Applying MFGM and Human Milk Science to Improve Nutrition for Infants and Toddlers

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Disclosures

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<tr>
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Learning Objectives

Describe how dairy-processing advances have isolated MFGM for supplementation to infant formula

Examine how purified MFGM from cow’s milk added to formula is similar in composition and bioactivity to human milk

MFGM, milk fat globule membrane.
Underutilized Dairy Components

• Lactose

• Minerals and minor components in milk, whey, and permeate

• Milk fat and phospholipids
  – Today, MFGM is what WHEY was in the ’80s

• To learn about processing advances, we need to know about MFGM origin, structure and composition
Ancel Keys on the cover of *TIME* magazine in 1961. He claimed that saturated fats in the US diet damaged arteries and led to coronary disease.

*TIME* magazine cover story in 2014. Scientists were wrong about saturated fats. They are not linked to higher risk for heart disease.
So... What Does MFGM Have to Do With Nutrition?

• Is MFGM similar to human and cows’ milk?

• What is the contribution of phospholipids to MFGM structure?

• How do we obtain MFGM for use in foods?

• What about inclusion in infant formula?
MFGM Components Found in Human and Bovine Milk and Their Potential Impact

Brain Function
- Choline
- Sphingomyelin
- Gangliosides
- Cholesterol
- Sialic acid
- Inositol
- Cerebrosides

Immune Defense
- Mucins
- Butyrophilin
- Lactadherin
- CD14
- TLR1
- TLR4
- Xanthine oxidase

CD14, cluster of differentiation 14; TLR, toll-like receptor.

Origin of MFGM

What is the Milk Fat Globule Membrane?

Lipid Droplet

MFGM, milk fat globule membrane.

APM, apical plasma membrane; BPM, basal plasma membrane; CLD, cytoplasmic lipid droplet; CR, cytoplasm residue; CM, casein micelle; GA, golgi apparatus; LD, lipid droplets; MLD, micro lipid droplets; RER, rough endoplasmic reticulum; SV, secretory vesicle.

Adapted from Mather and Keenan (1998).
Diagram of Synthesis and Secretion of Milk Constituents From Apical Membrane of the Epithelial Cell of a Mammary Gland

LD, lipid droplets; LG, lipid globules; MFG, milk fat globules; MP, milk protein, GA, golgi apparatus; GM, globule membrane.

Kinetics of milk lipid droplet transport, growth, and secretion revealed by intravital imaging: lipid droplet release is intermittently stimulated by oxytocin

Andrius Masedunskas, Yun Chen, Rebecca Stussman, Roberto Weigert, and Ian H. Mather

Laboratory of Cellular and Molecular Biology, Center for Cancer Research, National Cancer Institute, and Intracellular Membrane Trafficking Section, National Institute of Craniofacial and Dental Research, National Institutes of Health, Bethesda, MD 20892; Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742

Kinetics of Milk Lipid Droplet Transport, Growth, and Secretion Revealed by Intravital Imaging (cont)

The GO indicate that most conserved genes belong to the metabolic pathway for fat globules and MFGM production.

GO, gene ontology.
Industrial Processing to Obtain MFGM

- From cream
- From butter and buttermilk
- From whey
- Thermal treatments
Buttermilk Process

Cream

Buttermilk
31 million lb condensed/evap.
49 million lb dry

Butter
1.8 billion lb

Buttermilk From Cream or Whey
Separation of Cream From Skim Milk

Surplus standardized cream

Standardized milk

Whole milk

Skim milk

Control of cream fat content

Flow measurement

Flow measurement of remix cream

Flow measurement
Continuous Butter and Buttermilk Production
Spray Drier

To assay

Retentates from microfiltration

Products
Transmission Electron Micrograph of Buttermilk

Photo: Beth Fryksdale and R. Jiménez-Flores, 1998
MFGM From Whey

Ultrafiltration

Diafiltration Buffer  Retentate return  Valve to apply pressure  Retentate pressure

Feed Tank  Feed pressure

Feed Pump  Filtration Module  Filtrate stream

See https://www.youtube.com/watch?v=qEJVK0hhA0w

Adapted from EMD Millipore Technical Brief. No. TB032.
(A) Effect of continuous diafiltration on the conductivity of 5 x whey (□) and the extent of precipitation of MFGM at pH 4.2 (○). Other experimental conditions were as described in publication. Absorbance values were measured without dilution. The bars represent standard deviation (n=2). (B) Clarity of the supernatants as function of fold-diafiltration.
Laboratory and Industrial Scale Ultrafiltration

Laboratory

Pilot Plant

Industrial Scale
Confocal Laser Fluorescence Microscopy
CLSM to Probe Temperature Effects

**Figures:** Confocal laser scanning microscopy (CLSM) images of raw cream fat globules stained with Rd-DOPE and cooled at 4°C (left) or heated at 45°C for 10min (right)
Confocal Microscopy of MFGM and Bacterial Binding

Comparison

**Sphingolipids (30%)**
- Glucosylceramide (Glucer)
- Lactosylceramide (Lacer)
- Sphingomyelin (SM)

**Phospholipids (70%)**
- PE – Phosphatidylethanolamine
- PI – Phosphatidylinositol
- PS – Phosphatidylserine
- PC - Phosphatidylcholine

Unpublished results.
Microdomains Form in the Outer Leaflets of Biological Membranes

• Formation of disordered (Ld) and ordered (Lo) lipid phases

• Sphingomyelin (SM) and cholesterol rich

• Localization of glycated lipids likely in outer leaflet, but still debated

Lipid Composition of the MFGM

| Table 2. Content of main PLs in milk fat of different mammalian species. Values are expressed as percentage of total PLs |
|---|---|---|---|---|---|
| Species  | PE  | PI  | PS  | PC  | SM  |
| buffalo   | 24.5| 19.7| 6.6 | 24.3| 24.9|
| goat      | 31.7| 6.3 | 8.3 | 28.5| 25.2|
| ewe       | 34.4| 4.4 | 5.2 | 28.6| 27.4|
| human     | 21.3| 16.4|     | 19  | 43.3|
| donkey    | 60.2| 2.4 | 11.2| 17.3| 8.8 |
| mare      | 24.3| 8.5 | 10.6| 27.8| 28.9|
| human     | 21.7| 4.5 | 9.6 | 29  | 35.2|
| camel     | 34.3| 4.9 | 10.5| 22.1| 28.1|

| Table 3. Polar lipid content (g/100 g of fat) and phospholipids composition (percentage of total PLs) in milk fat of dairy products and by-products of butter-making process. |
|---|---|---|---|---|---|
| Matrix        | Polar lipids | PE  | PI  | PS  | PC  | SM |
| cream          | 0.86          | 42.7| 6.8 | 7.2 | 14.6| 28.6|
| butter         | 0.2           | 31  | 11.9| 15.3| 24.7| 17.1|
| buttermilk     | 4.49          | 33.5| 2.4 | 10.3| 35.5| 18.3|
| cow cream      | 0.17          |     |     |     |     |     |
| cow buttermilk | 0.17          | 38.7| 9.3 | 9.1 | 23.9| 18.9|
| cow butter serum| 0.88        | 27.2| 10.8| 7.2 | 29.8| 24.9|
| goat cream     | 0.2           |     |     |     |     |     |
| goat buttermilk| 0.19          | 35.2| 9.8 | 9.9 | 24.8| 20.3|
| goat butter serum| 1.01         | 27.1| 11.7| 8.2 | 26.2| 26.8|
| cream          | 5.65          | 26.7| 7.5 | 11.7| 26.5| 20.8|
| butter         | 5.31          | 17.1| 15.4| 11.3| 33.7| 21.8|
| buttermilk     | 12.4          | 17  | 7.1 | 8.1 | 46.1| 21.7|
| buttermilk     | 8.4           | 8.2 | 4.6 |     | 51.2| 27.6|

PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.
PF, P-face; EFeq, E-face equivalent.
Phospholipid and SM Digestion/Absorption

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Image Description</th>
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<tr>
<td>30 min</td>
<td>Microscopic view of phospholipid and SM digestion.</td>
</tr>
<tr>
<td>120 min</td>
<td>Further progression of digestion observed.</td>
</tr>
<tr>
<td>180 min</td>
<td>Final stage of digestion and absorption.</td>
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SM, sphingomyelin.

• Capture Images of MFGM
• Observe the distribution of Phospholipids and Glycoproteins

Photos courtesy of Johnny Vo
Buttermilk Powder Granule—Morphology

Untreated sample – 150 x confocal/laser

350 bar, 75°C – 150 x confocal/laser

Unpublished results.
Buttermilk: More Than a Source of Milk Phospholipids

Membrane-Rich Milk-Fat Diet Provides Protection Against Gastrointestinal Leakiness in Mice Treated With Lipopolysaccharide

Figure 1. A $2 \times 2$ experimental design of treatments was used for each time point (24 and 48 h). Mice ($n=24$) were randomly assigned to one of the following treatments: 1) control diet [American Institute of Nutrition (AIN)-76A], saline vehicle control injection ($n=6$); 2) control diet, LPS injection ($n=6$); 3) AIN-76A + milk fat diet, saline vehicle control injection ($n=6$); or 4) AIN-76A + milk fat diet, LPS injection ($n=6$).

Figure 2. Effect of experimental diets on consumption (A) and total weight gain (B). Values are means ($n=36$ control diet, $n=33$ milk fat diet) $\pm$ SD. Experimental diets did not significantly affect consumption or weight gain.

Membrane-Rich Milk-Fat Diet Provides Protection Against Gastrointestinal Leakiness (cont)

Although the diets used in this study were formulated to differ only in the fat source, the use of a complex material like MFGM makes it impossible to associate the stress protection with any single component.

Conversely, MFGM is hypothesized to contain many constituents with beneficial bioactivity (Spitsberg, 2005) and is available as an ingredient to potentially modulate the barrier properties of the gut without the necessity of isolating specific components.

LPS, lipopolysaccharide.

MFGM Supplementation in Formula Modulates the [Rat] Neonatal Gut Microbiome and Normalizes Intestinal Development

Figure 1. MFGM supplementation in formula normalizes intestinal architecture in a dose-dependent manner without affecting body weight at postnatal day 15.

MFGM supplementation protects the formula fed neonate intestine from *C. difficile* toxin-induced damage.
While Control diet (CTL) formula yielded significant deficits in intestinal development as compared to Mother’s Milk (MM) littermates, addition of MFGM to formula restored intestinal growth, Paneth and goblet cell numbers, and tight junction protein patterns to that of MM pups.

Supplementary Figure 2: MM, 6 g/L MFGM and CTL formula fed pups display similar CA-1 staining at small intestinal sites. Representative immunostaining of enterocyte marker CA-1 (red) and DNA (blue) in the jejunum (A) and ileum (C) of pups at pn day 15, semi-quantified for fluorescence intensity of CA-1 relative to DAPI in (B) and (D). n>5. The graphed data presented are the mean ± SEM. Original Magnification: 200X.

CTL, control; MM, Mother’s milk; MFGM, milk fat globule membrane
Dietary Prebiotics and Bioactive Milk Fractions Improve NREM Sleep, Enhance REM Sleep Rebound and Attenuate the Stress-Induced Decrease in Diurnal Temperature and Gut Microbial Alpha Diversity

![Graph showing colony-forming units](image)

**FIGURE 2 |** Test diet increases *Lactobacillus rhamnosus*. Data demonstrating, in a subset of rats, that consumption of the test diet significantly increased levels of *Lactobacillus rhamnosus* in fecal cultures at PND 52, when compared with those eating the control diet (#p < 0.05 vs. control diet).
Dietary Prebiotics and Bioactive Milk Fractions Improve Sleep

**Conclusion:**
The bioactive milk fractions, Lf and MFGM, not only help promote growth of beneficial gut microbial species (Chatterton et al, 2013; Timby et al, 2015), but they may affect brain function directly.*

Mimicking the Human MFGM Structure

Fig. 2. 2D (A and B) and 3D (C and D) CLSM images of HM. (A) The milk fat globules were stained with Nile Red (Red) and Fast Green FCF (green). (B) The MFGM was stained with Rd-DOPE: the yellow arrows are pointing at $I_0$ domains which are rich in sphingomyelin and cholesterol and tightly packed, preventing the insertion of the fluorescent Rd-DOPE. (C) The milk fat globules were stained with Nile Red (red) and the MFGM glycoproteins and glycolipids with WGA (green); the white arrows are pointing at globules showing fluorescence from the lectin outside of the $I_0$ domains, indicating that glycoproteins and glycolipids are not located inside the $I_0$ domains. (D) The milk fat globules were stained with filipin; the white arrows are pointing at round spots of filipin–cholesterol fluorescent complexes; filipin reorients cholesterol molecules in the membrane plane, which may affect the distribution of cholesterol within the MFGM. However, the filipin–cholesterol complexes followed a similar pattern to that of the $I_0$ domains. Scale bars: (A) 10 μm; (B) 5 μm; (C) $X=0–100$ μm; (D) $X$ and $Y=0–50$ μm.
Fig. 3. 2D (A and B) and 3D (C and D) CLSM images of Concept IMF. (A) The fat droplets were stained with Nile Red (Red) and Fast Green FCF (green); the yellow arrows are pointing at dense patches of interfacial proteins. (B) The surface of the fat droplets was stained with Rd-DPPE; as a fluorescent phospholipid analogue, Rd-DPPE indicates the location of phospholipids in a sample; the yellow arrows are pointing at fat droplets with a stained surface. The surface of all droplets showed a phospholipid coating. (C) The fat droplets were stained with Nile Red (red) and the interfacial glycoproteins and glycolipids with WGA (green); the white arrows are pointing at fluorescent patches of sugar residues from glycoproteins and/or glycolipids. (D) The fat droplets were stained with filipin; the white arrows are pointing at linear networks of filipin-cholesterol fluorescent complexes, indicating the presence of cholesterol at the surface of the droplets. Scale bars: (A and B) 10 μm; (C) X and Y = 0–140 μm; (D) X and Y = 0–100 μm.
Clinical Trials of MFGM

• **Sweden—Timby et al study:** n=160; <2 to 6 months

  A low-energy and low-protein infant formula with bovine MFGM or a standard formula was randomized until 6 months of age. Reference group: breastfed infants

  At 12 months, MFGM-supplemented group obtained significantly higher cognitive scores in Bayley-III compared to the standard formula group (105.8 ± 9.2 vs 101.8 ± 8.0, M ± SD)

  Scores of the MGFM group do not differ from breastfed group (106.4 ± 9.5)

  Decreased incidence of otitis media in infants <6 months

  No increased risk of skin reactions in the group supplemented with MGFM

**Study From Timby & Lönnnerdal**

MFGM, milk fat globule membrane formula supplemented formula; F, standard formula; MM, mother’s milk.

Study From Timby & Lönnerdal

MFGM+F, milk fat globule membrane formula supplemented formula; F, standard formula; MM, mother’s milk.

Infections Treated With Antibiotics or Hospitalization

Incidence 0–6 months

Note: Both EF and SF contained supplemental DHA.
MFGM+F, milk-fat globule membrane supplemented formula; F, standard formula; MM, mother’s milk.

Significance of the Bayley-III Score: What is the Gap in Past Studies?

- A meta-analysis examined the mean differences between breastfed and formula-fed groups for cognitive score.\(^1\)
- In multiple studies, the mean difference between groups was approximately 4 points—in line with the effect reported for MFGM.\(^2\)

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Adapted with permission of the *American Journal of Clinical Nutrition.*
Effect of Brown-Adipocyte Exosomes on Neural Growth

Effect of Dairy Phospholipids on Neuron Stimulation and Growth

A. Control cell growth
B. 1% Fetal Calf Serum
C. 75 µg PL
D. 150 µg PL

MFGM Key Points

- MFGM is a complex structure containing bioactive fats and proteins naturally produced in breast milk and bovine milk.
- Human and bovine MFGM are compositionally similar and share key bioactive components.
- Advances in processing technology allow isolation and concentration of MFGM from bovine milk.
- MFGM may bring formula composition and function closer to those of breast milk.
OPPORTUNITIES MULITPLY AS THEY ARE SEIZED

SUN TZU