Milk to the brain
– the influence of milk on brain development and brain function

Anne Staudt Kvistgaard, science and sales development manager, AFI

Esben Skipper Sørensen, associate professor, Department of Molecular Biology and Genetics, Aarhus University
Eat yourself smarter …

- Brain development and cognitive performance are central processes for human life
- Can these processes be optimized through nutrition?
- BRAINFOOD – is a popular belief that you can eat yourself more intelligent with fish, nuts, berries, spices etc. but how about milk?!
- In this presentation we will mainly focus on the development of the infant brain and how this can be influenced by milk components
- But there are also indications that certain milk components can have positive effects on cognitive health and performance in adults and elderly
AGENDA

- Developing solutions for early life nutrition
- Brain development in early life
- Milk components involved in brain development and function
  - Milk lipids
    - Phospholipids, sphingomyelin, gangliosides
- Market insight
- On-going research projects supported by the Danish Dairy Research Foundation
  - InfantBRAIN
  - BRAIN MILK
AFI solutions for early life nutrition
- Ensuring a healthy beginning

Solutions for the critical first 1000 days

**Pregnancy**
Providing building blocks ensuring a healthy pregnancy and start in life

**Preterm**
Ingredients ensuring nutrition for the premature infant

**Infant**
Ingredients ensuring optimal growth and development of the infant

**Toddler**
Solutions supporting toddlers fast growth and development
Observed differences comparing breastfed and formula fed

- Cognitive Development
- Metabolic programming
- Growth pattern
- Immune development
- Gut microflora
- Allergies
Cognition
From conception to adulthood

Tau et al 2010, Andersen et al 2003
Cognitive development

Infant cognitive development

Prenatal and infancy is a very critical period for cognitive development due to rapid neuronal growth and maturation

- The infant brain is at birth approx. 25% of its adult volume
- By the end of the first year the brain reaches 75% of its adult volume
- The remaining 25% of brain growth is achieved over the next few years
Cognition
Images of the growing brain

- First two years increase in brain size and white matter intensity
- First two years increase in the organisation of white matter structure
Breastfeeding is associated with significantly higher scores for cognitive development than formula feeding:
- 3.2 higher IQ score compared to formula feds
- 5.2 higher IQ score for low birth weight infants

Difference in cognitive development is present in early life and sustained through childhood and adolescence.

Small IQ impact at individual level
Large IQ impact at population level

Anderson et al. 1999, Michaelsen et al. 2009
What determines the IQ of infants?

Many factors influence a child’s intelligence

- Genetics
- Environment (stimulation, smoking etc.)
- Nutrition – and perhaps the way nutrition is provided?

Components in human milk stimulates early cognitive development

- Phospholipids
- Sphingomyelin
- Gangliosides
- And others ......
Milk Lipids
- In brief

• Lipids constitute a group of naturally occurring molecules including fats, sterols, triglycerides, phospholipids and others
• Major source of energy for the infant
• Occurs as globules emulsified in the aqueous phase (milk fat globules (MFG))
• MFG contains a core of triglycerides (98 %) surrounded by a membrane composed of phospholipids (PL) (0.2-1 %) and cholesterol
Bioactive Lipids
- Occurrence and sources

Function
- Lipid bilayer
- Metabolic pathways
- Sources of fatty acids in breast milk
- Cognitive development and abilities

Bioactive Lipids in biological membranes
- Phosphatidylcholine
- Phosphatidylethanolamine
- Phosphatidylinositol
- Phosphatidylserine
- Gangliosides
- Sphingomyelin

Sources
- Milk
- Egg
- Soy
- Meat
Humanisation
Cognitive development and abilities

- Components in human milk supporting cognitive development includes amongst others phospholipids, sphingomyelin and gangliosides

- These components play roles in the central nervous system development and myelination supporting efficient transmission of nerve impulses

- Compared to human milk, infant formulas are particular low in sphingomyelin and phosphatidylserine, the two PL missing in soy lecithin

Bovine milk is the only phospholipid source with a similar PL profile compared to human milk and provides a source of gangliosides

<table>
<thead>
<tr>
<th>% distribution of milk lipids in different sources (% of total milk lipids)</th>
<th>Human milk</th>
<th>Bovine Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sphingomyelin (SM)</td>
<td>29.7</td>
<td>19.9</td>
</tr>
<tr>
<td>Phosphatidylcholine (PC)</td>
<td>24.5</td>
<td>28.7</td>
</tr>
<tr>
<td>Phosphatidylethanolamine (PE)</td>
<td>18.3</td>
<td>31.4</td>
</tr>
<tr>
<td>Phosphatidylserine (PS)</td>
<td>8.1</td>
<td>11.2</td>
</tr>
<tr>
<td>Phosphatidylinositol (PI)</td>
<td>3.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Others</td>
<td>13.9</td>
<td>4.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gangliosides</th>
<th>Human milk</th>
<th>Bovine Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD3 (mg/L)</td>
<td>3.8 - 0.9²</td>
<td>3.3 - 2.4³</td>
</tr>
<tr>
<td>GM3 (mg/L)</td>
<td>4.3 - 9.8²</td>
<td>0.98 - 0.15³</td>
</tr>
</tbody>
</table>

GD3 and GM3: the two major gangliosides in human milk
Human milk gangliosides measured at day: 0-11, 30, 60 and 120
Bovine milk gangliosides measured at day: 2, 15 and 90. *15.2 mg/L at day 2.
The importance of dietary phospholipids and gangliosides for brain development is of clear interest due to the observation that breast fed infants have improved learning compared to formula fed infants.

- Phospholipids increased brain growth and improved learning and memory in neonatal piglets.
- Phospholipids supplemented piglets made quicker choices and moved a shorter distance.
- Supplementation with phospholipids resulted in changes in brain hippocampus phospholipid metabolites that may be consistent with an increased uptake of phospholipids.

**Dietary phospholipids increased brain growth and improved learning and memory.**
Effect of dietary phospholipids in a piglet study

Liu et al. 2014
Effect of dietary sphingomyelin on brain, myelin weights and myelin lipids

The data suggests that dietary sphingomyelin contribute to central nervous system myelination in developing rats.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Non-LCS</th>
<th>LCS</th>
<th>SM-LCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain wet wt (g)</td>
<td>1.67 ± 0.01a</td>
<td>1.55 ± 0.01b</td>
<td>1.64 ± 0.01a</td>
</tr>
<tr>
<td>Myelin dry wt (mg/brain)</td>
<td>24.4 ± 0.5a</td>
<td>12.9 ± 0.5b</td>
<td>21.7 ± 0.8b</td>
</tr>
<tr>
<td>Myelin total lipid content (mg/brain)</td>
<td>17.2 ± 0.5a</td>
<td>8.5 ± 0.2b</td>
<td>14.8 ± 0.4c</td>
</tr>
<tr>
<td>Myelin total lipid composition (%)</td>
<td>70.5 ± 1.8a</td>
<td>67.0 ± 4.3a</td>
<td>68.4 ± 2.2a</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM (n = 6).

Effect of dietary sphingomyelin
On central nervous system myelination

• Rat model with blocked sphingolipid biosynthesis
• Rat pups fed bovine sphingomyelin supplemented diet (0.8%) (control = 0 %) until 28 days of age
• The sphingomyelin supplemented group had higher levels of:
  • Brain wet wt
  • Myelin dry weight
  • Myelin total lipid content

Oshida et al. 2003

AARHUS UNIVERSITET

Oshida et al. 2003
Human study on sphingomyelin
Premature infants

- Double-blinded randomised, controlled, clinical trial (inclusion birth weight < 1500g)
- Supplementation to infant formulas of milk derived PLs versus Egg-Yolk derived PLs
- Follow-up evaluations at 3,6,9,12 and 18 months
- Test of neurodevelopment: “intelligence”, memory, facial recognition, vision

<table>
<thead>
<tr>
<th>Groups</th>
<th>SM % of total PL</th>
<th>Number of infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard formula</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Experimental formula</td>
<td>20</td>
<td>12</td>
</tr>
</tbody>
</table>

Study showed serum SM concentrations increased in the trial group. The neurodevelopment test scores were better in the trial group.

Milk phospholipid and sphingomyelin fortified milk has a positive association with neurobehavioral development of low-birth-weight infants.
Gangliosides
Major constituents of the brain

- Gangliosides are abundant in the brain and also highly expressed in nervous tissue\(^1\):
  - They are concentrated within the grey matter and constitute around 6-10% of the total human brain lipid mass\(^2,3\)
  - They are also enriched at the synaptic membrane of neurons where they are functionally involved in neurotransmission and synapse formation\(^1,4\)

Brain material from infants who died from sudden infant death syndrome (SIDS) showed that breast fed compared to formula fed infants have

Approx 30% higher ganglioside content
Approx 20% higher protein-bound sialic acids

Gangliosides
- Changes in membrane lipid profile

Rats fed a ganglioside-enriched lipid diet (0.02 % GG) significantly increased total ganglioside in the intestinal mucosa, plasma and brain.

Gangliosides fed at a physiological level will alter the membrane lipid profiles that influence membrane functions involved in a wide variety of cell functions during early development.

Park et al. 2005
Human study on ganglioside

- Double-blind, randomized, controlled, parallel group clinical trial (inclusion age 2-8 weeks)
- Griffith scales and serum gangliosides was measured before and after the intervention (24 weeks)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ganglioside (μg/ml)</th>
<th>Number of infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastfed</td>
<td>3.4-16.2</td>
<td>40</td>
</tr>
<tr>
<td>Standard formula</td>
<td>7.5</td>
<td>35</td>
</tr>
<tr>
<td>Supplemented formula</td>
<td>11-12</td>
<td>35</td>
</tr>
</tbody>
</table>

Supplementation of an infant formula with complex lipids to enhance ganglioside content appears to have a beneficial effect on cognitive development in healthy infants age 0-6 months.
Cognitive development
Infant clinical study on Lacprodan® MFGM-10

- Double-blind, randomised, controlled, parallel group clinical trial (inclusion age < 2 months)
- The Bayley Scale of Infant and Toddler Development, 3rd edition was measured at 12 months

MFGM-10 supplementation to infant formula narrows the gap in cognitive development between breastfed and formula fed infants

<table>
<thead>
<tr>
<th>Groups</th>
<th>PLs mg/100 ml</th>
<th>Number of infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastfed (BFR)</td>
<td>30</td>
<td>72</td>
</tr>
<tr>
<td>Standard formula (SF)</td>
<td>30</td>
<td>68</td>
</tr>
<tr>
<td>Experimental formula (EF)</td>
<td>70</td>
<td>73</td>
</tr>
</tbody>
</table>

Results of testing with the Bayley Scales of Infant and Toddler Development, Third Edition, for the EF, SF, and BFR groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cognitive</th>
<th>Motor</th>
<th>Fine motor</th>
<th>Gross motor</th>
<th>Verbal</th>
<th>Receptive</th>
<th>Expressive</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF</td>
<td>10.58 ± 0.4</td>
<td>96.2 ± 1.3</td>
<td>9.30 ± 0.2</td>
<td>9.30 ± 0.2</td>
<td>102.6 ± 1.2</td>
<td>10.46 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>10.3 ± 0.4</td>
<td>96.2 ± 1.3</td>
<td>9.70 ± 0.2</td>
<td>9.70 ± 0.2</td>
<td>102.5 ± 1.2</td>
<td>10.46 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>BFR</td>
<td>10.4 ± 0.4</td>
<td>100.2 ± 1.3</td>
<td>10.2 ± 1.2</td>
<td>10.2 ± 1.2</td>
<td>106.7 ± 1.2</td>
<td>10.76 ± 1.2</td>
<td></td>
</tr>
</tbody>
</table>

Timby et al. 2014a
Market insight
Focus on cognition

- Parents are constantly focused on their infants early development and the early nutritional impact on areas like brain development

- Therefore, when launching new products focus continues to be on cognitive development
On pack claims:

.......... MFGM brain nourishing lipids also found in breast milk, designed to support mental development. MFGM to foster cognitive development........

On pack claims:

Babysemp 1 contains a carefully selected blend of milk fats and vegetable oils called lipilact. Mixture contains .....and several substances found in membrane (MFGM), for example, phospholipids, gangliosides, cholesterol, and glycoproteins.
InfantBRAIN research project
2016-2020 Principal Investigator: Lars Hellgren, DTU – supported by DDRF and ”Innovationsfonden”

Hypotheses:

- The lipase specificity and efficacy have co-evolved with the milk-fat globule membrane composition, to maximize the efficacy of fatty-acid absorption - Deviations from this will impact the rate of lipid absorption
- Low absorption of long-chained polyunsaturated fatty acids (in particular DHA) from infant formulas limits brain development in early life.

InfantBRAIN aim at developing a new PL-ingredient
- optimize process, yield, economic costs
- document improved lipolysis upon emulsification
- document improved brain and cognitive development
Blood Brain Barrier (BBB) and milk peptides

- integrity is important in dementia and Alzheimer’s

- The brain is protected by the skull, the cerebrospinal fluid and the blood brain barrier (BBB).

- The integrity of the BBB is essential for the resistance against infections and intoxications, but it is also important in the protection against age-related neurological pathologies like dementia and Alzheimer’s disease.

- The BBB tightly regulates the transport of molecules to the brain and normally only molecules of <500 Da can traverse the BBB – though some active transport of larger molecules takes place.

In the MFF project ”BRAIN MILK“ the effect of milk peptides on the BBB is investigated.
Increased ERK phosphorylation and “leaking” blood-brain barrier are correlated with dementia and Alzheimer’s ... and also seen in athletes that receives blows to the head

In the MFF project brain milk we have shown that endogenous milk peptides are able to reduce ERK phosphorylation and to increase the integrity of the blood-brain barrier

Milk peptides could potentially support brain function
Thank you for your attention!

Anne Staudt Kvistgaard, science and sales development manager, AFI

Esben Skipper Sørensen, associate professor, Department of Molecular Biology and Genetics, Aarhus University