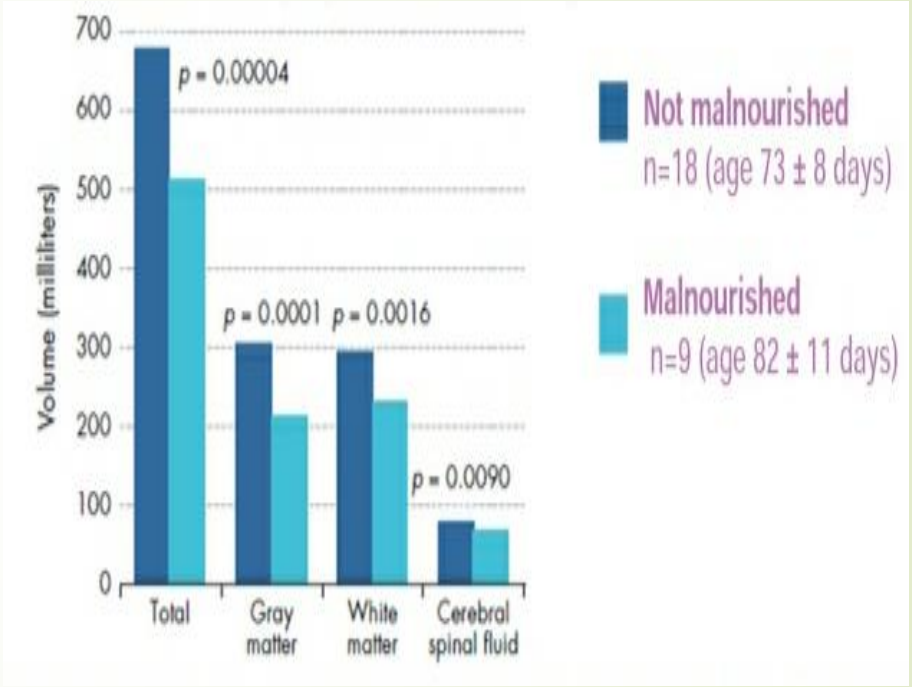


- 90 billion neurones
- 100 billion non-neuron cells
- 1 quadrillion synapses
- 100 km of nerves.

Leuret and Gratiolet, 1854; Kasthuri N, et al. Cell 2015;162:648–681; Wong A, et al. Front Neuroengineering 2013;6:1–22; 4. Ascoli G. N Eng J Med 2015;373:1170–2.

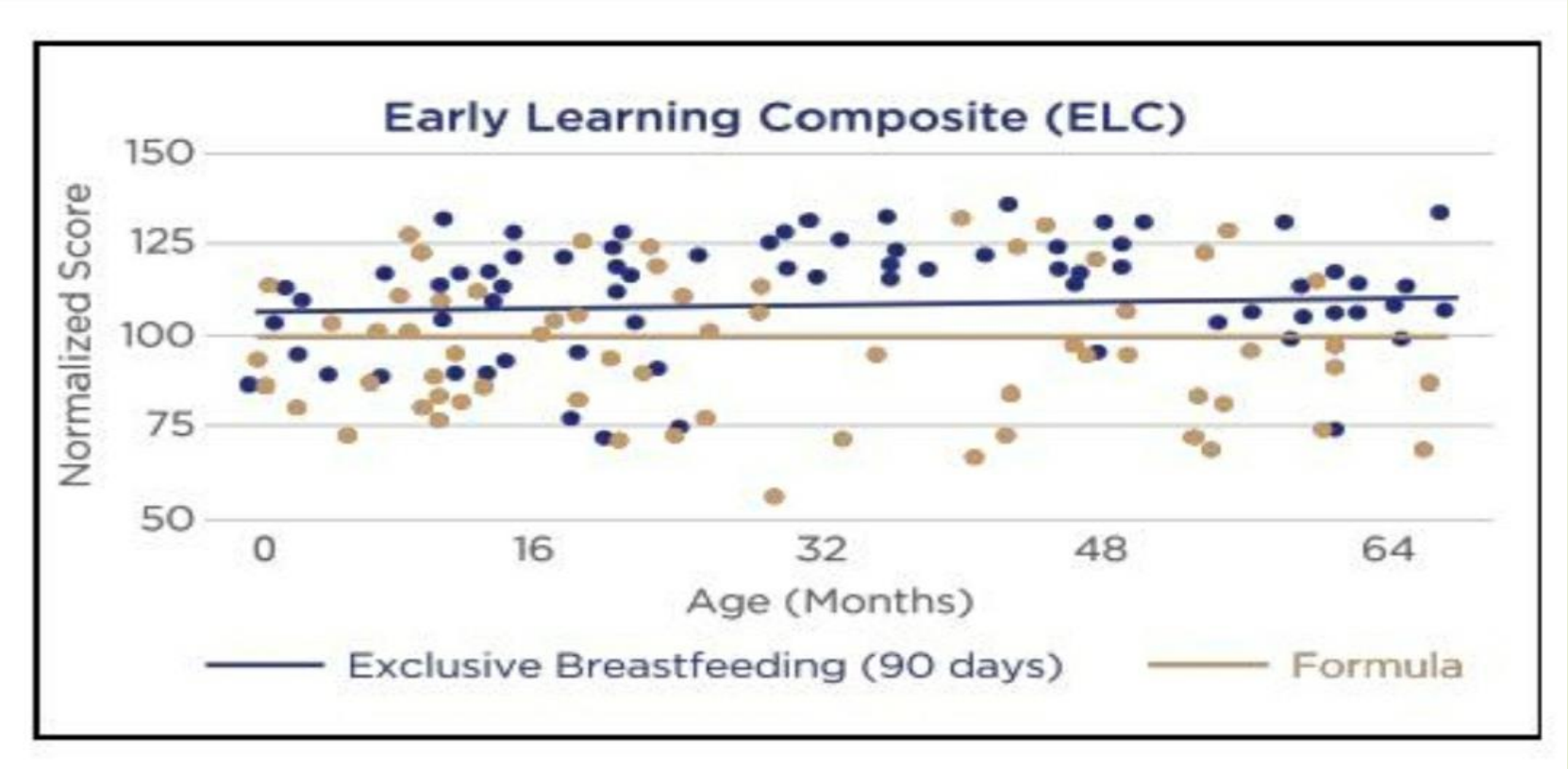
Key factors influencing brain development

- Gene expression (**nature**)
- Environmental factors (**nurture**)
  - Socioeconomic status
  - Social interactions
  - Urbanization
  - Pollution
  - Social mobility
  - Stress
  - **Nutrition and food**



Kolb B, Gibb R. J Can Acad Adolesc Psychiatry. 2011;20: 265-76; Hanson JL, et al. Plos One 2013;8:e80954; WDR team 2018, using data from Nelson and others (2017).

Early nutrition influences developmental myelination and cognition in infants and young children



Deoni et al. (2017)

What are HMOs?

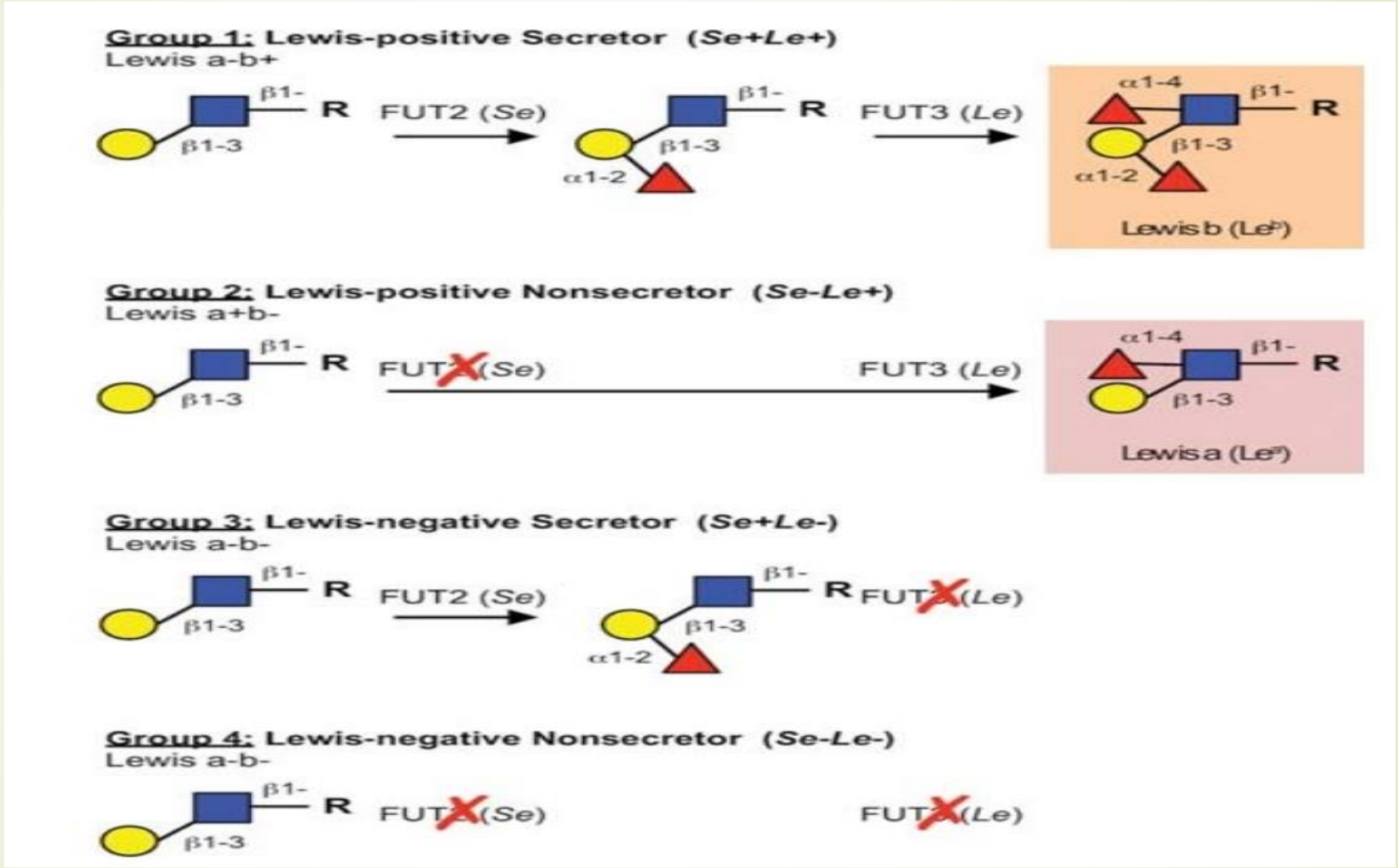
- Are complex sugars produced in large quantities in human milk
- They are unconjugated glycans
- They are undigestible or partially digestible
- Only 1%-2% of HMOs are absorbed in the gut
- They are processed in the colon by microbiota
- They are prebiotics
- Formerly called ‘bifidus-factor’ of ‘gynolactose’.

Composition of HMOs

- HMOs are composed of:
  - Five monosaccharides glucose
  - Galactose
  - N-acetylglucosamine
  - Fucose
  - Sialic acid (Sia)

Composition of HMOs

- HMO composition mirrors blood group characteristics.
- It depends on the expression glycosyltransferases.
- There are four milk groups based on the Secretor (Se) and Lewis (Le) blood group system
- This is determined by the activity of two gene loci encoding for the a1-2-fucoslyltransferase FUT2 (encoded by the Se gene) and the a1-3/4-fucosyltransferase FUT3.



Source: Bode L. Glycobiology 2012

Types of HMOs

1. **Non-fucosylated neutral (core) HMOs**
   - These HMOs are the foundations upon which other HMOs are built[3]
   - LNT is the most abundant representative in this category[4-1]
2. **Fucosylated neutral HMOs**
   - 2'FL is the most abundant fucosylated HMO[4-11]
   - DFL is among the 10 most abundant representatives in this group[4-11]
3. **Sialylated acidic HMOs**
   - 6'SL is the leading representative of this group[4-11]
   - 3'SL is an important representative as well[1]

DFL, difucosyllactose; FL, fucosyllactose; LNT, lacto-N-tetraose; SL, sialyllactose.

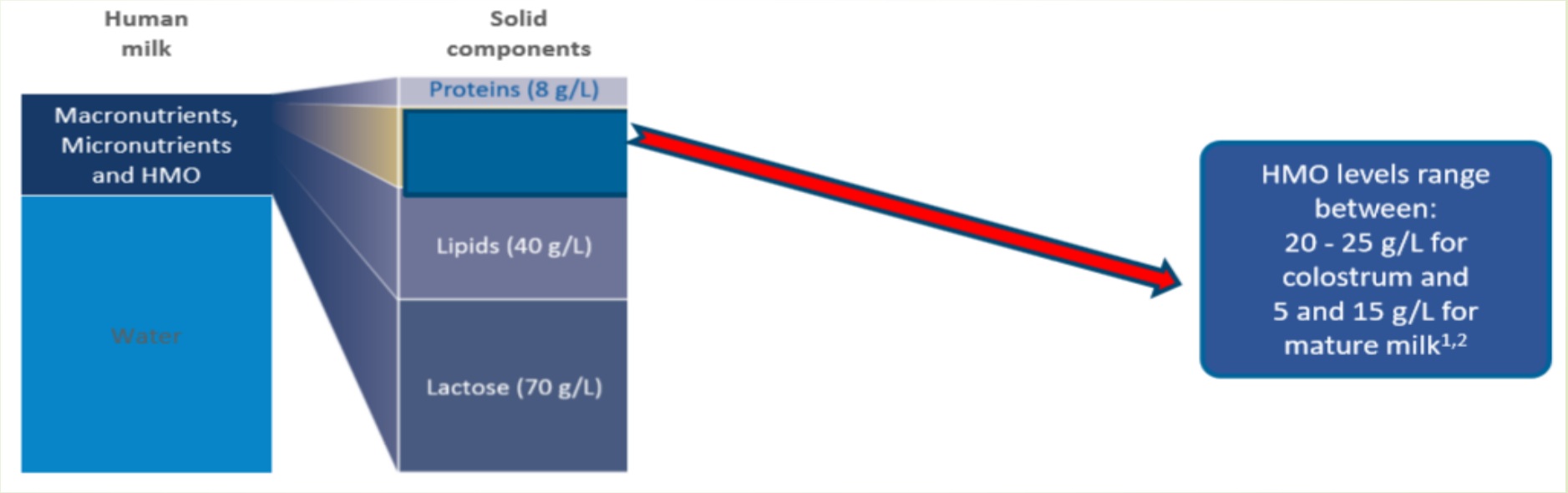
1. Donovan et al. 2016; 2. Zikovic et al. 2011; 3. Erney et al. J2000; 4. Hennet et al. 2014 ; 5. Bode. 2015; 6. Thurl et al. 2017; 7. Austin et al. 2016; 8. Sprenger N et al. 2017; 9. Samuel et al. 2019; 10. Austin et al. 2019; 11. Lefebvre et al. 2020.

Oligosaccharides in Human Milk

- Breast milk is the gold standard to provide the best nutrition to an infant from the start of life.
- The WHO and UNICEF recommend **EBF** for the first 6 months of life, the introduction of **CF** at the age of 6 months and continuation of BF for as long as possible. (WHO and UNICEF 2020)
- Breast milk is a comprehensive source of energy and nutrients, as well as bioactive components that are essential for the healthy growth and development of an infant. [Victora CG, Lancet. 2016]

Oligosaccharides in Human Milk

- HMOs are the third largest solid component in breast milk



Adapted from Anna Petherick, Nature volume 468, pages S5–S7 (23 December 2010); Zivkovic AM, et al. Proc Natl Acad Sci USA. 2011;108(Suppl. 1):4653–8; Austin S, et al. Nutrients 2016;8:pii: E346; Sprenger N, et al. PLoS One 2017;12:e0171814; Kunz C, et al. J Pediatr Gastroenterol Nutr 2017;64:789–98; Bode L. Glycobiology 2012;22:1147–1162, Samuel and Binia et al., 2019, Scientific Reports

Factors influencing HMOs by mothers

- Genetics
- Environmental factors:
  - Nutrition – less produced in undernourished mothers
- Colostrum contains 20–25 g/L of HMO
- Mature milk contains 5–15 g/L HMO
- Preterm milk – high amount of HMOs

Macronutrients and HMOs in mature human and bovine milk

Nutrient	Human	Bovine
Protein (g/L)	8	32
Fat (g/L)	41	37
Lactose (g/L)	70	48
Oligosaccharides (g/L)	5-15	0.05
Identified oligosaccharide	>100	40
% fucosylated	50-80	1
% sialylated	10-20	70

Source: Bode L. Glycobiology 2012

HMOs in infant formula

- Oligosaccharides for preterm infant formulas improved gastric emptying
- Some infant formulas contain non-HMOs such as galactooligosaccharides (GOS) and fructooligosaccharides (FOS).

Functions of HMOs

- Stimulates immune system directly
- Interact with other immune cells
- They are prebiotics – they promotes growth of specific microbiota in the colon - bifidobacteria
- Prevent pathogenic bacteria from binding to the cell receptors (2'-FL antiadhesion)
- Protect from NEC
- Promote development of infant's intestines
- Promote development of infant's brain

Functions of HMOs

- Prebiotics - They alter gut microbiota of the infant
  - (Defn.: a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confers benefits upon host well-being and health. (Gibson et al. 2004; Roberfroid 2007))
- Antiadhesive antimicrobials
  - Discourage adhesion of pathogenic organisms by giving competitive advantage to non-pathogens (microbiota)
  - Antiadhesive antimicrobials: Some HMOs resemble mucosal cell surface glycans, serve as soluble decoy receptors to prevent pathogen binding and reduce the risk of infections
- Directly modulate intestinal epithelial cell responses

Functions of HMOs

- Immune modulators
  - Sialylated HMOs reduce IL-4 production in patients with peanut allergy (Eiwegger et al. 2010).
- Natural protection against NEC
  - Breast-fed infants have 6–10-fold lower risk of developing NEC than formula-fed infants (Lucas and Cole 1990; Schanler et al. 2005; Sisk et al. 2007).

Functions of HMOs

- Nutrients for brain development
  - Breast-fed preterm infants have superior developmental scores at 18 months of age and higher intelligence quotients at the age of 7 (Lucas et al. 1990, 1992).
  - Brain development and cognition in part depend on Sia-containing gangliosides and poly-Sia containing glycoproteins (reviewed in Wang 2009).
  - Sialylated HMOs contribute to the majority of Sia in human milk.

The role of HMO on Cognition

ELSEVIER

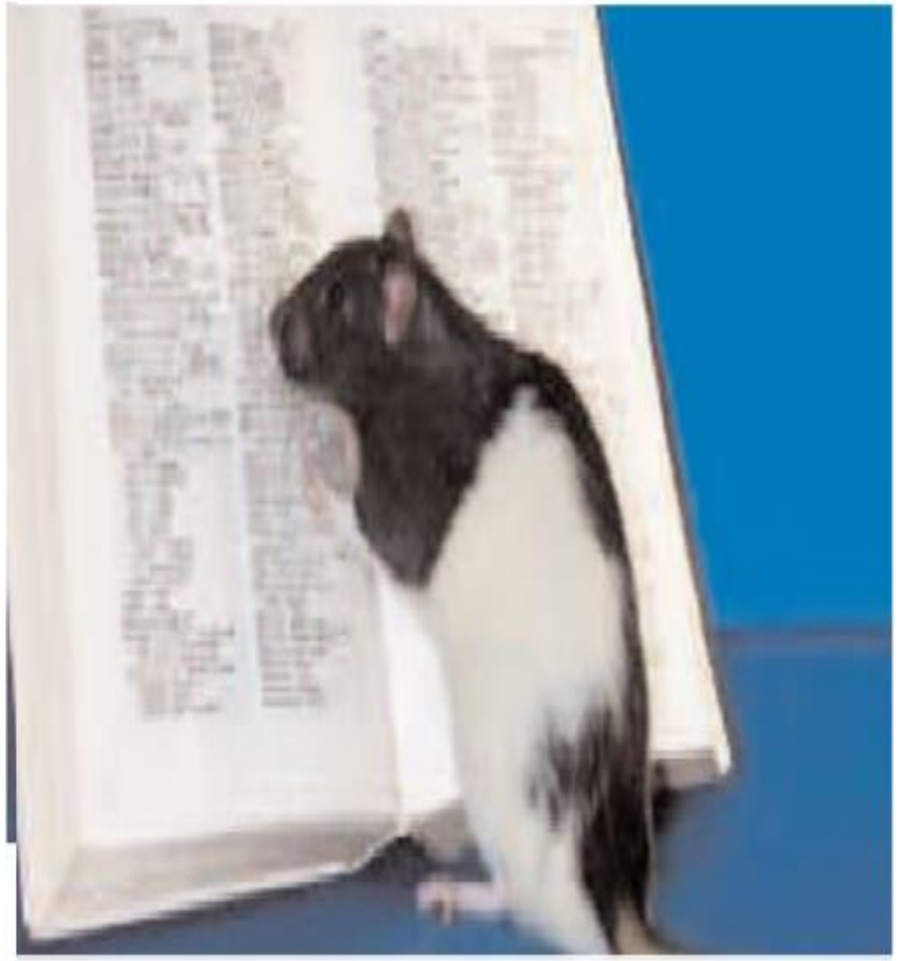
The Journal of Nutritional Biochemistry
Volume 31, May 2016, Pages 20-27

Oral supplementation of 2′-fucosyllactose during lactation improves memory and learning in rats ☆

Elena Oliveros [a] ✉, María Ramirez [a], Enrique Vazquez [a], Alejandro Barranco [a], Agnes Gruart [c], Jose Maria Delgado-Garcia [c], Rachael Buck [b], Ricardo Rueda [a], Maria J. Martin [a]

- Rat pups received oral supplements: 2'FL or water during lactation period
- Thereafter, rodent standard diet was given
- They were evaluated at 4-6 weeks and at 1 year using classical behavioral tests
- Results: Rats in 2'FL group performed better in the Novel Object Recognition and Y maze paradigms at 1 year
    - They had enhanced cognition

Learning



Memory

microorganisms

MDPI

Article

A Mediation Analysis to Identify Links between Gut Bacteria and Memory in Context of Human Milk Oligosaccharides

Stephen A. Fleming [1,*], Jonas Hauser [2], Jian Yan [3], Sharon M. Donovan [4,5], Mei Wang [4] and Ryan N. Dilger [1,5,6,7]

Microorganisms **2021**, *9*, 846. https://doi.org/10.3390/microorganisms9040846

- **Numerous bacterial genera in colon were related to short- and/or long-term memory.**
- Mediating variables frequently included GABAergic and glutamatergic hippocampal gene expression.
- Overall, this analysis identified multiple pathways between the gut and brain, with a focus on genes related to excitatory/inhibitory neurotransmission.

PLOS ONE

RESEARCH ARTICLE

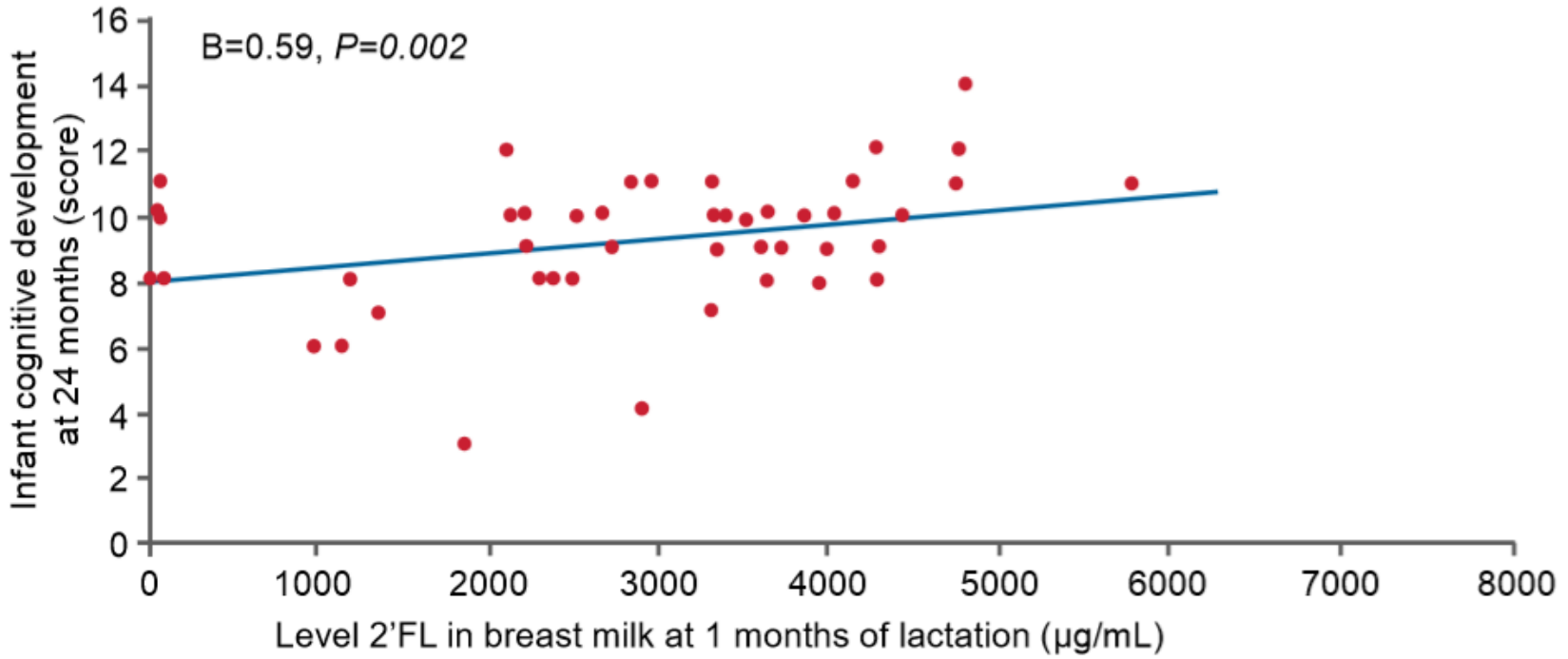
Human milk oligosaccharide 2′-fucosyllactose links feedings at 1 month to cognitive development at 24 months in infants of normal and overweight mothers

Paige K. Berger[1], Jasmine F. Plows[1], Roshonda B. Jones[1], Tanya L. Alderete[2], Chloe Yonemitsu[3], Marie Poulsen[4], Ji Hoon Ryoo[1], Bradley S. Peterson[1], Lars Bode[3], Michael I. Goran[1]*

1 Department of Pediatrics, The Saban Research Institute, Children's Hospital Los Angeles, Los Angeles, California, United States of America, **2** Department of Integrative Physiology, University of Colorado Boulder, Boulder, Colorado, United States of America, **3** Department of Pediatrics and Mother-Milk-Infant Center of Research Excellence, University of California, San Diego, La Jolla, California, United States of America, **4** University Center for Excellence in Developmental Disabilities, Children's Hospital Los Angeles, Los Angeles, California, United States of America

* mgoran@chla.usc.edu

2'FL in breast milk at 1 month is associated with better infant cognitive development at 24 months of age

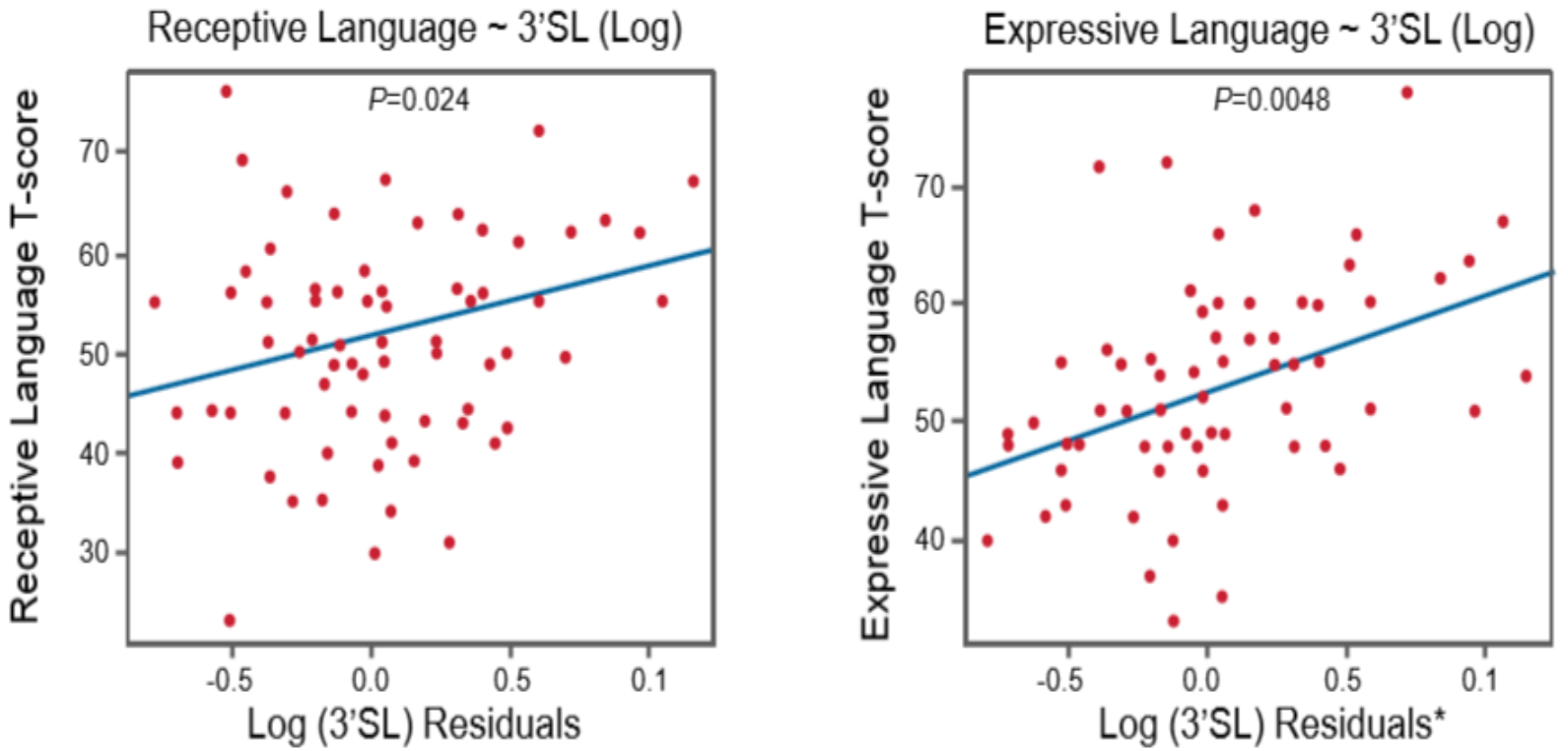


Adapted from Berger, et al. 2020.

SL, sialyllactose.

A Positive Correlation between Breast Milk 3'-Sialyllactose and Language Development during Early Infancy. Seoyoon Cho et al.

- 99 healthy children
- The Mullen Scales of Early Learning was administered to assess the child's cognitive development.
- Breast milk samples (n=191) were analyzed for specific HMOs: 2'FL, 3'FL, 3'SL, 6'SL, Lacto-N-tetraose(LNT), Lacto-N-neotetraose(LNNT), Lacto-N-fucopentaosel (LNFPI), and A-tetrasaccharide(A-Tetra).



*age-corrected 3'SL levels, expressed as difference to the population mean

Adapted from Cho, et al. 2020.

SL, sialyllactose.

Associations of human milk oligosaccharides and bioactive proteins with infant growth and development among Malawian mother-infant dyads

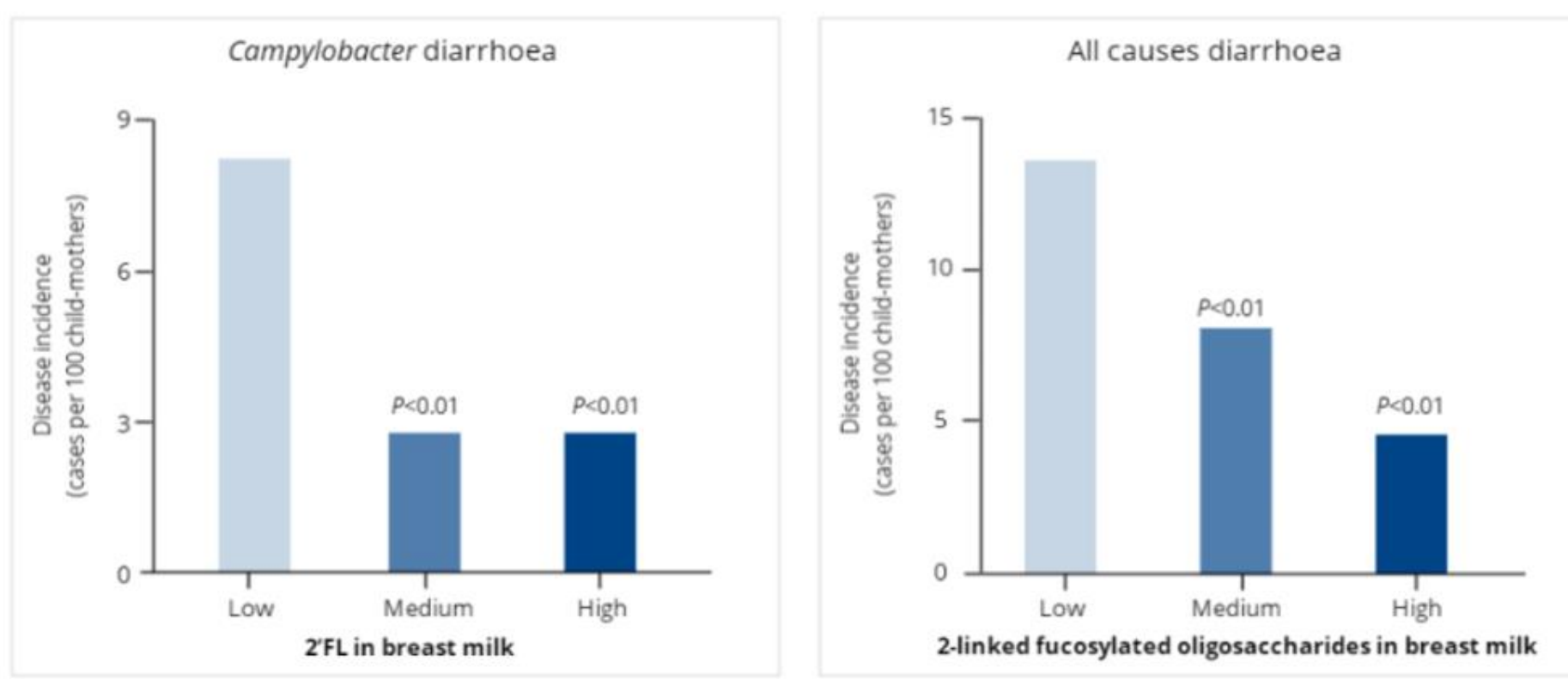
Josh M Jorgensen,[1] Rebecca Young,[1] Per Ashorn,[2,3] Ulla Ashorn,[2] David Chaima,[4] Jasmine CC Davis,[5] Elisha Goonatilleke,[5] Chiza Kumwenda,[4,6] Carlito B Lebrilla,[5,7] Kenneth Maleta,[4] Elizabeth L Prado,[1] John Sadalaki,[4] Sarah M Totten,[5] Lauren D Wu,[5] Angela M Zivkovic,[1,8] and Kathryn G Dewey[1]

Am J Clin Nutr 2021;113:209–220.

- 659 Breast milk samples were analyzed at 6 mo
- HMOs, and 6 bioactive proteins (lactalbumin, lactoferrin, lysozyme, antitrypsin, IgA, and osteopontin).
- Associations of the relative abundances of HMOs and concentrations of bioactive proteins with infant growth from 6 to 12 mo were examined.
- Ability to stand or walk alone at 12 mo, and motor and language skills, socio-emotional development, executive function, and working memory at 18 mo.
- Analyses were adjusted for covariates and multiple hypothesis testing

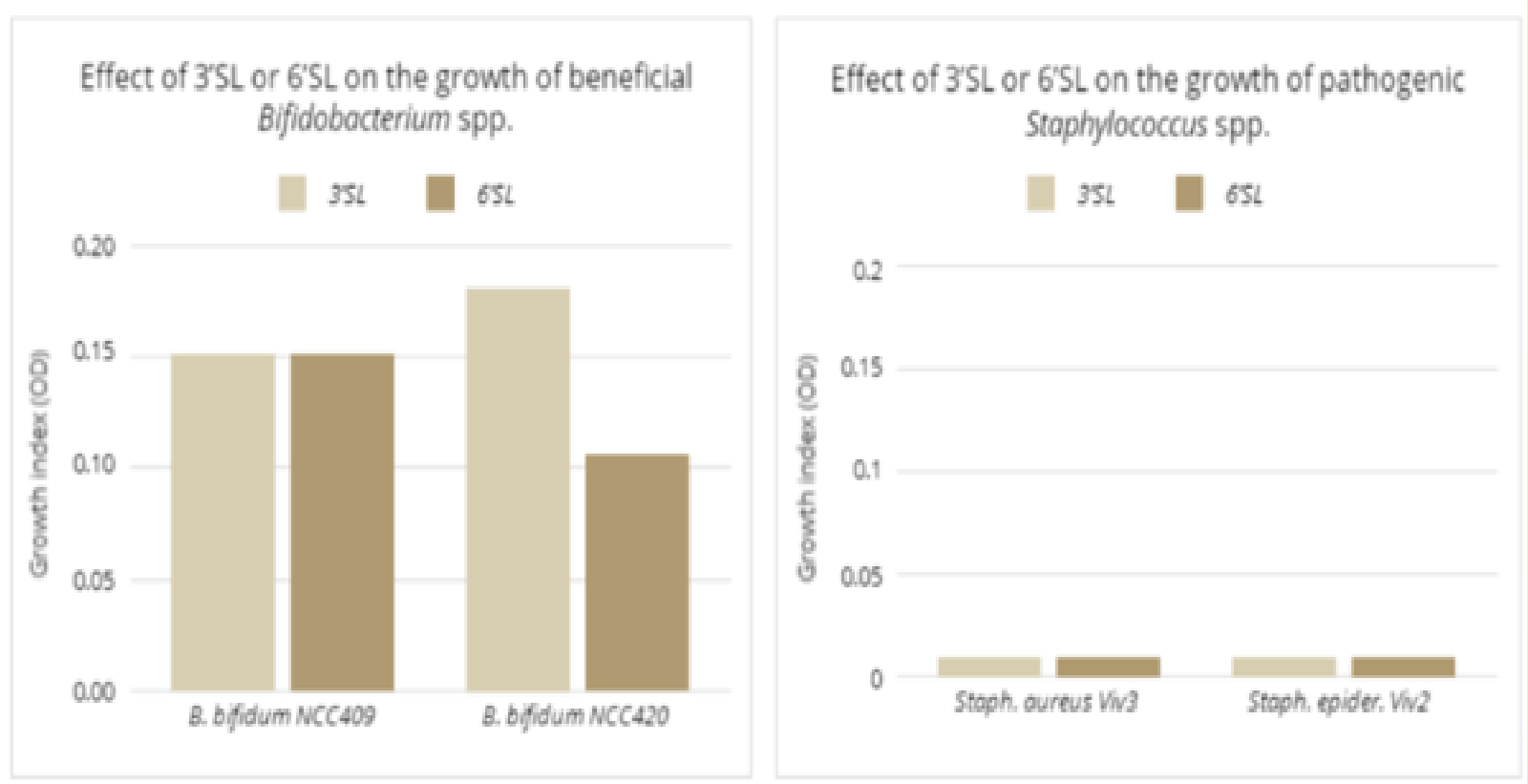
- **Relative abundance of fucosylated and sialylated HMOs with language at 18 mo (P < 0.001 and P=0.033, respectively)**
- **Relative abundances of several individual HMOs were associated with growth and development.**
- Positive associations of absolute abundance of HMOs with LAZ (P=0.035)

2FL in human milk reduce the risk of infectious diarrhoea



Adapted from Morrow et al., 2004

HMOs Support the growth of beneficial gut bacteria



Key Messages

- The first years of life are a rapid & dynamic period for brain maturation
- Most brain processes during that period are focused on connecting the brain, e.g. myelination
- Many factors, including nutrition, influence brain growth and myelination
- HMOs are prebiotics, and they promote growth of intestinal microbiota
- HMOs are important in cognitive development in children.

References

- Piirainen L, Kekkonen RA, Kajander K, Ahlroos T, Tynkkynen S, Nevala R, et al. In school-aged children a combination of galacto-oligosaccharides and Lactobacillus GG increases bifidobacteria more than Lactobacillus GG on its own. Ann Nutr Metab. 2008;52:204–8
- Jorgensen JM. et al. Associations of human milk oligosaccharides and bioactive proteins with infant growth and development among Malawian mother-infant dyads. Am J Clin Nutr 2021;113:209–220.
- Seoyoon Cho et al. A Positive Correlation between Breast Milk 3'-Sialyllactose and Language Development during Early Infancy.
- Silbereis JC, et al., Neuron 2016;89:248–68

- World Health Organisation (WHO) and United Nations International Children's Emergency Fund (UNICEF). Infant and young child feeding. https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding. Published August 2020.
- Victora CG, Bahl R, Barros AJ, et al; Lancet Breastfeeding Series Group. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet. 2016;387(10017):475-90.
- Bode L. Human milk oligosaccharides: Every baby needs a sugar mama. Glycobiology 2012;22 (9):1147–1162. doi:10.1093/glycob/cws074
- Donovan SM, Comstock SS. Human milk oligosaccharides influence neonatal mucosal and systemic immunity. *Ann Nutr Metab*. 2016;69 Suppl 2:42-51.
- Zivkovic AM, German JB, Lebrilla CB, Mills DA. Human milk glycobiome and its impact on the infant gastrointestinal microbiota. *Proc Natl Acad Sci U S A*. 2011;108 Suppl 1:4653-4658.

- 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.
- Hennet T, Weiss A, Borsig L. Decoding breast milk oligosaccharides. *Swiss Med Wkly*. 2014;144:w13927.
- Bode L. The functional biology of human milk oligosaccharides. *Early Hum Dev*. 2015;91(11):619-622.
- Thurl S, Munzert M, Boehm G, et al. Systematic review of the concentrations of oligosaccharides in human milk. *Nutrition Reviews*. 2017;75(11):920-933.
- 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;8(6).
- Sprenger N, Lee LY, De Castro CA, et al. 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.
- Samuel TM, Binia A, de Castro CA, et al. Impact of maternal characteristics on human milk oligosaccharide composition over the first 4 months of lactation in a cohort of healthy European mothers. *Scientific Reports*. 2019;9(1):1-10.
- Austin S, De Castro CA, Sprenger N, Binia A, et al. Human milk oligosaccharides in the milk of mothers delivering term versus preterm infants. *Nutrients*. 2019;11(6):1282.
- Lefebvre G, Shevlyakova M, Charpagne A, et al. Time of lactation and maternal fucosyltransferase genetic polymorphisms determine the variability in human milk oligosaccharides. *Front Nutr*. 2020;7:574459. doi: 10.3389/fnut.2020.574459. eCollection 2020.

Thank You for Listening