Milk to the brain

– the influence of milk on brain development and brain function

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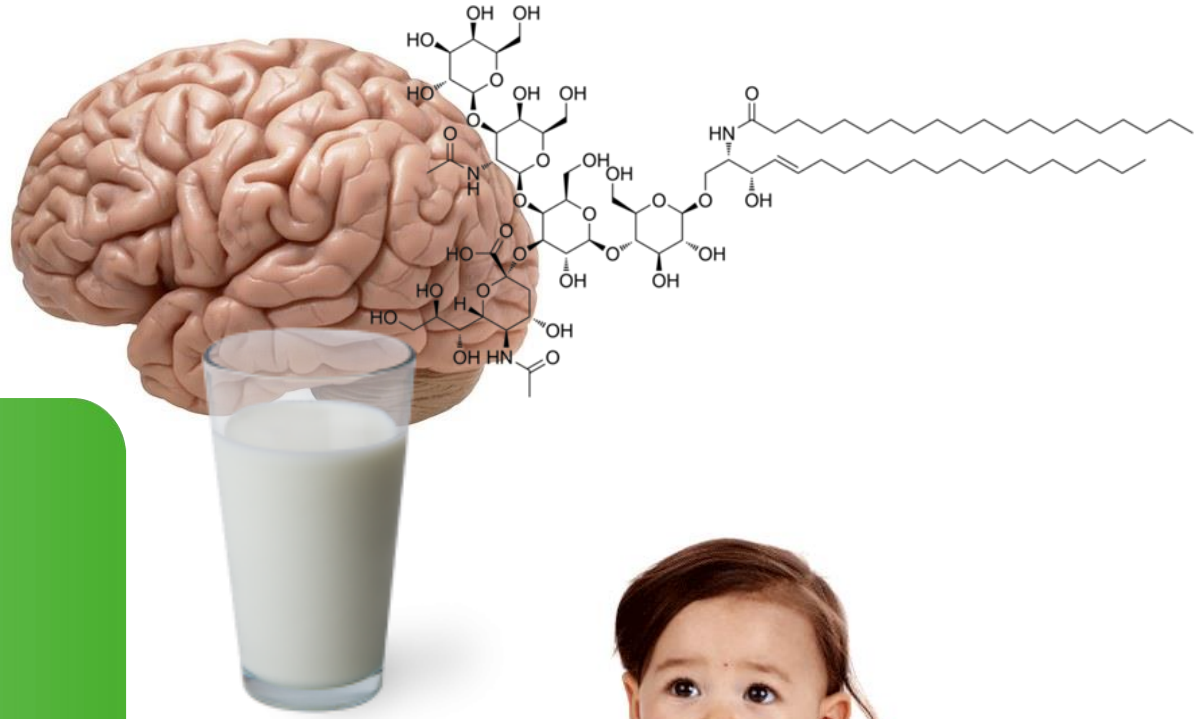
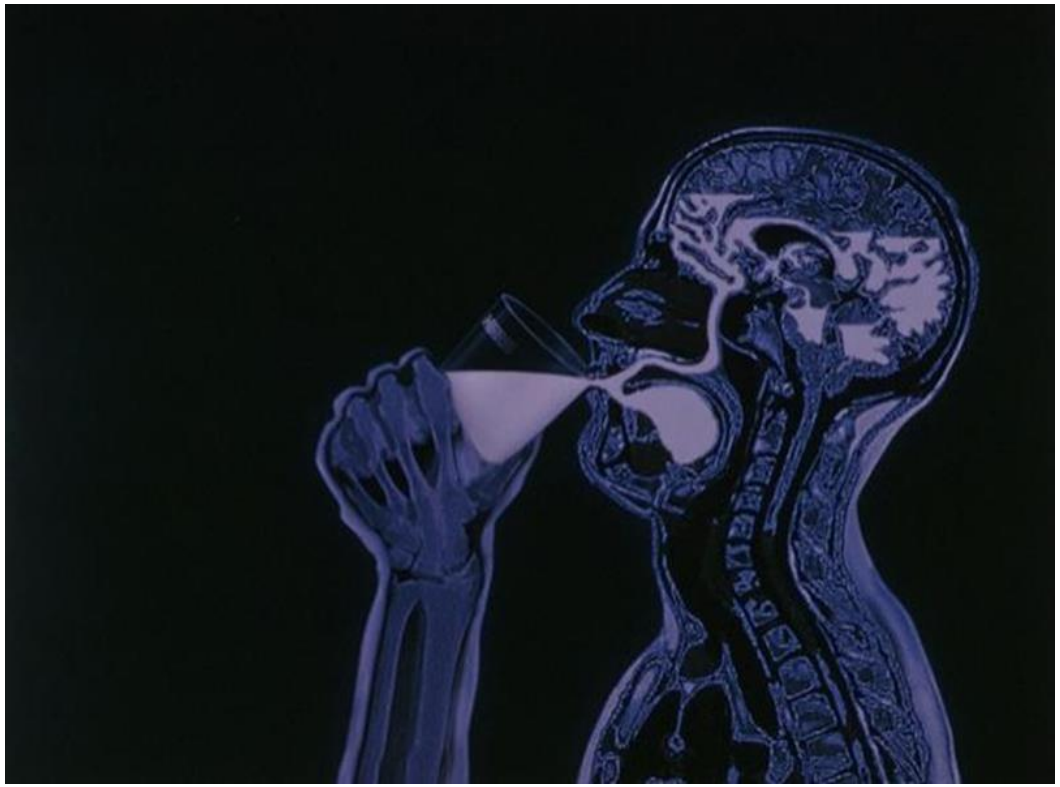


Eat yourself smarter …

- 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



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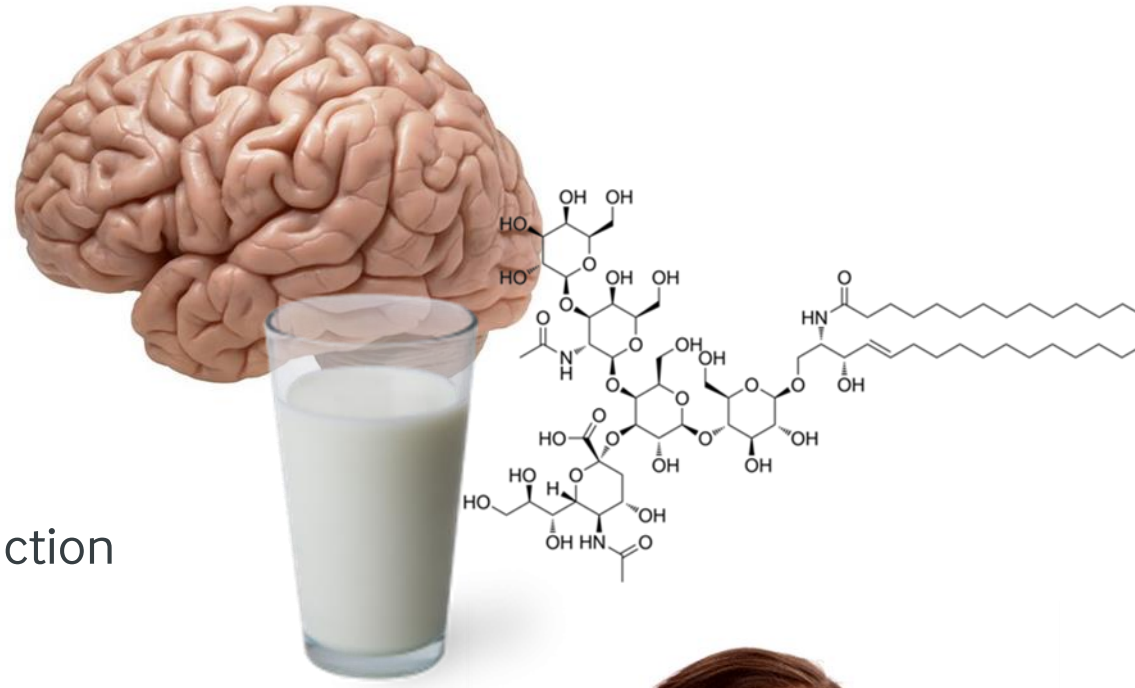
AGENDA



- 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

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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

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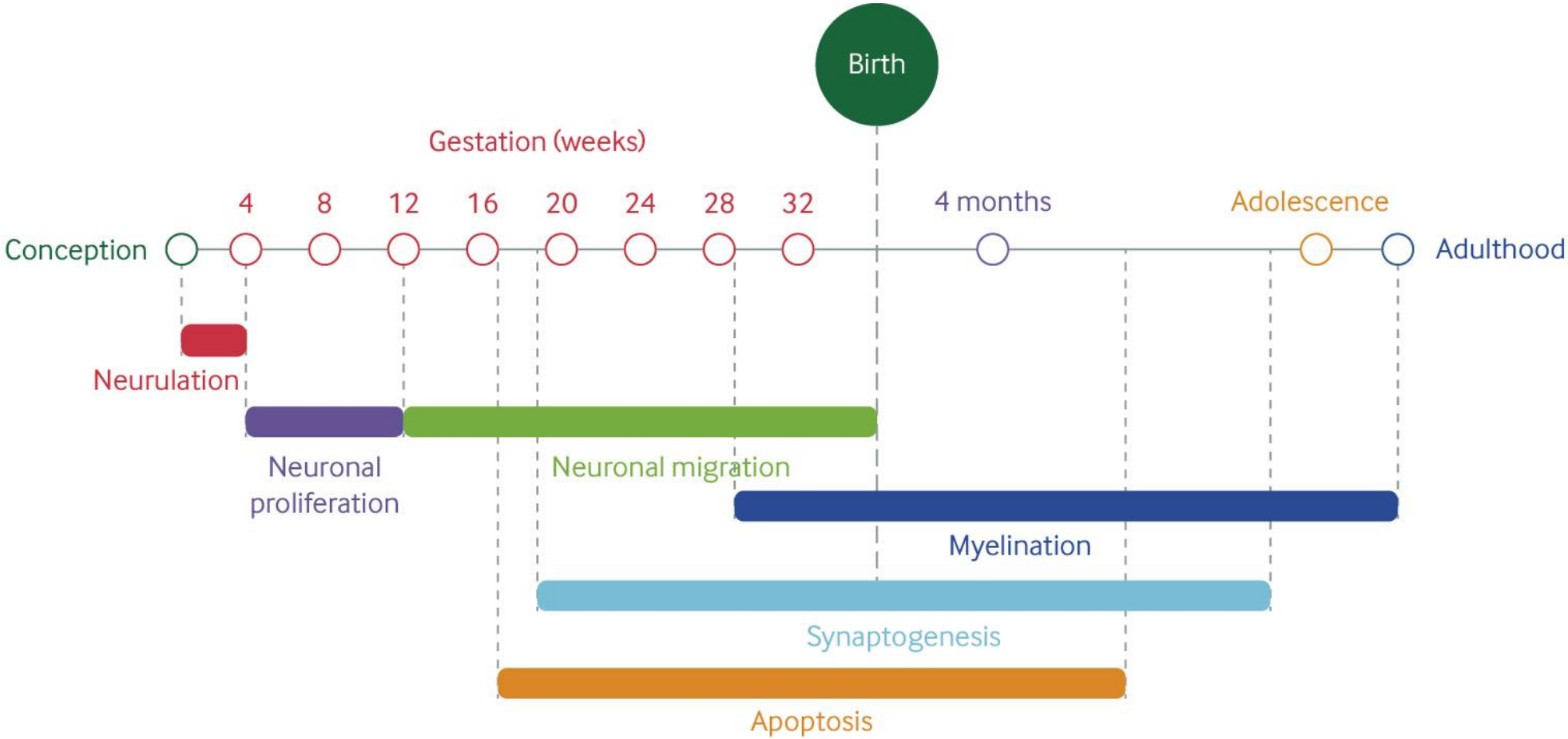
Cognition
From conception to adulthood



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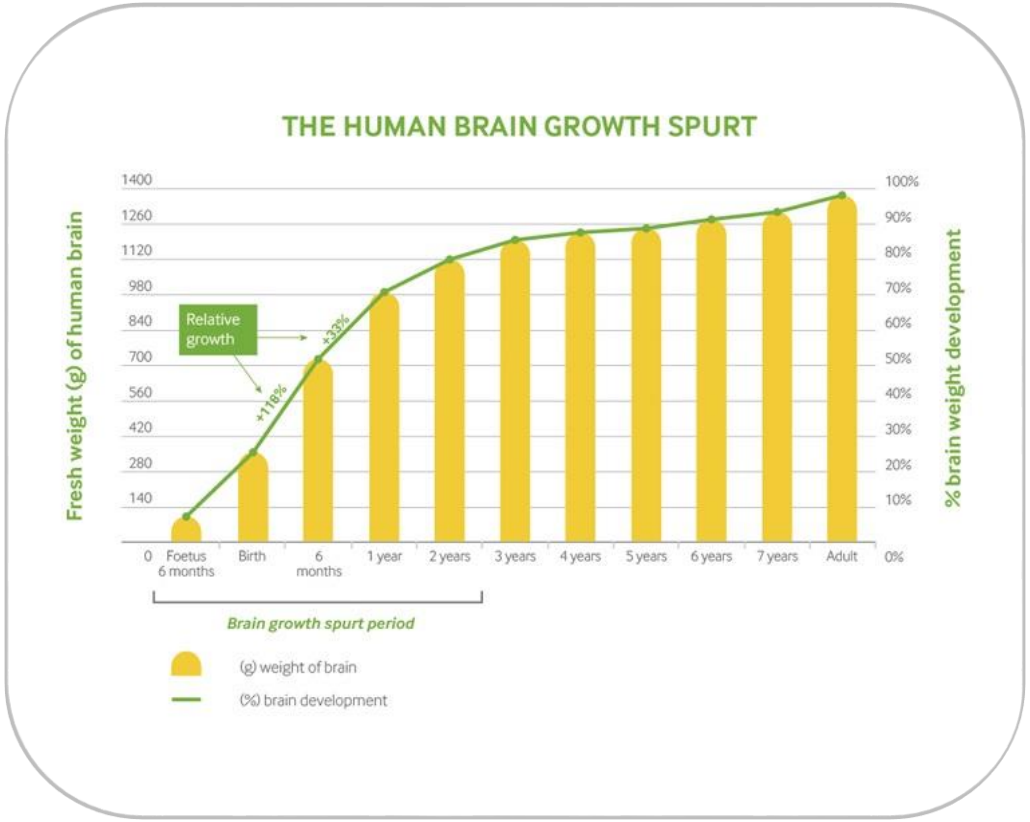
Tau et al 2010, Andersen et al 2003

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



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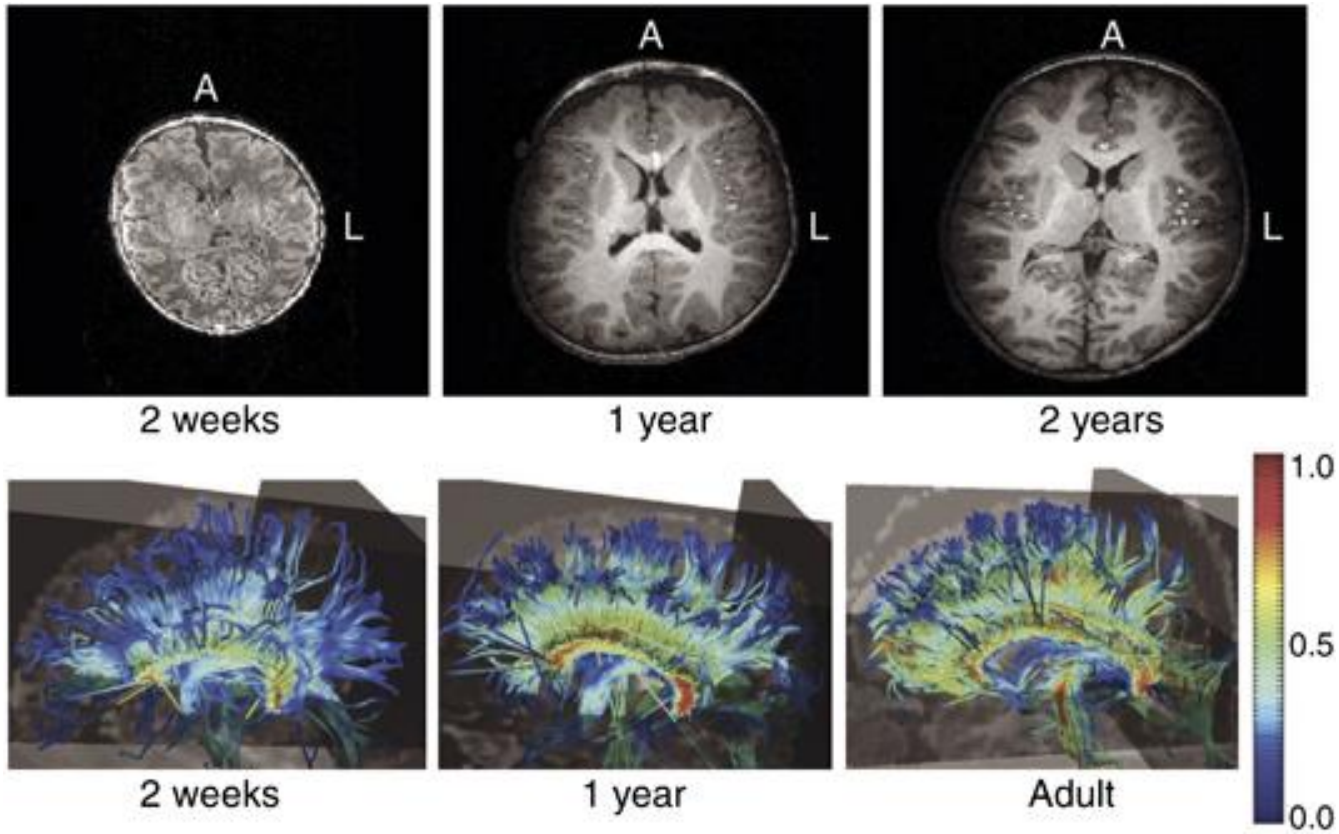
Lauritsen et al 2001, [1]Wang et al. 2003

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



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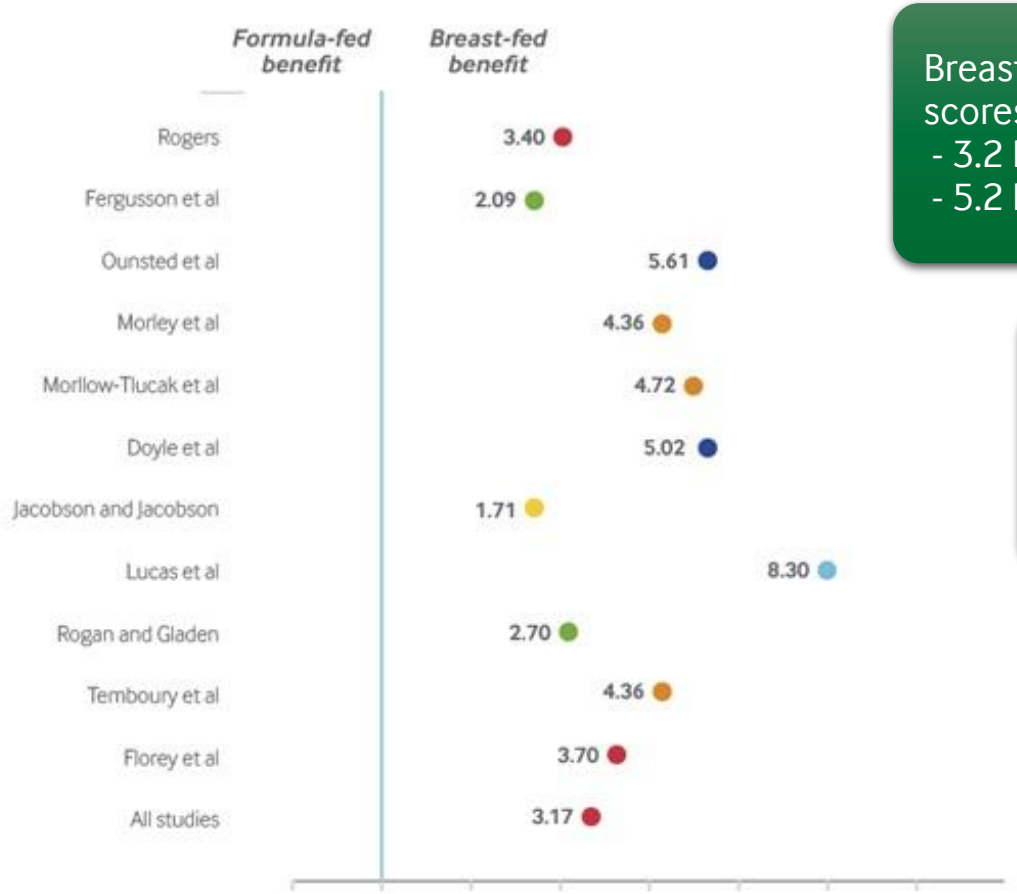
Tau et al 2010, Andersen et al 2003

Cognitive development

Meta-analysis



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

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Anderson et al. 1999, Michaelsen et al. 2009

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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 .....

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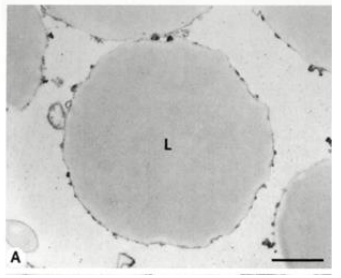
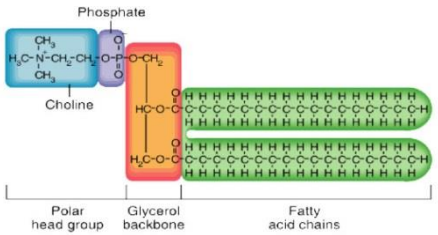
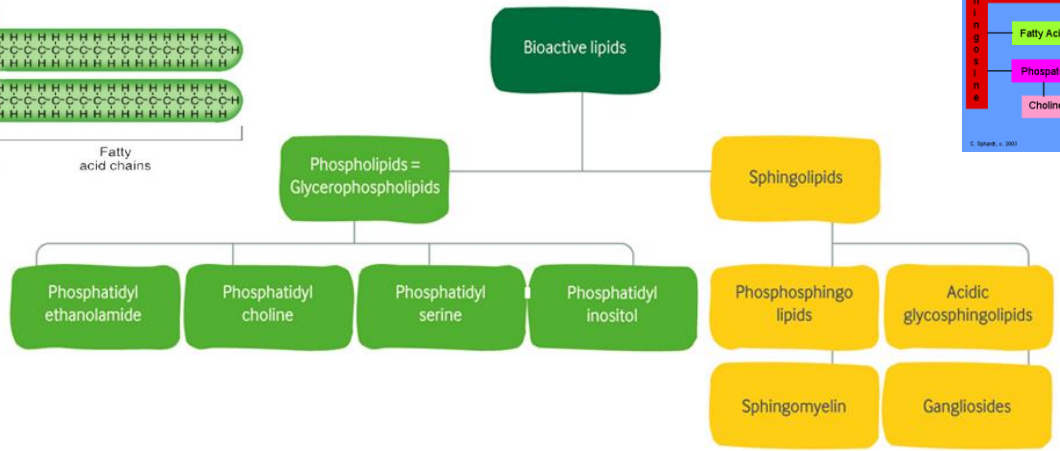
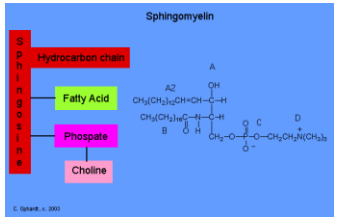
Milk Lipids
- In brief

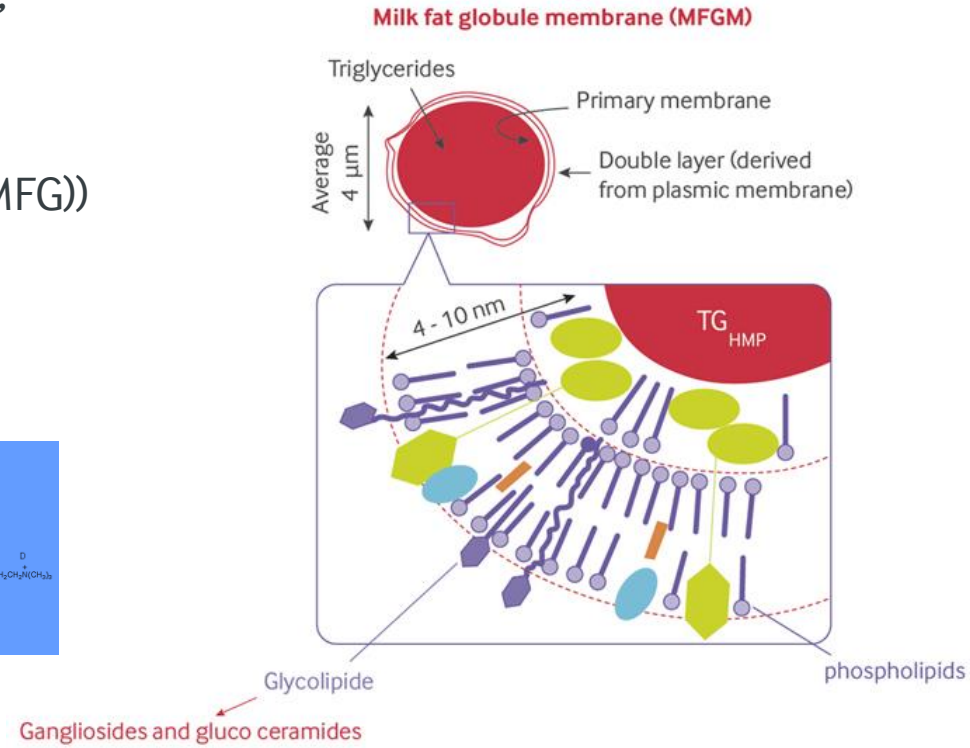


- Lipids constitute a group of naturally occurring molecules including fats, sterols, triglycerides, phospholipids and others
- Major source of energy for the infant
- Occurs as globules emulsified in the aqueous phase (milk fat globules (MFG))
- MFG contains a core of triglycerides (98 %) surrounded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









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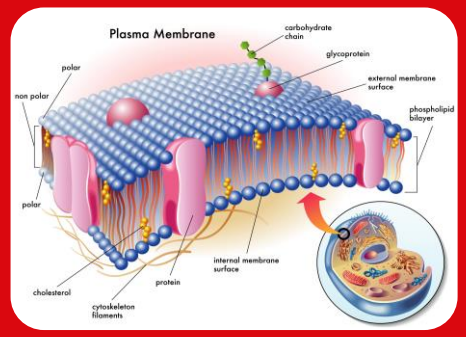
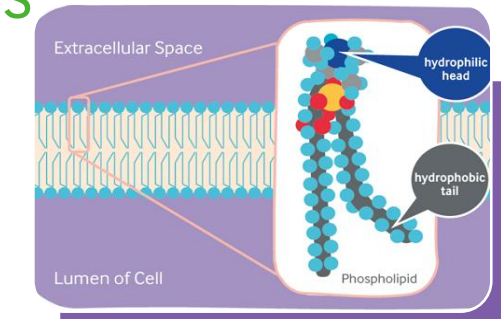
Bioactive Lipids

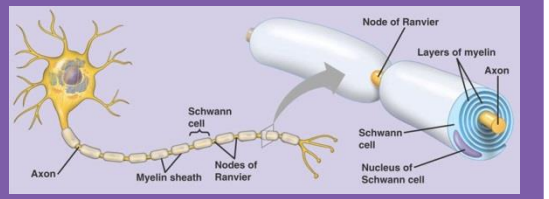
- Occurence and sources



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

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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

% distribution of milk lipids in different sources (% of total milk lipids)	Human milk[1]	Bovine Milk[1]
Sphingomyelin (SM)	29.7	19.9
Phosphatidylcholine (PC)	24.5	28.7
Phosphatidylethanolamine (PE)	18.3	31.4
Phosphatidylserine (PS)	8.1	11.2
Phosphatidylinositole (PI)	3.8	3.6
Others	13.9	4.5
Gangliosides		
GD3 (mg/L)	3.8 - 0.9[2]	3.3 - 2.4[3]
GM3 (mg/L)	4.3 - 9.8[2]	0.98 - 0.15[3]

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.

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Zeisel et al. 1986, Vance et al. 2000, Hirabayashi et al. 2008, [1]Garcia et al 2012, [2]Giuffrida et al. 2014, [3]Lee et al. 2013

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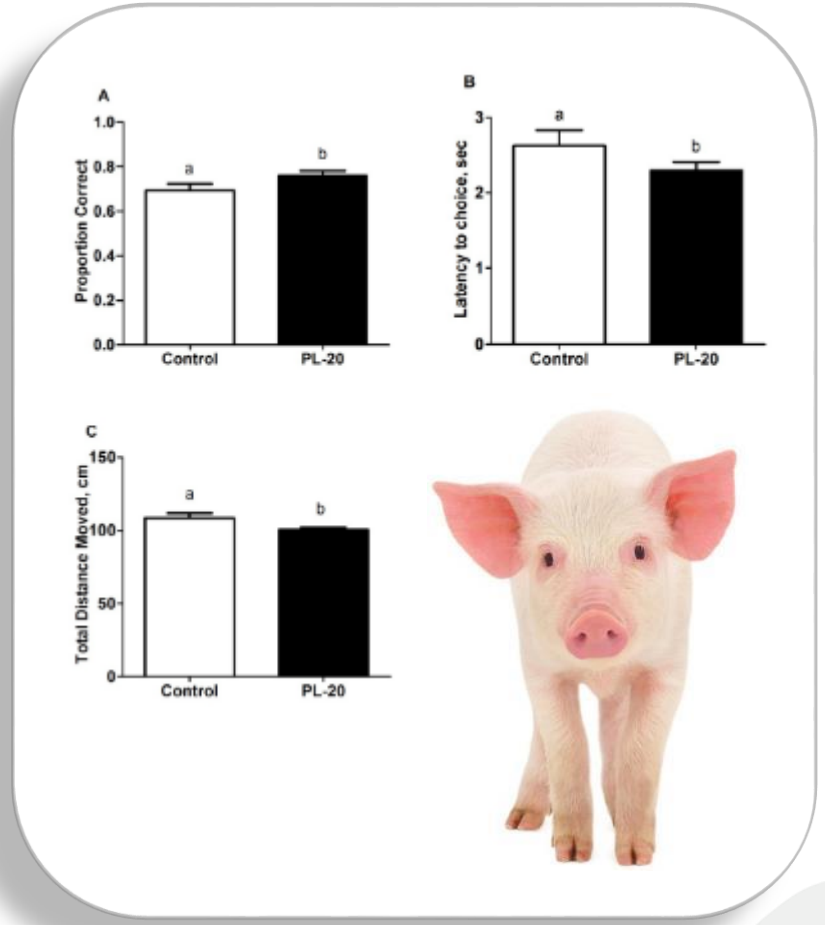
Effect of dietary phospholipids in a piglet study

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



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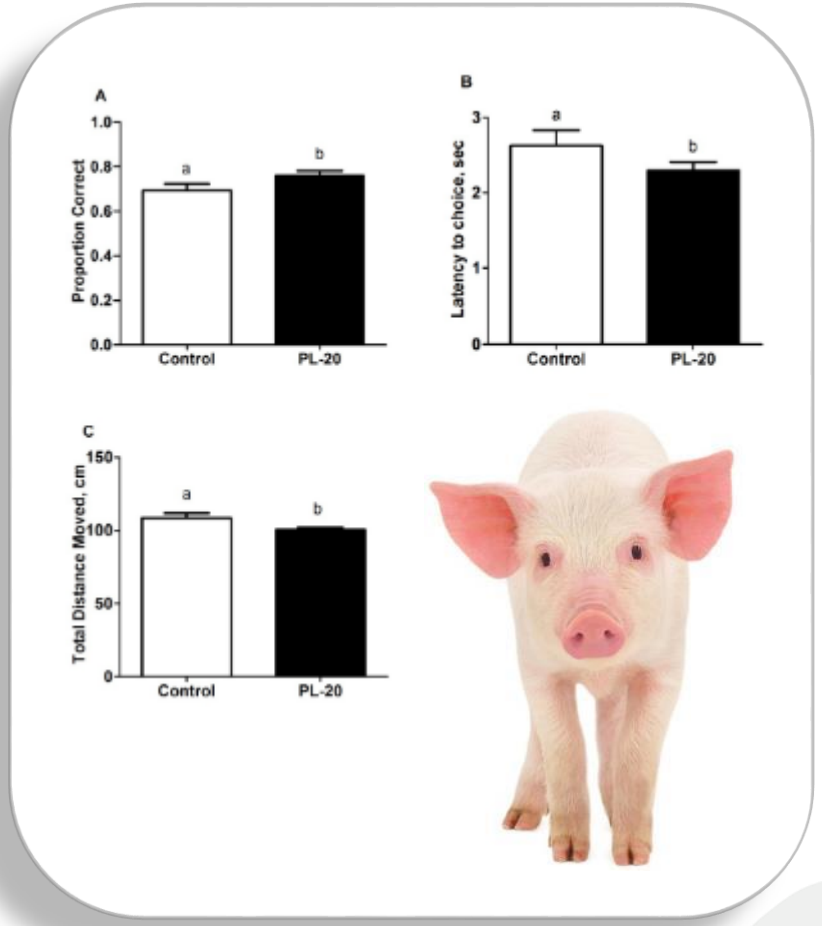
Liu et al. 2014

Effect of dietary phospholipids in a piglet study



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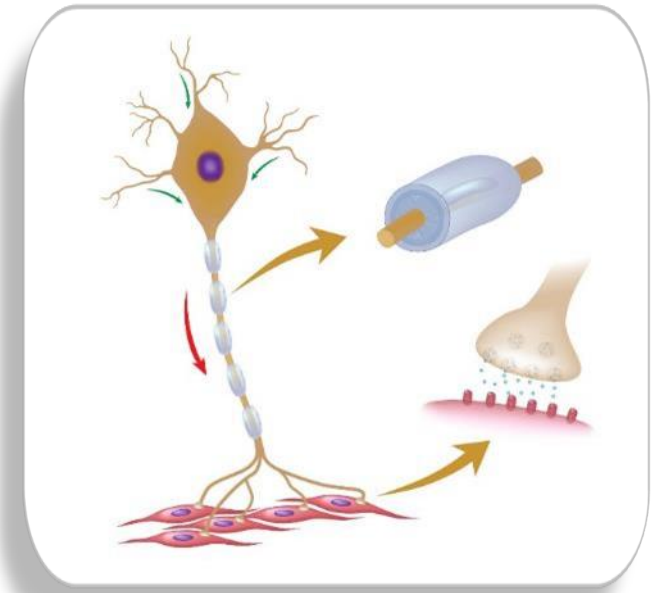
Liu et al. 2014

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

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

Effect of dietary sphingomyelin on brain, myelin weights and myelin lipids

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

Values are the mean ± SEM ($n = 6$).

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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

Groups	SM % of total PL	Number of infants
Standard formula	13	12
Experimental formula	20	12

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

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Tanaka et al. 2012

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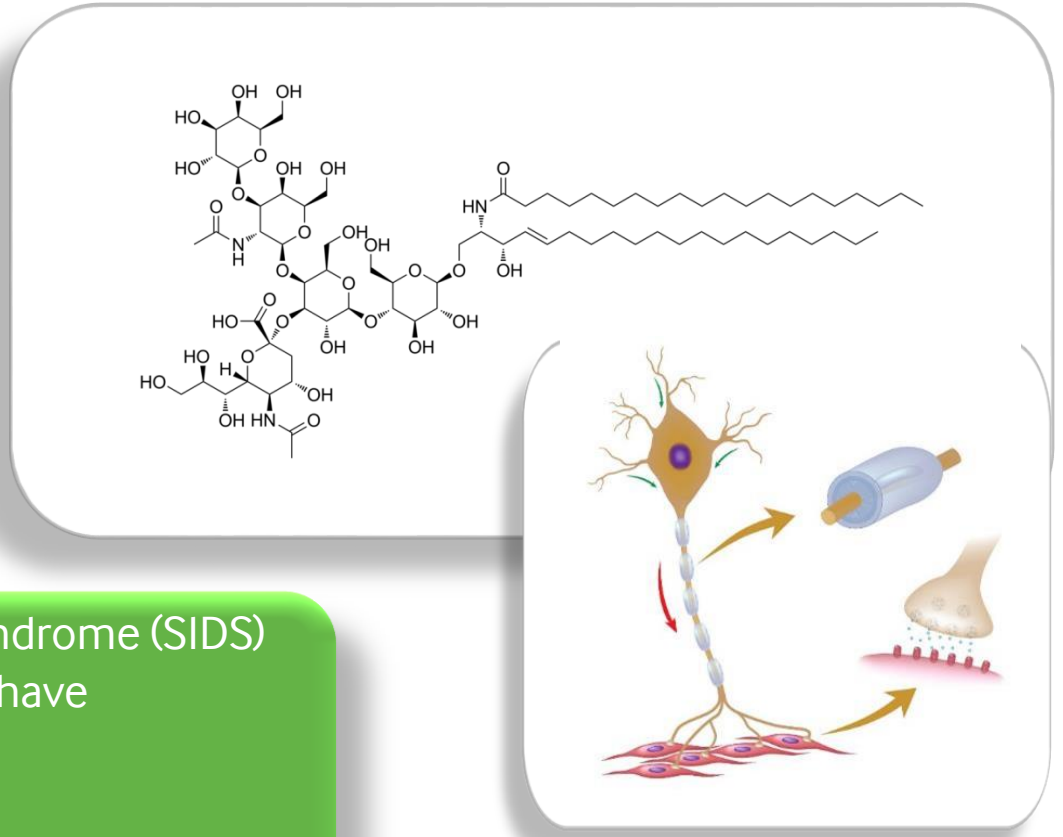
Gangliosides
Major constituents of the brain

- 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

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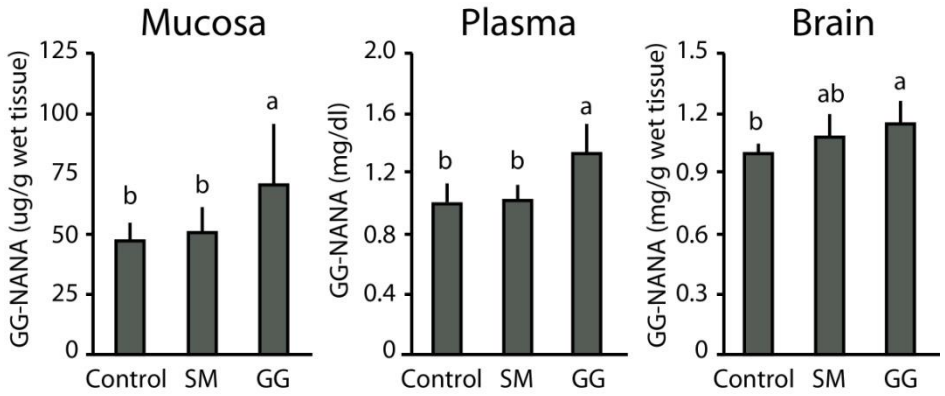
[1]McJarrow et al. 2009, [2]Ledeen et al. 1982, [3]Wang et al. 2009, [4]Palmano et al. 2015, [5]Gurnida et al. 2012

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)

Groups	Ganglioside µg/ml	Number of infants
Breastfed	3.4-16.2	40
Standard formula	7.5	35
Supplemented formula	11-12	35

Griffith Scale outputs of 6 month-old infants in treatment group, control group and reference group. Data are presented as means of raw scores and 95% confidence intervals.

Griffith Scale	P-value	Supplemented formula[a] (treatment group) (n = 29)	Standard formula[a] (control group) (n = 30)	Breast-fed[b] (reference group) (n = 32)
Locomotor IQ	0.225	120.0 (114.3–123.2)	117.2 (111.1–123.2)	113.7 (110.9–116.5)
Personal–social IQ	0.368	121.2 (115.1–127.4)	119.0 (112.5–125.5)	115.4 (112.0–118.8)
Hearing and speech IQ	0.114	120.3 (114.7–126.0)	116.7 (110.7–122.7)	115.1 (112.1–118.1)
Hand and eye coordination IQ	0.006	129.5 (123.0–136.0)	122.0 (115.1–128.9)	123.9 (120.3–127.6)
Performance IQ	<0.001	131.1 (125.7–136.5)	123.2 (117.5–128.9)	127.8 (124.9–130.8)
General IQ	0.041	125.4 (119.7–131.1)	120.6 (114.6–126.7)	120.0 (116.8–123.2)

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

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Gurnida et al. 2012

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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

Results of testing with the Bayley Scales of Infant and Toddler Development, Third Edition, for the EF, SF, and BFR groups[1]

				P (adjusted *P*)[2]		
	EF (n = 71)	SF (n = 64)	BFR (n = 70)	EF compared with SF	EF compared with BFR	SF compared with BFR
Cognitive	105.8 ± 9.2[3]	101.8 ± 8.0	106.4 ± 9.5	0.008 (0.008)	0.73 (0.35)	0.003 (0.029)
Motor	98.6 ± 9.3	98.2 ± 9.0	100.2 ± 7.2	0.81 (0.80)	0.25 (0.24)	0.16 (0.34)
Fine motor[4]	9,69 ± 1.55	9.77 ± 1.63	10.24 ± 1.27	0.78 (0.93)	0.022 (0.20)	0.060 (0.37)
Gross motor[4]	9.72 ± 2.39	9.58 ± 1.93	9.76 ± 1.92	0.71 (0.80)	0.92 (0.41)	0.59 (0.48)
Verbal	102.6 ± 10.4	102.5 ± 8.9	106.7 ± 10.7	0.93 (0.92)	0.022 (0.025)	0.014 (0.029)
Receptive[4]	10.48 ± 2.61	10.41 ± 2.28	11.47 ± 2.53	0.86 (0.88)	0.023 (0.029)	0.012 (0.017)
Expressive[4]	10.51 ± 1.66	10.53 ± 1.41	10.76 ± 1.57	0.93 (0.97)	0.36 (0.26)	0.39 (0.48)

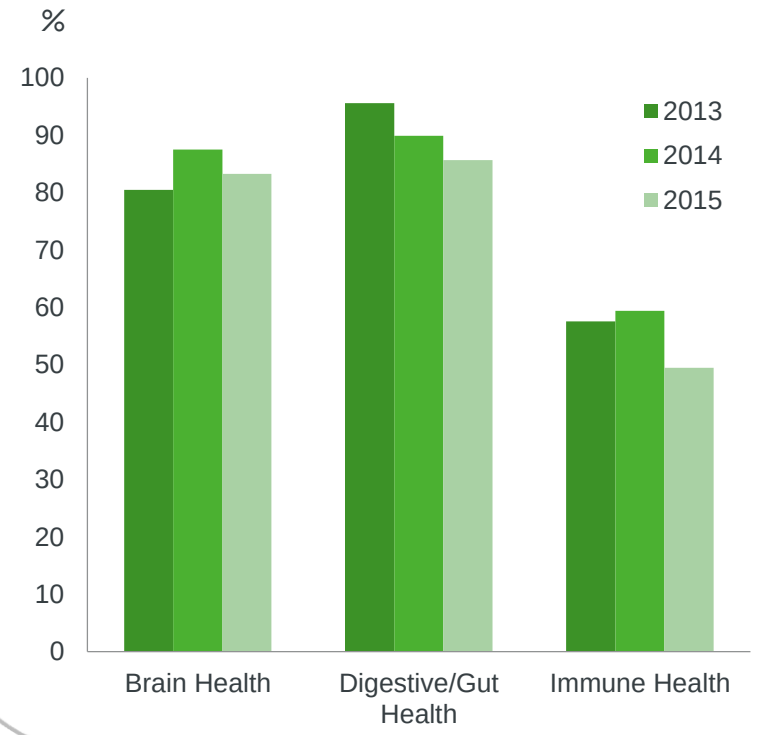
MFGM-10 supplementation to infant formula narrows the gap in cognitive development between breastfed and formula fed infants

Groups	PLs mg/100 ml	Number of infants
Breastfed (BFR)	30	72
Standard formula (SF)	30	68
Experimental formula (EF)	70	73

Market insight
Focus on cognition

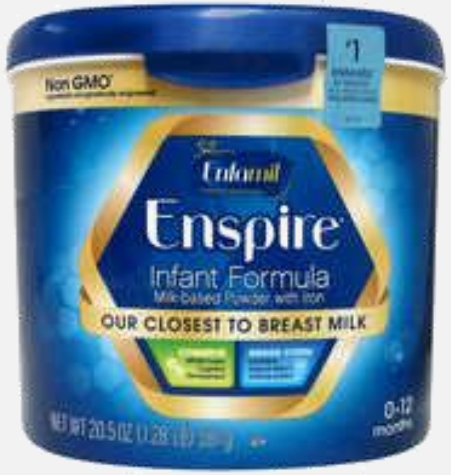
- Parents are constantly focused on their infants early development and the early nutritional impact on areas like brain development
- Therefore, when launching new products focus continues to be on cognitive development

New product launches for infant formula (0-1 year) containing most commonly used health claims



Source: Innova

Product examples



On pack claims:

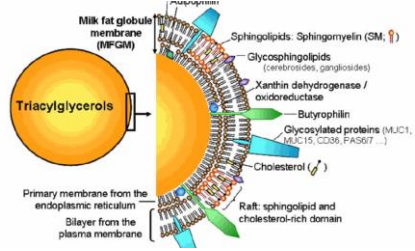
..... MFGM brain nourishing lipids also found in breast milk, designed to support mental development. MFGM to foster cognitive development.....

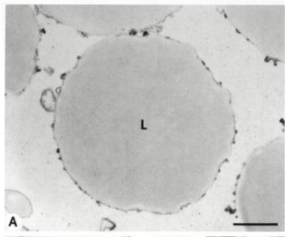
On pack claims:

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.

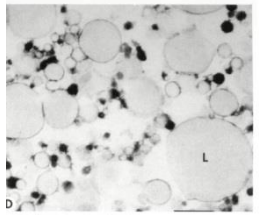
InfantBRAIN research project

2016-2020 Principal Investigator: Lars Hellgren, DTU – supported by DDRF and "Innovationsfonden"





MFG: 1-10 µm
Emulsifier: MFGM



EM micrographs from Armand *et al* 1996

Lipid droplets: 0.1-0.8 µm
Emulsifier: Milk proteins&Soy lecithin

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

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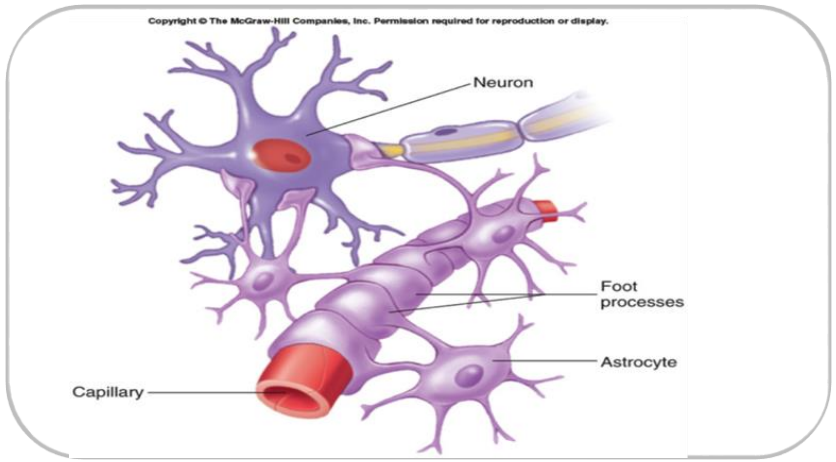
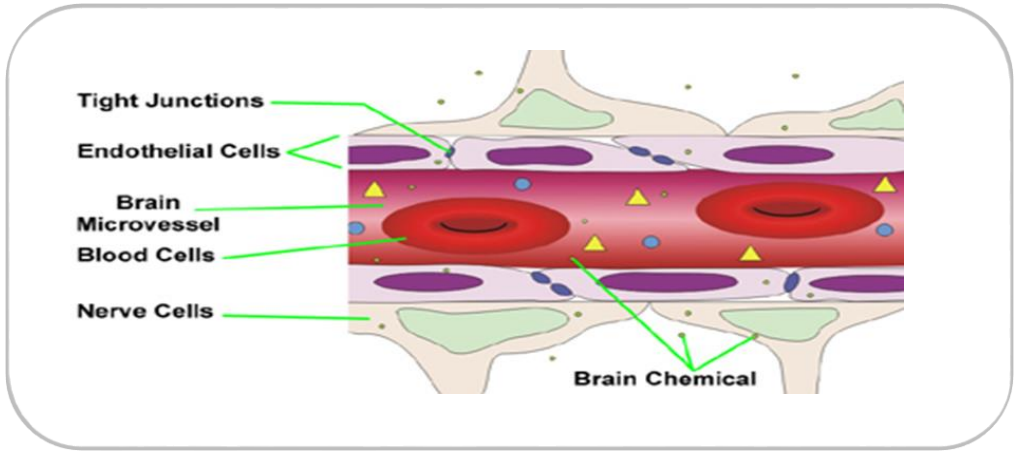
Blood Brain Barrier (BBB) and milk peptides

-integrity is important in dementia and Alzheimer's



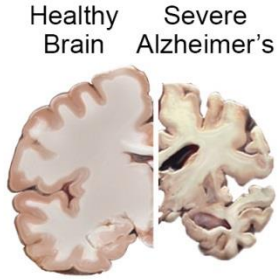


- The brain is protected by the skull, the cerebrospinal fluid and the blood brain barrier (BBB).
- The integrity of the BBB is essential for the resistance against infections and intoxications, but it is also important in the protection against age-related neurological pathologies like dementia and 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

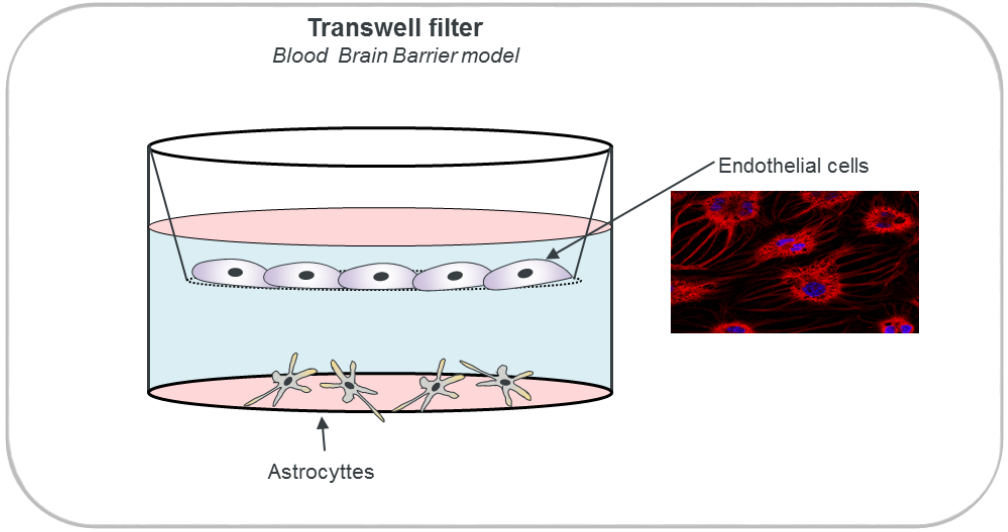
BRAIN MILK research project

2015-2017 Principal Investigator: Esben Skipper Sørensen, AU - supported by DDRF

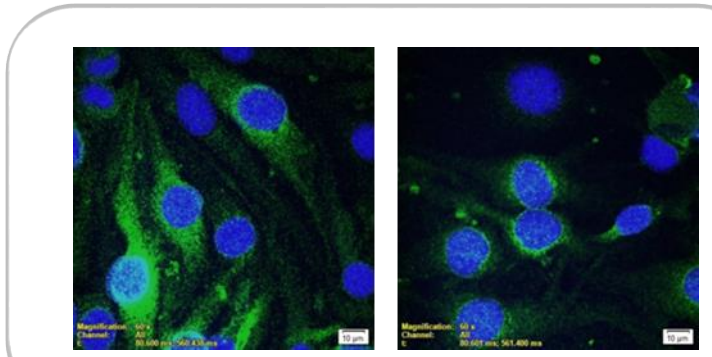


Increased ERK phosphorylation and "leaking" blood-brain barrier are correlated with dementia and Alzheimer's ... and also seen in athletes that receives blows to the head

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 potentiallysupport brain function



Thank you for your attention!

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