Human Milk Oligosaccharides (HMOs) and Brain Development: Key Perspectives

The New Perspective in Nutrition, Learning and Development.
Outline of Presentation

• Overview of the brain structure and functions
• Brain development in early childhood
• Infant nutrition in learning
• Human Milk Oligosaccharides (HMOs)
• HMOs in brain development: New perspectives
• Conclusions
Brain Structure

- Neurons (nerve cells)
- 100 billion neurons (100,000,000,000)
- 100 km of nerves
- 100 billion non-neuron cells (100,000,000,000)
- 1 quadrillion synapses (1,000,000,000,000,000)
- 600 km of blood vessels

Kasthuri N, et al. 2015

FUNCTIONS OF THE BRAIN

FRONTAL LOBE
• Attention and concentration
• Organization
• Expressive language
• Motor planning and initiation
• Personality
• Problem solving
• Emotion

BRAIN STEM
• Breathing
• Arousal and consciousness
• Heart rate
• Sleep and wake cycle
FUNCTIONS OF THE BRAIN

TEMPORAL LOBE
- Learning and Memory
- Understanding language (receptive language)
- Sequencing
- Hearing
- Organization

OCCIPITAL LOBE
- Vision

PARIETAL LOBE
- Sense of touch
- Spatial perception
- Differentiation
- Visual perception

TEMPORAL LOBE
- Balance
- Skilled motor activity
- Co-ordination
Functions of the various parts of the brain

Proper connectivity is key for proper brain and cognitive functions

The Development of the Child Nervous System

- The foetal nervous system is one of the first systems to develop and the last to be completed after birth.
- The process generates the most complex structures within the embryo.
- The brain weighs 300-350 grams at birth and contains 100 billion neurons.
Development of The Child Nervous System

- At birth, the average baby’s brain is about a quarter of adult brain.
- Doubles in the first year.
  - Critical period
  - Undergoes rapid growth in the first 6 months of postnatal life
- 80% of adult size by age 3.
- 90% – nearly full grown – by age 5 to 6.
Timeline of key human neurodevelopmental processes

- After birth, billions of neurons get connected by synaptogenesis: 700,000 synapses/second are formed
- Myelination develops rapidly to enhance neuron communication throughout the entire brain.
- These early experience-dependent processes underlie the plasticity & capacity for adaptation that is the hallmark of early brain development.

Adapted from Silbereis JC, et al., Neuron 2016;89:248–68

Brain Development in Children

Cerebral volume (% of adult volume) by:
- ~36% 2–4 weeks
- ~72% 1 year
- ~80% 3 years
- ~90%–92% 9 years

Timeline of major events in brain development
- Conception
- 4 weeks
- 8 weeks
- 12 weeks
- 16 weeks
- 20 weeks
- 24 weeks
- 28 weeks
- 32 weeks
- Birth
- 4 months
- Adolescence
- Adulthood

Neurulation
Neuronal proliferation
Neural migration
Myelination
Synaptogenesis
Apoptosis

The brain volume achieved at the end of the first year is an important indicator of later intelligence.

Brain Development

NATURE
Genetic expression

NUDURE
Environmental factors

90% Brain development before age 5
10% Brain development after age 5
Key Factors Influencing Brain Development

NATURE AND NURTURE

• Nature (Gene Expression)

• Nurture (Environmental factors): The developing brain is vulnerable to modification by environmental factors
  - Socioeconomic status
  - Stress
  - Pollution
  - Urbanization
  - Social Interactions
  - Nutrition
Interplay between structural functional development and nutrition

Nutritional sensitivity

Right parietal white matter

<table>
<thead>
<tr>
<th>Age (corrected days)</th>
<th>MWF</th>
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<tbody>
<tr>
<td>0</td>
<td>0.05</td>
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<tr>
<td>375</td>
<td>0.14</td>
</tr>
<tr>
<td>750</td>
<td>0.18</td>
</tr>
<tr>
<td>1900</td>
<td>0.18</td>
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n = 62
n = 28
n = 39

Predictability

Correlation between MWF and the cognitive development. N. Schneider & S. Deoni, personal communication

<table>
<thead>
<tr>
<th>MWF (4 months of age)</th>
<th>ELC (2 years of age)</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>45</td>
<td>105</td>
</tr>
<tr>
<td>60</td>
<td>140</td>
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R² = 0.4057

Pro-Myelin Nutrients: Sphingomyelin, DHA/ARA, Choline, Vit B9, Vit B12, Iron

Association between sphingomyelin/iron and myelin in 0 - 5 year old children. S. Deoni, personal communication

ARA, arachidonic acid; DHA, docosahexaenoic acid; MWF, myelin water fraction.

Key Nutrients Supporting Brain Growth and Development

- Choline
- Lutein
- Sphingomyelin
- DHA
- Iodine
- HMOs
Structure of HMOs: Fucosylated and Sialylated

Building blocks and main structures

- Glucose
- Galactose
- N-acetylgalactosamine
- Fucose
- Sialic acid

Lactose core

Gal → Glu

Core structures

Gal → GlcNAc

n = 0-15

Terminal positions

Small HMOs Examples

- 2'FL (trisaccharides)
- LNnT (tetrasaccharides)

Large HMOs Examples

- LNnD
  - n = 0-3

Small HMOs such as 2'FL and LNnT form the largest group of HMOs in human milk.1-3

2'FL, 2'-fucosyllactose; LNnD, lacto-N-neo-difuco-hexaose; LNnT, lacto-N-neotetraose.
HUMAN MILK OLIGOSACCHARIDES (HMOs)

HMOs are the third largest solid component in breast milk

HMO levels range between: 20 - 25 g/L for colostrum and 5 and 15 g/L for mature milk\(^1,2\)

The HMO fraction is larger than that of proteins and can therefore be considered a key component of human breast milk.\(^1,2\)
### Difference between Human and Bovine Milk

<table>
<thead>
<tr>
<th></th>
<th>Human Milk</th>
<th>Bovine Milk</th>
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<tbody>
<tr>
<td>Oligosaccharides (g/L)</td>
<td>10-15</td>
<td>~0.05</td>
</tr>
<tr>
<td>Number of identified</td>
<td>&gt;200</td>
<td>~40</td>
</tr>
<tr>
<td>oligosaccharides</td>
<td></td>
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</tbody>
</table>

- % neutral fucosylated: 50%-80% (Human), ~1% (Bovine)
- % sialylated acidic:   10%-20% (Human), ~70% (Bovine)

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**Bar Chart:**
- **Human**:
  - Lactose: 65
  - Proteins: 10
  - Lipids: 35
- **Bovine**:
  - Lactose: 45
  - Proteins: 35
  - Lipids: 35

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**Diagram:**

- Human milk compared to Bovine milk in terms of Oligosaccharides, Lactose, Proteins, and Lipids.
- Human milk has a higher number of oligosaccharides and a different distribution of components compared to Bovine milk.
Metabolism of HMOs

- Infant gut **cannot** digest HMOs because of lack of the necessary enzymes
- Reaches the lower gut unchanged
- Only $\approx 1\text{-}2\%$ of HMOs are absorbed in the gut and enter the systemic circulation
- The rest remain in the gut,
  - Modulates gut microbiota
  - Metabolize into SCFA, GABA etc and participate in the **Gut-Brain Cross Talk**

Adapted from Rudloff S et al., 2012.
SCFA cross the BBB to modulate structural and functional aspects of the Brain, affect the immune response via regulation of dendritic and T-cell function, as well as via inhibition of cytokine production.
2´FL promotes the growth of specific bifidobacteria

- HMOs such as 2´FL and 3´FL were able to promote the growth of bifidobacterial strains and to suppress potentially pathogenic bacteria.
- When supplemented with 2´FL, the bifidobacterial strains multiplied abundantly and produced ample SCFA.

B. longum ATCC15697, B. infantis; FL, fucosylated; FOS, fructo-oligosaccharide; HMO, human milk oligosaccharide; LDFT, Lactodifucotetraose; SCFA, short-chain fatty acid.
Oral supplementation of 2′-fucosyllactose during lactation improves memory and learning in rats

Elena Oliveros a, María Ramírez a, Enrique Vazquez a, Alejandro Barranco a, Agnes Gruart c, Jose María Delgado-Garcia c, Rachael Buck b, Ricardo Rueda a, Maria J. Martín a
Learning

Rat pups received an oral supplementation of 2'-FL or water (control group) during the lactation period. Thereafter, maintained on a rodent standard diet. Evaluated at age 4-6 weeks and 1 year, using classical behavioral tests. Both groups showed similar behavior when assessed just after weaning (age 4-6 weeks). At age 1 year, 2'-FL rats performed significantly better in the Novel Object Recognition compared to controls.
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. Berger¹, Jasmine F. Plows¹, Roshonda B. Jones¹, Tanya L. Alderete², Chloe Yonemitsu³, Marie Poulsen⁴, Ji Hoon Ryoo¹, Bradley S. Peterson¹, Lars Bode³, Michael I. Goran¹*
Objective

- To determine the impact of 2’FL from breast milk feeding on infant cognitive development at 24 months of age relative to maternal obesity and breast milk feeding frequency.

Materials and Methods

- Hispanic mother-infant pairs (n = 50) were recruited across the spectrum of pre-pregnancy BMI.
- Breast milk was collected at 1 and 6 months, and feedings/day were reported.
- Nineteen HMOs were analyzed using high-performance liquid chromatography, with initial interest in 2’FL.
- Infant cognitive development score was assessed with the Bayley-III Scale at 24 months.
Maternal pre-pregnancy BMI related to infant cognitive development score at 24 months of age
Breastfeeding frequency at 1 month is associated with better infant cognitive development at 24 months of age.
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 were included in this analysis. The Mullen Scales of Early Learning was administered to assess the child’s cognitive development. The concurrent breast milk samples (n=191) were obtained and analyzed for specific HMOs including 2’FL, 3’FL, 3’SL, 6’SL, Lacto-N-tetraose (LNT), Lacto- N-neotetraose (LNNT), Lacto-N-fucopentaose I (LNFPI), and A-tetrasaccharide (A-Tetra).
3’SL in breast milk is positively associated with language development in early childhood

Expressive Language ~ 3’SL (Log)

Receptive Language ~ 3’SL (Log)

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


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 Dewey1

Sialyllated and fucosylated HMOs are both associated with language development

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 Chattoo,4 Jasmine CC Davis,5 Elitha Geona, Chizimbabwe,5,6 Carlos B Lebrilla,5,7 Kenneth Muleta,8 Elizabeth I. Prado,1 John Sadoulaki,3 Sarah M Forsten,1 Lauren D Wu,9 Angela M Zinkovic,9,10 and Kathryn G Dewey1

- Malawian mother-infant pairs (N = 869)
- Breast milk HMO content at 6 months of age
- A checklist based on the MacArthur–Bates Communicative Development Inventory used for language evaluation
Summary: HMOs and Brain Health

HMOs Support cognitive and language development

- **2'FL**: Associated with better cognitive development
  - Improves memory and learning
  - Mother-Infant cohort study

- **3'SL**: Associated with language development
  - Mother-Infant cohort study

- **3'SL + 6'SL**: Improves executive function and learning
  - Role in cognitive function
  - Pre-clinical trial

- **6'SL**: Improves memory function
  - Improves attention and reduces impulsivity
  - Pre-clinical trial

FL, fucosyllactose; SL, sialyllactose.
Key Messages

• There is rapid, dynamic and complex brain growth and development in the first years of life

• Genetic and environmental (nutrition) factors have been shown to modify this process

• Breastmilk, rich in nutrients such as HMOs is associated with improved brain development and cognition.

• Infants fed with HMOs supplemented formulae show comparable cognitive outcomes to breastfed infants.


THANK YOU