Human Complex Milk Lipids: Concentrations, benefits and the implications for Paediatric Nutrition

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Overview

- Importance of structure in milk
- Some of the new insights lipidomics has brought
- Importance of lipidomics in health as well as disease.
Programme

• Structure of milk fat globule membrane
  – Secretion
  – Complex lipids

• Human breast milk complex lipids
  – Measurement by mass spectrometry
    • Gangliosides, phospholipids

• Benefits of milk fat globule membrane

• Comparison to infant formula
Milk fat globule structure and function: nanoscience comes to milk production

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The milk fat globule membrane (MFGM)
Secretion of coalescing microlipid droplets

Structure of the human milk fat globule membrane (MFGM).

Complex lipid structures

Polar head group
- Glycerol
- Sphingosine

Alcohol
- Glycerol: Phosphate
- Sphingosine: Phosphate

Fatty acid chains

Glycerophospholipid
- Sphingomyelin
- Glycosphingolipid

Phospholipids
- Sphingolipids

Note similarities in the shape of the molecules
Glycosphingolipid structures

- **Polar head group (Sugars)**
  - NANA, sialic acid
  - Glucose
  - Galactose

- **Alcohol**
  - Sphingosine
  - Lactosylceramide

- **Fatty acid chains**
  - GM3
  - GD3

**Gangliosides**
Human breast milk complex lipids
HPLC-MS analysis of Complex Lipids

• HPLC separation (amino-propyl column).
  – Separation based on polar head group

• High Resolution Mass spectrometry-accurate mass extraction\(^1\)
  – Quantification and characterisation GA

• Multiple reaction monitoring (MRM)\(^2, 3\)
  – Quantification of GA

• Neutral loss/Precursor ion monitoring\(^4\)
  – Quantification of glycerophospholipids and sphingomyelin

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MS analysis of membrane proteins

• Membrane proteins were quantified using the absolute quantification (AQUA) technique

• This was adapted for simultaneous analysis of the six most abundant MFGM proteins
  – fatty acid binding protein, butyrophilin, PAS 6/7, adipophilin, xanthine oxidase, and mucin by unique peptides after enzyme cleavage


Detailed studies of human breast milk

When examining the data on breast milk, we observed a significant increase in the concentration of certain lipids. These findings have important implications for infant nutrition and health. In this study, we explore the lactational changes in concentration and distribution of ganglioside molecular species in human breast milk from Chinese mothers.

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Determination of ganglioside concentrations in breast milk and serum from Malaysian mothers using a high performance liquid chromatography-mass spectrometry-multiple reaction monitoring method

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Gross composition

- Gross composition of the breast milk was also measured especially the fat content.
- Much of the literature has expressed the complex lipid as a percentage of total lipid, rather than of total breast milk.
- However, the fat content in breast milk has very large variation.
MS² spectra HBM
GM3 m/z 1179.8469 d36:1 [M-H]−

![Graph showing MS² spectra for GM3 with m/z values and their corresponding intensities.](image-url)
MS² spectra HBM
GD3 m/z 762.9476 d40:1 [M-2H]²⁻
Range of molecular species for GM3 and GD3

Blue line shows the distribution of the amplitude of the molecular species
HBM
Sphingomyelin and Phosphatidylserine

- These two phospholipids are abundant in human milk
Changes in gangliosides concentration during lactation
Molecular species of GM3 and GD3
GM3 and GD3 species changes over lactation (individual molecular masses)

**Black** is relative concentration

**Red** is the absolute concentration

For instance the trend in the species within the group are not the same and often quite divergent.

Dynamic nature of the composition during lactation
Benefits of MFGM Components
Benefits of MFGM

• MFGM membrane is more than just a physical barrier between fat and the aqueous solution.
• The complexity of the structure suggests this...
Complex lipids have multiple benefits delivered compositionally and structurally

**Brain development**

Naturally occurring complex lipids are essential for young brain development
- Essential component of neural cell membranes
- Promote neuronal migration and differentiation
- Dietary sources may play a role in supplying to the foetus and neonate

**Growth & development**

Complex lipids assist maturation of the guts nutritional absorption function
- Promote mucus layer integrity
- Promote tight junction formation and maintenance

**Immunity (including allergy)**

Complex lipids aid in optimising the developing immune system
- Balances lymphocyte populations and promotes other immune cell function maturation
- Promotes good balance of gut microflora

**Anti-infection**

Complex lipids provide protection against pathogens
- Inhibit binding of pathogens to mucus and enterocytes
- Promote anti-infective gut microflora
THE BRAIN IS RICH IN COMPLEX LIPIDS

- Phospholipids
  - 32% of myelin (nerve coating)
  - 20% of white matter
  - 20% of grey matter

- Gangliosides increase as the infant ages to make up about 1% of the brain.
Infant formula Composition
Similarity of MFGM in Bovine and Human Milk

Comparison of Phospholipid species (as % of total PL) between different sources

- Phosphatidylcholine (PC)
- Phosphatidyethanolamine (PE)
- Phosphatidylinositol (PI)
- Phosphatidylserine (PS)
- Sphingomyelin (SM)

Gangliosides in milk

- Bovine milk provides the closest phospholipid (PL) profile to human breast milk
- Bovine milk provides the only viable source of gangliosides (GA)

Complex lipids in Infant formula

• Infant formula has the milkfat removed and replaced by vegetable oil and so low in MFGM components

• Infant formula does contain some phospholipids due to phospholipids carried through with the skim and whey
  – Also there are phospholipids from added lecithin

• However in comparison to human milk, infant formula is particularly lower in the sphingomyelin and phosphatidylserine, the two phospholipids missing from lecithin

• Thus there is a gap in these phospholipids between infant formula and human milk

• For the same reason gangliosides are lower in infant formula and because there are no gangliosides in lecithin the gap is larger
Fat in infant formulas and milk

• **Current formulas**
  – Protein stabilised droplets

• **Human milk**
  – Phospholipids
  – Complex membrane
  – Complete package
Effect of droplet coating on lipolysis

The effect of lactoferrin on physical changes in phospholipid stabilised emulsions during neonatal in vitro gastric digestion: Does synergism of pepsin and lipase promote lipolysis in protein-stabilised emulsions?
Food Hydrocolloids Volume 43, January 2015, Pages 785-793
doi:10.1016/j.foodhyd.2014.08.010
Fat droplets in milks

Human milk

Cow milk

Formula milk
Supplementation of infant formula
Published clinical trials
Cognition

Pilot study: Supplemented formula fed infants at 24 weeks had a cognitive Griffith score closer to breast milk group than the control infant formula group.
Cognition


Neurodevelopment, nutrition, and growth until 12 mo of age in infants fed a low-energy, low-protein formula supplemented with bovine milk fat globule membranes: a randomized controlled trial\(^1\)\(^–3\)

*Niklas Timby, Erik Domellöf, Olle Hernell, Bo Lönnerdal, and Magnus Domellöf*

MFGM supplementation to infant formula narrows the gap in cognitive development test (Baileys) between breastfed and formula-fed infants at 6 months (26 weeks)
Febrile episodes

Regular consumption of formula enriched with a MFGM product by preschool children was safe, well tolerated, and, based on per-protocol analysis, is associated with a significant decrease in the number of short febrile episodes and leads to improved behavioral regulation.
Summary
Summary

• Human MFGM has a complex structure
  – More than just a coating for the fat to separate it from the aqueous solution
    • Range of functions and benefits

• Infant formula has a lower concentration of the MFGM components
  – Skim and whey are the main dairy components

• Future of infant formula
  – Infants of mothers that are unable to breastfeed may be able to get more of the benefits of breastfeeding by increased concentrations of MFGM components
Thank you