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Title	Dairy Foods: A Matrix for Human Health and Precision Nutrition —The relevance of a potential bioactive ingredient; The milk fat globule membrane
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Publication date	2025
Original Citation	Wilmot, L., Miller, C., Patil, I., Kelly, A. L. and Jimenez-Flores, R. (2024) 'The relevance of a potential bioactive ingredient: The milk fat globule membrane', Journal of Dairy Science, 108(4), pp. 3109-3134. DOI: 10.3168/jds.2024-25412
Type of publication	Article (peer reviewed)
Link to publisher's version	10.3168/jds.2024-25412
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Download date	2026-10-17 10:03:27
Item downloaded from	https://hdl.handle.net/10468/17316

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Coláiste na hOllscoile Corcaigh

J. Dairy Sci. 108:3109–3134
https://doi.org/10.3168/jds.2024-25412
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Dairy Foods: A Matrix for Human Health and Precision Nutrition— The relevance of a potential bioactive ingredient; The milk fat globule membrane

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ABSTRACT

The milk fat globule membrane (MFGM) is a complex structure that surrounds the surface of fat globules in the milk of mammals. The MFGM is rich in bioactive compounds such as phospholipids, glycoproteins, and sphingolipids. Recent research highlights its important role in human health, particularly in infant nutrition, where it contributes to cognitive development, immune function, and gastrointestinal health. This review article examines the variability in commercial MFGM ingredients derived from dairy sources, detailing the impact of processes used to extrapolate the bioactive rich fractions from the MFGM. The potential applications of MFGM in food products, especially infant formulas, are emphasized, showcasing its ability to improve nutritional outcomes. Furthermore, the review discusses clinical studies that demonstrate the health benefits associated with MFGM supplementation, including enhanced cognitive performance and reduced incidence of infections in infants. Some of the underlying mechanisms behind the health-enhancing effects are elucidated in this review. Overall, this review underscores the importance of MFGM as a valuable bioactive ingredient in promoting health and development in early life nutrition.
Key words: MFGM, dairy matrix, human health, microbiome

OVERVIEW OF THE MFGM

Formation, Sources, and Structure

The milk fat globule membrane (**MFGM**) is the surrounding membrane of the triacyl glyceride–dense fat globules in milk. The native MFGM is a tri-layer structure formed during the biosynthesis of lipids in the rough endoplasmic reticulum of lactocytes within the mammary gland. It is composed of a phospholipid tri-layer, cholesterol, many proteins, glycosylated proteins, and various lipids (Brink and Lönnerdal, 2020). The 2 main sources of MFGM are cream-derived during butter production and whey-derived from cheese manufacturing. The use of MFGM ingredients in nutritional products, especially in infant nutrition, has received great attention in recent years. Today, a range of MFGM-enriched ingredients are commercially available from various suppliers across the globe. The basic components of the tri-layer, phospholipids, and proteins have been shown to exert bioactive effects in various models including in humans. It is not yet known how the differences in the composition of such complex ingredients impact the bioactive potential of MFGM within the dairy matrix. The MFGM becomes more complex as a result of processing as it loses its native tri-layer structure and MFGM fragments are formed. This review aims to bring to light the relevance for the bioactive effects of MFGM ingredients on human health.

Composition of the MFGM

Lipid Composition. The main phospholipids identified in the MFGM are phosphatidylserine (**PS**), phosphatidylinositol (**PI**), phosphatidylethanolamine (**PE**), and phosphatidylcholine (**PC**; Brink and Lönnerdal, 2020; Brink et al., 2020; Thum et al., 2023). Other lipids, such sphingolipids, cholesterol, and gangliosides, are also present in MFGM. Phosphatidylcholine, PE, and sphingomyelin (**SM**) are the most abundant lipids present in bovine MFGM (Thum et al., 2023). These phospholipids are important for the stability of the milk fat globule, which is achieved by forming an emulsion to protect against enzymatic degradation and prevent globules from coalescence. The lipid structure is held together mostly by SM, which is highly saturated in comparison to the phospholipids; this sphingolipid may be important in neonatal digestion for delivery of other key components such as ceramides and sphingosine (Lopez et al., 2019, 2023). The concentration of the phospholipid classes within MFGM sources are report-

Received July 9, 2024.
Accepted September 20, 2024.
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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

ed inconsistently in the literature, with some reporting concentration per total phospholipids, per total fat, or per 100 g of product (de Boer, 2014; Price et al., 2018; Calvo et al., 2020). Therefore, the values reported in Table 1 should be interpreted with caution.

Protein Composition. The protein composition of MFGM ingredients and bovine milk is quite diverse as shown in Table 2, and there have been various qualitative and quantitative proteomic studies identifying proteins in the MFGM fraction (Reinhardt and Lippolis, 2006; Affolter et al., 2010; Lu et al., 2016; Yang et al., 2018; Brink et al., 2020; Ebhardt et al., 2022). The figures reported in the literature should be carefully considered, however, as there are major differences not only in the material composition, but the analytical techniques used.

A total of 120 bovine MFGM proteins in milk were identified by Reinhardt and Lippolis (2006) via one-dimensional electrophoresis and in-gel trypsin digestion followed by analysis using liquid chromatography tandem MS. In contrast, another study identified 156 proteins in the MFGM fraction using dimethyl-labeling nano liquid chromatography-linear trap quadrupole-Orbitrap (ThermoFisher Scientific)-tandem MS (Lu et al., 2016). Yang et al. (2018) compared sample preparation methods to quantify MFGM proteins in milk and found that the relative abundance of proteins in MFGM varied between methods used to quantify MFGM proteins; notably, they concluded that S-Trap and filter-aided sample preparation methods yielded the highest number of proteins from MFGM.

Affolter et al. (2010) and Brink et al. (2020) both determined the relative abundance of MFGM proteins in more complex matrixes (i.e., MFGM-enriched commercial ingredients). Affolter et al. (2010) used an LC-MS/MS-based shotgun approach and label-free proteomic approach with linear trap quadrupole-XL-Orbitrap MS/MS and reported a difference in abundance between a cream-based MFGM ingredient (buttermilk powder) and a whey-based MFGM ingredient (whey protein concentrate [**WPC**]) of 133 and 244 proteins, respectively. Conversely, Brink et al. (2020) used S-Trap with LC-MS/MS to identify 679 total proteins in sweet butter milk powder. Furthermore, Brink et al. (2020) concluded that there was a varied relative abundance of proteins between all materials analyzed, but significantly more were found in the whey-based MFGM ingredient than in the cream-based ingredient and commercially produced premium infant formula. More recently, Ebhardt et al. (2022) developed a reductionist approach for a targeted proteomics method for profiling important milk and MFGM proteins in commercially available MFGM materials and other dairy-based ingredients. Although there is currently no standard analytical method for analysis of MFGM proteins in infant nutrition, Fong and Norris (2009) have published a method for the absolute quantification of 6 MFGM proteins in dairy products using selected reaction monitoring MS. A more recent development by Bär et al. (2019) used a multiple reaction monitoring method using liquid chromatography MS to quantify 20 milk proteins, including MFGM proteins, whey proteins and caseins in different dairy products. This is promising for future developments for the quantification of MFGM and milk proteins in more complex food matrixes such as infant formula products.

The location of MFGM proteins varies; some are found in the inner monolayer membrane and others are in the outer bilayer membrane. In addition, several proteins are loosely bound to the membrane while others are embedded within it. The major MFGM proteins are butyrophilin (BTN), mucin-1 (MUC1), adipophilin (ADPH), fatty acid binding protein (FABP), periodic acid Schiff (PAS) 6/7, lactadherin, CD36, xanthine dehydrogenase/oxidase (XDH/XO), and PAS III. As mentioned, the abundance of the MFGM proteins differs between sources, as well as in commercial infant formulas (Ji et al., 2019; Brink et al., 2020). The protein fraction of MFGM is known to influence the structure and stability of MFGM; furthermore, some of these proteins have significant functions in gastroduodenal digestion (Lu et al., 2016; Fontecha et al., 2020; Sun et al., 2023). Adipophilin, located within the inner monolayer, has a role in regulation of lipolysis by controlling access of proteins to milk fat globules (El-Loly, 2011; Manoni et al., 2020). Butyrophilin is the most abundant bovine MFGM protein and is also a transmembrane protein that is affiliated with the immunoglobulin superfamily (Manoni et al., 2020). It should be noted that the protein *BTN1A1* is also found in human MFGM; the function of *BTN1A1* is to control the synthesis of lipid droplets via a process involving the membrane phospholipid of MFGM (Manoni et al., 2020). Mucin-1 is a digestion-resistant and highly glycosylated glycoprotein found in the outer coat of milk fat globules; these characteristics make MUC1 available to function as a decoy receptor for pathogens (Manoni et al., 2020). Xanthine dehydrogenase/oxidase is uniquely situated in the intermembrane space between the monolayer and the bilayer of the MFGM to form a 3-way structure with ADPH and BTN; as a result, this redox enzyme connects the inner and outer membrane. Xanthine dehydrogenase/oxidase has a local function in the gastrointestinal tract by producing reactive oxygen species and reactive nitrogen species (El-Loly, 2011); these both have bactericidal properties, but like MUC1, xanthine dehydrogenase may also act as a decoy. Fatty acid binding protein transports fatty acids across the capillary endothelium to reach the cytoplasm of mammary endothelial cells, where they diffuse through the membrane (Manoni et al., 2020). Like XDH/XO, FABP

Table 1. Phospholipid composition (mean ± SD) of dairy-based MFGM sources in the literature

Item	PC	PE	PI	PS	SM	Total phospholipids	Reference
g/100 g							
Human milk	0.00415–0.00588	0.00563–0.0121	0.0015–0.00465	0.001–0.00497	0.00474–0.0126	0.0182–0.0242	Claumarchirant et al., 2016; Giuffrida et al., 2016; Yang et al., 2022
Raw bovine milk	0.0192–0.0432	0.0268–0.0464	0.0006–0.0136	0.0028–0.0161	0.0051–0.0287	NA[1]	Ortega-Anaya and Jimenez-Flores, 2019
α-LA-enriched WPC	0.0056 ± 0.13	0.00314 ± 0.18	0.00197 ± 0.31	ND[1]	0.00514 ± 0.52	15.9 ± 1.14	Ponchon et al., 2024
MFGM-enriched WPC	1.78 ± 62.2	1.79 ± 61.9	0.362 ± 11.0	0.606 ± 27	1.72 ± 101	6.25 ± 246	Ponchon et al., 2024
α-LA and MFGM-enriched WPC	1.22 ± 45.5	1.46 ± 45.5	0.365 ± 20.4	0.564 ± 40.8	0.974 ± 141	4.59 ± 162	Ponchon et al., 2024
Buttermilk	4.74 ± 0.56	3.44 ± 0.37	0.95 ± 0.52	3.28 ± 1.17	2.09 ± 1.16	14.5 ± 3.58	Calvo et al., 2020
MFGM isolate from buttermilk	13.83	13.07	0.47	5.53	7.41	40.3	Calvo et al., 2020
Acid whey MFGM[2]	6.88 ± 0.01	11.4 ± 0.28	1.26 ± 0.03	1.00 ± 0.14	4.82 ± 0.66	25.4 ± 0.78	Kosmerl et al., 2023b
Buttermilk powder	44.3	7.3	7.1	4.0	23.9	NA	Gallier et al., 2010b
Whey buttermilk	6.26 ± 3.32	3.19 ± 1.50	0.94 ± 0.10	0.93 ± 0.40	3.48 ± 1.71	14.8 ± 6.61	Costa et al., 2010
Supercritical fluid extracted whey buttermilk	26.7 ± 5.46	13.5 ± 2.51	2.74 ± 1.41	3.19 ± 2.11	14.2 ± 3.00	60.6 ± 11.1	Costa et al., 2010
Liquid WPPC	31.0 ± 0.7	47.8 ± 1.4	5.1 ± 0.2	8.2 ± 0.1	7.9 ± 0.1	29.1 ± 0.7[3]	Price et al., 2018
Whey protein phospholipid from whey serum	14.6	10.1	1.82	1.36	10.7	38.6	Lee et al., 2020
% of total polar lipids							
Buttermilk[4]	19.0 ± 0.45	42.9 ± 0.25	8.91 ± 0.27	8.55 ± 0.12	16.4 ± 0.50	NA	Rombaut et al., 2005
% of fatty acid content							
WPPC	~0.9	~0.9	0.21–0.33	0.21–0.33	0.1	2.45	Ozturk et al., 2022
% of total phospholipids							
Sweet whey	25	25	7	12	24	7.5	de Boer, 2014
Butter serum from anhydrous milk fat[2]	24.9 ± 1.5	29.2 ± 1.1	9.0 ± 0.8	10.1 ± 0.7	26.8 ± 0.2	0.95 ± 0.08	Britten et al., 2008
Goat milk butter serum[2]	26.2 ± 1.5	27.1 ± 1.5	11.7 ± 2.5	8.2 ± 1.4	26.8 ± 1.6	NA	Lamothe et al., 2008

[1]NA = not available; ND = not detected.

[2]SE or SD not stated.

[3]% of total fat.

[4]Mean ± SE.

Table 2. Properties of principal MFGM proteins

Protein	MW (kDa)	Physiological mechanism	Bioactivity shown in vivo and in vitro	Reference
Mucin I (MUC1)	160–200	Decoy receptor for pathogen Immune protection	Antibacterial Protects against rotavirus Improved barrier function	El-Loly, 2011; Liu et al., 2012; Kosmerl et al., 2024
Xanthine oxidase (XO)	146–155	Produces reactive oxygen species Produces reactive nitrogen species	Antioxidant Antimicrobial	Harrison, 2006; Ozturk et al., 2022
PAS III	94–100	Lubrication	Protective barrier, potential antimicrobial	Riccio, 2004
CD36	76–78	—	Anti-inflammatory	Chatterton et al., 2013
Butyrophilin (BTN)	66–67	Lipid droplet synthesis	Immune regulation Influence for pathogenesis of autistic behavior	Vojdani et al., 2002; Ammann, Cooke and Trowsdale, 2013; Arnett and Viney, 2014
Adipophilin	52	Lipolysis regulation	—	Redwan et al., 2020
PAS 6/7 (lactadherin)	47–59	—	Anti-infective for viral infection of gut	Newburg et al., 1998; Kvistgaard et al., 2004
Proteose peptone 3	18–34	—	—	—
FABP	13–15	Fatty acid transportation	Breast cancer indicator and inhibition	Hancke et al., 2010

proteins are found in the intermembrane space between the monolayer and bilayer.

CHANGES IN THE MFGM DURING PROCESSING

The impact of processing on the structure and composition of the MFGM has been studied, and the effects of those processing methods on losses or changes in certain components of the MFGM have been elucidated.

Heating

Heating of milk (above 60°C) is known to cause association of major whey proteins β-lactoglobulin and α-LA with MFGM via disulfide bond linkages, and this association increases with rising temperature and increasing time. Milk fat globule membrane proteins containing several disulfide and sulfhydryl groups may associate with β-lactoglobulin through their free thiol groups. Most MFGM proteins associate with either the serum proteins or other MFGM proteins. However, the intensity of PAS 7 bands in SDS-PAGE gels was found to be reduced for MFGM material isolated after heating milk above 65°C as reported by Ye et al. (2004). Other MFGM proteins, such as xanthine oxidase and butyrophilin, were retained with MFGM material following heat treatment (Ye et al., 2004). Similar results were reported by Lee and Sherbon (2002), where heating whole milk at 80°C for 3 to 18 min showed incorporation of whey protein, especially β-lactoglobulin on the MFGM. Their results also reported reduction of free sulfhydryl groups and increase in disulfide groups due to heating, suggesting association of β-lactoglobulin with membrane proteins. When homogenization was used along with heat treatment, there was a significant increase in the total protein content on the membrane as compared with milk that was only homogenized. This increase was due to probable adsorption of κ-casein and α-LA complexes formed during heating in the milk serum. For only homogenized milk, a significant amount of casein was adsorbed to the membrane. Heating after homogenization caused whey proteins to be deposited onto the membrane with no significant increase in the caseins (Lee and Sherbon, 2002). A study on whole milk subjected to common industrial heat treatment of low temperature-long time and high temperature-short time pasteurizations, sterilization, and ultraheat treatment concluded that heat treatment had minimal effect on composition of the major lipid components in milk (Gathercole et al., 2017).

Nonthermal Heating Technologies

To examine the effect of various nonthermal technologies on milk fat globules (**MFG**) and MFGM for evaluation of the quality and functionality of cheese making milk, Garcia-Amezquita et al. (2009) subjected whole milk to different thermal treatments: (1) low temperature, long time (**LTLT**), 63°C ± 0.5°C for 30 min, 97°C ± 0.5°C for 7 min; (2) extreme thermal control, 97°C ± 0.5°C for 7 min; (3) high-pressure processing (**HPP**), pressures of 400 and 500 MPa, 5 time periods of processing (come-up time, 5, 10, 15, and 20 min), temperature of 20°C ± 2°C; and (4) pulsed electric field (**PEF**), 42 kV/cm (8, 16, 24 pulses), 36 kV/cm (24, 40, 64 pulses), temperature not exceeding 25°C. The LTLT milk had a significant increase in the volume percentage of particles in the range of 5 to 11 μm range (large particles), but a zeta-potential similar to that of raw milk. This result suggested clumping of MFG due to association of whey proteins and casein micelles after

heat treatment on the surface of the MFGM rather than coalescence. This association might have resulted in the increase in volume percentage of the larger particles and the average value of the ratio of mass to surface charge did not change significantly, suggesting that the fat globules did not undergo membrane damage. The milk treated with PEF had a MFG size distribution similar to that of LTLT treatment, which suggested that PEF treatment might be preserving the integrity of the MFG. The HPP treatment at 400 MPa resulted in an increase in the diameter of the fat globules in case of longer time, probably because lower pressures required more time for flocculation. In the case of processing at 500 MPa for longer treatment times, the diameter increased in the beginning, followed by a decrease most likely due to destabilization. The suggested MFGM interactions in HPP-treated milk were termed flocculation rather than coalescence owing to the interactions between whey proteins and MFGM, along with the high zeta-potential value, and the MFGM was believed to not be disrupted (Garcia-Amezquita et al., 2009). Higher absolute zeta-potential values denote greater stability and indicate flocculation rather than coalescence.

Fresh milk was treated with shear homogenization (preheated milk at 50°C and homogenized at 20 MPa for 3 times) and compared with an equivalent ultrasonication treatment in a study conducted by Liu et al. (2021) to study the comparative retention of MFGM proteins in these processes. Cream washing was used to isolate MFGM material. The size of MFG was found to be reduced after both the treatments. Shear homogenization was suspected to have extensive re-coalescence due to short treatment period and lost more MFGM proteins. An SDS-PAGE analysis of the isolated material showed no bands for XDH/XO and faint bands for CD36 and BTN proteins in shear-homogenized milk MFGM isolate, with relatively lower losses of MFGM proteins reported for the ultrasonication treatment. Casein bands were also identified in both the treated samples. Proteomic analysis revealed losses of MFGM proteins from the treated samples as compared with raw milk, with more losses detected in the homogenized milk sample. The study concluded that ultrasonication treatment might have had a lesser decrease in the abundance of MFGM proteins due to a constant acoustic cavitation force (Liu et al., 2021). Structural and compositional changes in MFGM due to PEF followed by centrifugation and washing were examined by Walkling-Ribeiro et al. (2024). Whole raw milk was treated with PEF of 1-microsecond square-wave pulses at 150 Hz with intensities of 9, 15, 21, 27 kV/cm for a treatment time of 66 µs. After milk was treated with PEF, it was subjected to cream separation and subsequent washings of cream (3 times in 10 volumes of simulated milk ultrafiltrate at pH 6.5) and centrifugation to yield isolated MFG. The PEF facilitated electro-permeabilization of MFGM. This step was followed by restructuring due to fat-protein aggregation between MFG, which likely led to an increase in the fat content of cream. The changes occurring either due to the separation process along with PEF treatment, or due to either of the processes, were indicative of the structural modifications in MFGM and its protein profiles. Cream obtained from PEF-treated milk had some changes in MFGM proteins like XDH/XO, PAS III, lactoferrin, CD36, butyrophilin, PAS 6/7, and ADPH for certain intensities of treatment as observed in SDS-PAGE gels. The changes in intensities of these protein bands at various stages of treatments suggested the effects of temperature and PEF treatment to either mildly, partially, or completely electroporate the MFGM. These might have resulted in structural changes and fat-protein matrix modifications that could be reflected as interactions of MFGM components with the matrix elements, hinting at the intactness of the native-like structure of the MFGM, the efficiency and efficacy of extraction, and losses of desirable components during processing (Walkling-Ribeiro et al., 2024).

Washing Procedures for Isolation of MFGM

Zheng et al. (2013) studied the effect of washing procedures on the retention and release of different MFGM proteins, and the possible reasons for the losses of certain MFGM proteins. Milk fat globule membrane material was derived using centrifugal washing for isolation of cream, centrifugation, and collection of the top layer of MFG (this process was one washing). Three washing methods were applied (**M1**, 3,000 × *g*, 5 min, cream + 3 washes; **M2**, 3,750 × *g*, 15 min, one wash; and **M3**, 15,000 × *g*, 20 min, cream + 3 washes). Results and previous data indicated that CD36 had the strongest affinity or binding of proteins to the MFGM, most likely from stretching of hydrophobic amino acids serving as transmembrane anchors leading to an increase in affinity to MFGM as the exoplasmic hydrophobic pockets expand. Because ADPH is associated in the inner layer of protein under the MFGM bilayer, it would be comparatively stable after the washing processes. The PAS 6/7 proteins are associated with the membrane as exoplasmic, peripheral proteins that are bound to anionic phospholipids in the membrane through the C-terminus, hence the affinity might depend on the number of phospholipids retained during the washing steps. MUC15 is exoplasmic and weakly bound to the MFGM. The results interpreted that MUC1, MUC15, XDH/XO, and BTN proteins were weakly bound proteins to the MFGM, hence they were lost in the M1 and M3 washing processes. Xanthine dehydrogenase/oxidase located in the protein coat was re-

leased due to structural alteration of MFGM, and BTN is bound to XDH/XO, therefore it was also lost to some extent. The fatty acid binding protein (FABP) is associated with CD36 probably by interprotein binding, but some FABP proteins are also present in the noncomplexed form, which was deduced due to its varying depletion levels in some of the washing processes (Zheng et al., 2013). Raw milk was processed to make cream, buttermilk, and isolate MFGM material with cream washing steps by Le et al. (2013). They observed that 3 cream washings resulted in the loss of some MFGM proteins. Based on the total protein content and proteomic approach, the recommendation was that washing processes could be limited to 2 cycles to isolate MFGM material with minimal losses. They also reported that proteins minimally lost in the washing processes are those that are located toward the inner side of the membrane, which bind strongly to the neutral core lipids. In contrast, transmembrane proteins, which are loosely bound to the membrane, are more prone to losses during the washing steps. Mechanical impact could also be a reason for losses of fragments of damaged MFGM owing to excessive washing steps, and the membrane damage can be indicated by the coalescence of fat globules. In addition to obtaining and analyzing the aforementioned products, commercial buttermilk powder was reconstituted to make buttermilk, and sodium citrate was added to it for dissociation of casein micelles, after which it was passed through cross-flow microfiltration and 3 cycles of diafiltration to obtain concentrated MFGM material. According to proteomics analysis, microfiltration coupled with addition of sodium citrate yielded more MFGM proteins as compared with the buttermilk from cream separation (Le et al., 2013). The changes occurring during washing of cream in the MFGM and its proteins were studied by Holzmüller et al. (2016). Particle size of the MFG increased with consecutive washing steps, indicating coalescence of fat globules and loss of MFGM proteins. Peripheral MFGM proteins such as PAS 6/7 as well as integral proteins such as XDH/XO and BTN were found to be depleted after higher number of washing steps, suggesting loss of MFGM fragments rather than individual components of the membrane. Shear forces occurring in the cream separator and the loss of milk serum proteins from the surfaces of the MFG due to washing contributed to an increase in their particle size. The type of washing solutions and their electrolytic properties also influenced coalescence in the MFG, as washing solution with higher ionic strength disturbed the stability of the electrochemical double layer of the fat globules, resulting in coalescence. The presence of stabilizing milk proteins such as caseins and whey proteins adsorbing to damaged membranes provided stability and resistance to increase in the globule size (Holzmüller et al., 2016).

Observation of Structural Changes Using Microscopy Techniques

Milk fat globule membrane from raw milk and cream, processed (pasteurized and homogenized) milk and cream, and reconstituted spray-dried buttermilk was studied using confocal laser scanning microscopy (**CLSM**) by Gallier et al. (2010a) to understand the effects of processes such as centrifugation, churning, pasteurization, and homogenization on MFGM. Total lipid extraction was conducted using Folch extraction and the Bitman procedure to separate phospholipids (**PL**). Fluorescent probes with affinities to phospholipids such as PE, PC, SM, and to proteins were used to observe changes in structures of the MFG. Centrifugation is known to cause losses of PL into the serum, especially SM and PC, as was compared with their content in raw milk and raw cream, because they are located on the outer side of the MFGM bilayer. Phosphatidylethanolamine is more hydrophobic, so it was likely carried over to the butter phase. More lysoPC was found in buttermilk than cream or raw milk, suggesting degradation during butter manufacture, processing, or storage of buttermilk powder. Gallier et al. (2010a) reported that CLSM images indicated lateral heterogeneity in the MFGM, with observation of liquid-ordered and -disordered phases. The surface of raw cream MFG had fewer Fast Green–stained proteins (casein micelles) compared with a higher protein-to-lipid ratio in processed cream MFGM due to attachment of caseins (and probably whey proteins, as well), indicating changes in functionality of processed cream (Gallier et al., 2010a). Balasuriya et al. (2012) analyzed the effects of processing on changes in MFGM using atomic force microscopy for samples of raw milk, raw ultrafiltrate retentate, cheese milk, homogenized milk, and cream. The authors reported that the native state of the MFGM (considered for raw milk) was observed as rough globules with presence of small and large domains; homogenized milk showed a smooth and flat MFG surface, probably because of removal, deposition, or association of material and had the highest amount of absorbed protein among the samples; cream also seemed to have a smooth membrane surface, but higher magnification showed some lateral heterogeneity in cream MFGM, due to lower shear force of centrifugation to obtain cream in comparison to the homogenized milk sample. Larger globules formed by coalescence cause changes in the MFGM, as the same amount of MFGM covers a larger surface area. High filtration pressure and shear forces in the ultrafiltrate retentate sample resulted in adsorption or deposition of milk proteins onto MFG, which formed a gel-like structure of proteins. The authors also observed that cheese milk MFG had a globular appearance, and the more ordered globular appearance than raw milk MFGM was probably due to some aggregated whey proteins.

Young's modulus studies showed that the ultrafiltrate retentate with the most elastic MFGM resonated to a porous structure like a protein gel on their surface, which suggested superficial changes in the membrane, whereas cream and homogenized milk had the stiffest MFGM, which correlated with the high mass of casein micelles and whey proteins attached to the membrane surface (Balasuriya et al., 2012). Gallier et al. (2010c) conducted a study on the composition and monolayer structure of the PL using Langmuir film balance, epifluorescence microscopy, and atomic force microscopy. Monolayer films at various pressures and temperatures, changes in physical behavior, phase separation, and changes in the size and shape of liquid-ordered (saturated PL; SM) and liquid-disordered (unsaturated PL; PE, PI, PC, PS) domains were used to study changes in MFGM due to processing. Phospholipids were obtained from buttermilk powder, raw milk, processed milk, and raw cream using the Folch lipid extraction technique. It was observed that processing affected the composition and fatty acid profile of the phospholipids, causing changes in the lipid-lipid interactions in different matrixes ultimately leading to physicochemical changes in the MFGM. Fluorescence microscopy helped in understanding and visualizing phase separation in phospholipid films as liquid-ordered and disordered phases. Fluorescence microscopy coupled with compression isotherms suggested that phospholipids in the mixtures were in a heterogeneous distribution. The differences in the phospholipids profile and fatty acid distribution resulted in different physical and morphological behavior of each of the samples, with the phospholipids mixture in the buttermilk powder sample being the most distinct, owing to all the processing methods it was subjected to causing changes in physicochemical properties (Gallier et al., 2010c).

Centrifugation is known to cause losses in SM, which is an important phospholipid for biological and structural health benefits. Spray drying (especially during manufacture of buttermilk powder) is known to cause losses in the PE content (Gallier et al., 2010b).

Compositional Differences in Commercially Available MFGM Ingredients

Few studies have characterized the composition of different commercially available MFGM-enriched ingredients. As already highlighted, the composition of such ingredients can affect the fate of the MFGM in terms of its nutritional and biological properties. Most whey-derived MFGM ingredients have a milk fat composition of ~10% to 20%. One study reported variability between the fat and protein compositions for different dairy-based MFGM ingredients (Ozturk et al., 2022). This study alone shows the complexity and variation of the commercially produced MFGM ingredients, which gives further validation for the requirement to characterize these ingredients. Ozturk et al. (2022) also reported and compared the relative abundance of proteins present in MFGM ingredients with those reported by Brink et al. (2020). There was little variability between the total relative amount of MFGM proteins in whey protein phospholipid concentrate (**WPPC**) and cream-derived MFGM materials (22.81%–26.39%). However, there was significantly less total relative MFGM proteins in the Lacprodan MFGM-10 (Arla Foods) ingredient, a whey-derived MFGM material. Compositional differences between commercial infant formula types were also evaluated; notably, low amounts of the MFGM proteins were present overall in both premium infant formula and standard infant formula compared with commercially available MFGM ingredients (Brink et al., 2020). This difference may have been due to further processing of the MFGM fragments during infant formula manufacture. There was also great variation observed across levels of individual milk proteins, such a lactoferrin, β-casein, and β-lactoglobulin. A study by Le Berre et al. (2023) assessed the global levels of glycosylation of commercial MFGM-enriched WPC and compared them with other WPC and found differences in glycosylation between MFGM sources; however, a co-enriched α-LA and MFGM WPC had similar global levels of glycosylation to the MFGM-enriched WPC. This finding is significant for the design of infant formula; whey protein and MFGM glycoprotein glycosylation is associated with bioactivities, and supplementation with a MFGM-enriched WPC could therefore improve the bioactive profile of the formula. Furthermore, the co-enriched WPC is a desirable ingredient for infant nutrition design, as it has reduced levels of β-lactoglobulin and is enriched with MFGM components.

Ebhardt et al. (2022) has also presented the great variability of the proteome among commercially available MFGM ingredients, and even between batches of the same material. In the context of infant nutrition, dose-response effect studies with different commercial MFGM batches and bioactivities should be conducted to determine the minimum concentration of bioactive proteins or lipids that are required to have a beneficial effect in vitro. As already emphasized, given the variation between commercially available MFGM ingredients in the literature, quantitative methods for MFGM proteins need to be developed to investigate this variability. Furthermore, this will enable food regulatory bodies to establish a more standardized addition rate of MFGM to infant formulas and for infant formula manufacturers to formulate products with sufficient amounts of MFGM to achieve possible health benefits for the neonate.

The variation in the composition of the MFGM fraction is largely due to milk from different species, breeds,

lactation stages, diets, and possibly geographical regions (Thum et al., 2023). These environmental factors, combined with the processing methods used to enrich cream- or whey-derived MFGM ingredients, contribute to variability in composition observed by Brink et al. (2020) and Ozturk et al. (2022). Furthermore, the language used to characterize an MFGM ingredient is unclear in the literature. Some groups have referred to MFGM ingredients as "phospholipid-enriched" or "milk polar lipid enriched" MFGM ingredients (Tomé-Carneiro et al., 2018; Lee et al., 2020; Palmano et al., 2020; Rocha-Mendoza et al., 2020; Ortega-Anaya, Marciniak and Jiménez-Flores, 2021). Such materials have been extracted by technologies such as supercritical fluid extraction (**SFE**) whereby the nonpolar lipid fraction was removed while the polar lipids were retained (Astaire et al., 2003). However, the protein profile of the buttermilk starting material was only determined during the microfiltration step of the 2-step MFGM isolation process. In other studies, which used supercritical fluid extraction to enrich MFGM fragments, it was shown that SFE increased the proteins slightly (Spence et al., 2009); the results were consistent with that of Costa et al. (2010). Although they both achieved an improved phospholipid profile for MFGM sourced from buttermilk, these studies undermine the significance of the protein profile of these ingredients. Therefore, caution should be taken when MFGM is characterized in this way, as phospholipids can also be found in other food sources such as soy lecithin, a commonly used emulsifier in the food industry. Additionally, the MFGM fraction is a combination of phospholipids, MFGM proteins, and glycoproteins. At present, there is strong evidence from many reviews suggesting that the polar lipids from milk and milk-based products could provide many biological benefits (Lordan and Zabetakis, 2017; Verardo et al., 2017; Lordan et al., 2020; Pawar et al., 2023). However, the protein and glycoprotein fractions of MFGM should not be overlooked from a nutritional and bioactive perspective.

REGULATORY CHALLENGES

Although the phospholipid concentrations of MFGM ingredients range between sources, they are relatively similar in ratio composition. The characterization and regulation of MFGM ingredients is ongoing. Some regions are drafting guidelines to help food manufacturers to characterize commercially produced protein powders with an enriched MFGM fraction. These ingredients are relatively new in infant nutrition formulations, and they have therefore not been fully characterized. At present, the US Food and Drug Administration, the European Food Safety Authority, and Food Safety Authority New Zealand do not regulate the use of MFGM ingredients as novel food ingredients for use in infant nutrition. However, the American Dairy Products Institute has published standards for infant formula grade WPPC ingredients, which states that there should be a minimum 12% milk fat with 4% total phospholipid, 50% protein, and a maximum of 8% ash and 6% moisture (ADPI, 2023). Fontecha et al. (2020) outlined the regulatory challenges associated with characterizing novel infant nutrition ingredients like MFGM-enriched ingredients in more detail.

The MFGM and Gut Health

Bifidobacterium. *Bifidobacterium* are gram-positive, nonmotile bacteria associated with the microbiome of breast-fed infants. They are the first and most abundant species present in the gastrointestinal tract of infants. They are responsible for prevention of disease like type 1 diabetes, allergenicity, cardiometabolic diseases, and cystic fibrosis due to their complex metabolic activity (Zhao et al., 2022; Kosmerl et al., 2023a). *Bifidobacterium* viability and proliferation in the harsh environment of the intestinal tract is essential for their colonization and beneficial health impacts. One way to improve *Bifidobacterium* viability is through the addition of MFGM. One study by Kosmerl et al. (2023a) treated *Bifidobacterium longum* ssp. *infantis* with milk phospholipids and exposed the bacteria to a simulated in vitro digestion compared with culture without milk phospholipids. They demonstrated significantly improved survivability following the intestinal phase, signifying that the phospholipids mitigated some of the bile-induced stress on the bacteria. Another study by Zhang et al. (2024) also demonstrated the ability of MFGM to increase cell survival and restore cell integrity, viability, and metabolic function when in the presence of bile salts. The restored viability was displayed through increased gene expression of nutrient transporters and metabolism (Zhang et al., 2024). This result demonstrates a mechanism by which MFGM can protect and promote metabolic pathway activity of *Bifidobacterium* when exposed to the harsh environment of the gastrointestinal tract to increase the survival and colonization in the microbiota. Another potential mechanism by which milk phospholipids enhance *Bifidobacterium* colonization in the gastrointestinal tract is through alteration of the bacterial surface properties via direct interactions between the bacteria and the MFGM. These changes include a more negative surface charge and a thicker outer layer following treatment with milk phospholipids (Kosmerl et al., 2023a). Interaction with the milk phospholipids at the cell surface led to the enhancement of bound polysaccharide production and surface protein changes, which could play a role in the protection from bile-induced stress and provide desirable functional properties of the

bacteria. An increase in membrane-bound polysaccharides has the potential to interact with intestinal epithelial cells and the mucus layer, providing a greater chance for bacterial attachment and colonization.

Another study uncovered that specific components of the MFGM may be responsible for promoting the growth of *Bifidobacterium* in the gut (Zhao et al., 2022). Lactadherin, sialic acid, and phospholipids are abundant in MFGM and together demonstrate positive correlations with an increase in *Bifidobacterium* and a negative correlation with harmful pathogens in infant stool samples. Taken together, these studies elucidate potential mechanisms by which MFGM enhances and restores *Bifidobacterium* survival and metabolic function through digestion and bile salt exposure, along with altering of the surface proteins and polysaccharides, which could promote attachment and colonization in the gastrointestinal tract.

Lactic Acid Bacteria. Lactic acid bacteria (**LAB**) are a common class of probiotics used in food products for fermentation. These bacteria produce many compounds through carbohydrate metabolism, which give fermented products flavor and texture (Kosmerl et al., 2021). Other metabolites produced by LAB are neuroactive compounds such as dopamine and serotonin, which can influence behavior as impacted by brain stimuli through the gut-brain axis (Oleskin et al., 2014). These compounds were found by Oleskin et al. (2014) in fermented dairy products containing probiotic LAB, which may translate to neurotransmitter production in the gastrointestinal tract when LAB are consumed with dairy products. Lactic acid bacteria are nutritionally fastidious, and milk is a nutrient-rich growth medium that allows for successful LAB growth. However, LAB can adapt their metabolism based on their environment; for example, they can increase exopolysaccharide production for their own protection in harsh conditions of bile salt exposure (Zhang et al., 2020). A study by Zhang et al. (2020) demonstrated the ability of MFGM to increase the survival and significantly enhance biofilm formation of *Lactobacillus rhamnosus* when under bile stress and when ingested by mice. *Lactobacillus rhamnosus* supplemented with MFGM was able to successfully survive and colonize the intestine of the mice compared with no MFGM. Lactic acid bacteria are not only found in food, but are a major resident of the gastrointestinal tract. To be considered a probiotic, these bacteria must be able to competitively adhere to intestinal components for colonization, preventing pathogen adhesion and promoting a healthy microbiota (Ortega-Anaya et al., 2021). Lactic acid bacteria are primarily gram-positive, providing exposure of teichoic acids, polysaccharides, and surface-layer proteins from the cell wall, which serve as the main mechanism for adhesion to the intestinal epithelia and other nutrients, such as MFGM. When grown in the presence of MFGM or milk phospholipids, LAB increase their expression of surface-binding genes, exemplifying their ability to alter their metabolism in different environments (Rocha-Mendoza et al., 2020; Ortega-Anaya et al., 2021). Interaction with the MFGM phospholipids increases the electronegative surface potential, most likely due to the phospholipid polar head group interacting with surface proteins on the bacterial cell; the interaction between MFGM phospholipids and bacterial surface proteins is correlated with increased adhesion to intestinal cells (Rocha-Mendoza et al., 2020; Ortega-Anaya et al., 2021). Increased adhesion to intestinal epithelial cells and the mucosa lining of the intestinal tract by LAB supplemented with MFGM is not only initiating colonization of beneficial bacteria but also the attenuation of pathogen adhesion, reduction of chronic disease, symbiosis resolving gut distress and inflammation, along with improved digestion and metabolism.

Intestinal Cell Culture In Vitro. A common method for measuring nutrient interaction in the gastrointestinal tract is with in vitro cell culture models. To better understand the mechanisms behind MFGM interaction and function following ingestion, cell lines like Caco-2, HT29-MTX, and MA104 are typically used. One such mechanism is cell membrane alteration; the MFGM can fuse with the Caco-2 intestinal cell membrane via polar lipid interactions and influence the lipid profile of the cell (Martínez-Sánchez et al., 2023). The epithelial cells can alter lipid metabolism based on the absorption of nutrients rich in lipids like MFGM; for example, accumulation of triacyl glycerides and synthesis of polar lipids following MFGM interaction reflects the important role of the enterocyte functionality to reorganize incoming lipids into chylomicrons for secretion into the lymphatic system (Martínez-Sánchez et al., 2023). Triacyl glycerides must be packaged inside a polar lipid membrane forming the chylomicron, whereby the polar lipids are the target for membrane-membrane interactions to secrete the chylomicron. The lipid profile alteration of the Caco-2 cells following MFGM interaction exemplifies stimulation of the endothelial cell functionality. MFGM not only stimulates endothelial cell use and functionality of lipids, but can also reduce intestinal permeability through increasing the expression of tight junction proteins (Jiang et al., 2022b). Treatment of Caco-2 cells with MFGM significantly increased the transepithelial electrical resistance and enhanced the transcription of tight junction proteins, suggesting that MFGM can strengthen the intestinal barrier and subsequently prevent barrier dysfunctional-related diseases (Jiang et al., 2022b). Another physical mechanism for the role of MFGM in intestinal barrier protection and strengthening is through fortification of the mucosal layer to prevent injury and disease (Kosmerl et al., 2024). Pretreatment of Caco-2/HT29-MTX cells with MFGM before challenge with dex-

tran sulfate sodium increased transepithelial electrical resistance, inhibited mucus layer permeability, allowed for mucin protein production homeostasis, and visually fortified the mucin layer (Kosmerl et al., 2024). These studies demonstrate the protective effects of MFGM on intestinal cell permeability and prevention of intestinal barrier-related disease.

Milk fat globule membrane can also inhibit the pathologies resulting from bacterial or viral infection. Guerin et al. (2018) demonstrates the ability of MFGM to bind to bacteria; this study elucidates the mechanism by which the bacterial pili establish contact points with the MFGM, most likely at glycoproteins serving as pseudoreceptors recognized by the bacteria. This pseudorecognition functions as a competitive binding mechanism that prevents adhesion of the bacteria to Caco-2 cells. This finding could potentially prevent pathogen adhesion to intestinal cells with the ingestion of MFGM. In a study by Monaco et al. (2021), a WPC with MFGM was significantly more effective at reducing infection by rotavirus than a WPC without MFGM. A greater reduction in infectivity was observed for both intestinal Caco-2 cells and kidney MA104 cells, for both porcine and human rotavirus, and at various concentrations of WPC with MFGM compared with WPC without MFGM. A similar study by Fuller et al. (2013) demonstrated the ability of both a buttermilk and whey cream-derived MFGM to inhibit the infectivity of rotavirus in a dose-dependent manner on MA104 cells. Furthermore, Fuller et al. (2013) elucidated that the nonpolar fraction of the MFGM is the major component responsible for the antiviral activity, revealing a mechanism by which MFGM can prevent both pathogen adhesion in a competitive binding manner and rotavirus infection due to the nonpolar lipid antiviral activity to inhibit these infectious pathologies.

MFGM and Animal Studies

Brain Health. Oxidative stress naturally becomes more abundant with age, specifically with cell age. As brain cells age, memory and learning skills decline because brain atrophy occurs. Milk fat globule membrane is known to promote neurodevelopment and cognitive abilities in infants, but it can also improve cognitive impairment due to stress and age (Zhou et al., 2023). One study by Zhou et al. (2023) measured learning and spatial memory in mice after intragastric injection of MFGM for 18 weeks using a Morris water maze test and hippocampal tissue analysis. The mice exposed to MFGM demonstrated greater spatial memory and cognition by taking less time to complete the Morris water maze test and crossing over the point of interest more often than the control group not exposed to MFGM. To elucidate the mechanism by which MFGM is enhancing cognition, Zhou et al. (2023) found that MFGM reduces the loss of neural cell numbers, enhances the neuron intensity, and promotes a more even distribution and regular morphology of neural cells in the hippocampus. Along with increases in neural cell number, MFGM was found to cause differentially expressed proteins mainly involved in signaling pathways such as neuroactive ligand-receptor interaction, calcium signaling, complement and coagulation cascades, and neurotransmitter synapses, which could be directly related to improved learning and memory in the brain (Zhou et al., 2023). Milk fat globule membrane improved spatial learning and cognition in mice through enhancement of both neurogenesis and synaptogenesis. To further measure the ability of MFGM to improve cognitive function and prevent brain aging, a study by Unno et al. (2022) used mice that were prone to accumulation of amyloid-β at an early stage in the brain that corresponded to cognitive decline. Ingestion of MFGM led to an increase in lipocalin 2 secreted from astrocytes to regulate brain age, along with enhancement of repressor element 1-silencing transcription factor, which leads to increased neural differentiation to suppress memory decline. There was also an upregulation of transforming growth factor β1, which promotes the activation of cyclic adenosine monophosphate or cAMP responsive element binding protein (also known as CREBP), leading to more neurogenesis to increase brain function (Unno et al., 2022). Because these mice had accelerated brain aging, another marker was increased lifespan, and this increase was partially due to the suppression of brain inflammation exemplified by no elevation of serum endotoxin levels in the mice. Milk fat globule membrane demonstrates its ability to enhance cognitive function through reduction in neural apoptosis and promotion of neurogenesis.

Gastrointestinal Health. Gastrointestinal tract health is dependent on many factors including inflammatory status, abundance and strength of tight junction proteins, the mucus layer integrity, the microbiome, and metabolites produced by commensal bacteria. Intestinal permeability and compromised gut barrier integrity are the main causes for inflammation, which results in disease. One study by Snow et al. (2011) demonstrated the protective effect of MFGM in mice challenged with LPS, where the MFGM group showed significantly lower concentrations of inflammatory cytokines therefore reducing gut permeability and leakiness compared with the control group, which died following LPS challenge. Two other studies conducted on mice treated with MFGM and challenged with dextran sulfate sodium exemplified reduced levels of cytokines, which aided in amelioration of colitis-like pathology; this amelioration can be attributed to the MFGM sphingolipids that demonstrate anti-inflammatory effects in the intestine (Wang et al., 2019; Wu et al., 2022). Reduction of disease pathology can also prevent

colitis-induced secondary liver injury via reduction in inflammatory markers and liver enzymes while also increasing glutathione antioxidant concentration. Wu et al. (2022) demonstrated that MFGM induced changes in gene expression related to mitochondrial morphogenesis and antioxidant enzymes as the mechanism behind attenuation of liver injury. Another mechanism for reduction of intestinal disorders like colitis and irritable bowel disease due to gut permeability is through fortification of the mucosal layer and strengthening of tight junctions. Both Wang et al. (2019) and Wu et al. (2022) demonstrated that mice challenged with dextran sulfate sodium and fed MFGM had a decreased disease activity index score related to colitis. This decrease can be attributed to an upregulation of epithelial cell proliferation that resulted in longer colon length and significant amelioration of goblet cell loss. Goblet cells are responsible for mucus secretion; therefore, increased expression of mucin proteins was observed in both studies, which allows for increased barrier strength to prevent a disease state. Along with mucins, the entire mucosal architecture, density, and epithelial cell crypt was improved by MFGM in the colitis models owing to the upregulation of cell proliferation (Le Huërou-Luron et al., 2018; Wang et al., 2019). The mechanism by which MFGM upregulates goblet cell proliferation and modulates differentiation is through balancing the overactive Notch pathway; by reverting the pathway to nearly normal levels, it allows for acceleration and restitution of goblet cells to relieve induced colitis associated with goblet cell depletion (Wang et al., 2019).

Strengthening of tight junction proteins is also an essential aspect of alleviating intestinal disease. Jiang et al. (2022b) demonstrates significant increases in 5 tight junction proteins and their transcripts in a dose-dependent manner in rats fed MFGM. An increase in tight junction proteins not only indicates a reduction of intestinal permeability but also is suggestive of greater intestinal cell differentiation as was observed in rats fed MFGM where goblet, enteroendocrine, and Paneth intestinal cell types were all upregulated (Jiang et al., 2022b). The mechanism by which MFGM may be promoting intestinal cell differentiation is through activation of signaling pathways. Milk fat globule membrane is partially resistant to digestion due to its complex composition; therefore, it can activate multiple cellular signaling pathways such as the PI3K/Akt/mTOR, MAPK, and MLCK, which promote intestinal cell differentiation and allow for increased tight junction protein expression for enhancement of intestinal barrier strength (Jiang et al., 2022b). Another study by Berding et al. (2016) fed piglets MFGM and observed greater intestinal development as indicated by an increase in ileal villus length and height. An increase in villus length and height allows for a greater intestinal surface area for more nutrient absorption. Berding et al. (2016) also observed an increase in tyrosine hydroxylase and vasoactive intestinal peptide which are neurotransmitters that stimulate the intestinal neuromuscular layer, via the enteric nervous system, to increase absorptive capacity through improvements in digestive ability and gut motility. These piglets also showed faster growth, which may suggest that the increase in absorption surface area and digestive capacity allows the piglets greater ability to use the nutrients.

The microbiome plays a great role in gastrointestinal health. Wu et al. (2022), Yu et al. (2021), and Berding et al. (2016) found that MFGM increased the abundance of *Parabacteroides*, *Firmicutes*, *Bifidobacterium*, and anti-inflammatory bacteria. *Firmicutes* are the main butyrate-producing group of bacteria known to colonize the intestinal tract and a lack of *Firmicutes* in the microbiome is associated with various intestinal disease states. An increase in *Firmicutes* with *Bifidobacterium*, anti-inflammatory bacteria, and *Parabacteroides* families allows for healthy modulation of the microbiota and protection against pathogens and disease. This result was evident as piglets fed MFGM exhibited no urinary or intestinal tract infections where the control group did; this lack of infections can be attributed to *Parabacteroides* along with the MUC1 and gangliosides present in MFGM, which have anti-pathogenic activity (Berding et al., 2016). Yu et al. (2021) found that the increase in *Firmicutes* resulted in a great amount of butyrate, which is an important energy source for epithelial cells and aids with inflammatory inhibition. The observed actions of butyrate on the intestine led to an increase in mucin protein production to activate the NLRP6 inflammasome pathway, which enhances the mucus barrier. Together, these findings suggest that MFGM can help maintain intestinal homeostasis.

Immune Health. Several biologically active components present in milk have immunomodulatory properties, including the MFGM. Regulation of the immune system is the balance between both suppression and enhancement of various actions in response to the environment (Zanabria et al., 2014). A study conducted on piglets fed a formula with milk fat emulsified with MFGM and milk proteins demonstrated accelerated immune maturation, enhancement of immune function, and increased presence of immunoreactive peptides (Le Huërou-Luron et al., 2018). Milk fat globule membrane is resistant to digestion, which allowed for greater immunoreactive β-lactoglobulin, casein residuals, and short- and medium-chain fatty acids in the intestine. These intact proteins and fatty acids promote maturation of the immune system through sustaining IFN-γ production by T helper cells as part of cellular immunity, which directly accelerates immune system maturation (Zanabria

Table 3. Fat and protein composition of different MFGM sources[1]

Material	Fat, %	Protein, %	Reference
MFGM-10	17.4	72.6	Chitchumroonchokchai et al., 2023
WPPC	24.4	62.7	Ozturk et al., 2022
MFGM WPC	17.6	73.3	Ponchon et al., 2024
α-LA and MFGM-enriched WPC	10.8	80.7	Ponchon et al., 2024
Sweet whey	16	73	de Boer, 2014
Buttermilk	7–13	30	de Boer, 2014
MFGM WPC	18	80	Sun et al., 2023
MFGM isolate (buttermilk isolate)	20	68	Snow et al., 2011

[1]MFGM = milk fat globule membrane; WPC = whey protein concentrate; WPPC = whey protein phospholipid concentrate.

et al., 2014; Le Huërou-Luron et al., 2018). The piglets also exemplified greater proliferation and inflammatory function in mesenteric lymph nodes giving them a cellular function similar to that of suckling piglets (Le Huërou-Luron et al., 2018). Another study conducted on mice splenocytes demonstrated the ability of MFGM to prevent immunological overreaction in preactivated immune cells (Zanabria et al., 2014). When immune cells are activated, they begin to proliferate and exert an immune response through secretion of cytokines; MFGM addition to activated cells attenuated and reduced proliferation back to baseline levels, decreased apoptosis of the cells, and worked as an anti-inflammatory agent to modulate the immune response to stimulation (Zanabria et al., 2014). Milk fat globule membrane contains proteins that can bind immune stimulating mitogens and prevent immune cell activation, resulting in less cytokine production for immune response ablation. Through MFGM protein and lipid action, the immune system can be matured and regulated, which is an essential function for breast-fed infants.

Clinical Evidence for the Bioactive Effects of the MFGM

Several clinical studies have established the effect of MFGM-enriched infant formulas on different types of development including neurodevelopment, intestinal development, and microbiota modulation in various infant cohorts. A number of clinical studies have focused on the use of the whey-derived MFGM-10 ingredient in infant nutrition. One can refer to Table 3 for details on the fat and protein composition of this MFGM ingredient.

The Impact of MFGM on Neurodevelopment and Cognitive Function. The influence of MFGM on cognitive development in infants has been a key focus area of research on MFGM ingredients, as their components, mainly phospholipids like SM, are associated with the structural and cognitive development of the brain. The composition of the brain is mostly lipids (60%) including cholesterol and phospholipids, which are also components found in the MFGM (O'Brien and Sampson, 1965; Lopez, 2020). Li et al. (2019) studied the impact of stage 1 and 2 MFGM and lactoferrin-fortified infant formulas on 233 infants; the control group (n = 228) was given standard formula, but there was no reference group of breast-fed infants used in this study. Language, motor and cognitive development, and attention were measured up to 545 d of age. The results showed that at 12 mo, the infants fed the formula with added MFGM and lactoferrin had higher neurodevelopmental scores than the control group, especially in cognitive, language, and motor scores. These results were attributed to contribution of phospholipids in MFGM, and similar results were observed in vivo in a rat pup model (Brink et al., 2019). There have also been some in vivo studies on the role of lactoferrin in cognitive function, so the combination of MFGM and lactoferrin may have attributed to the positive outcomes on neurodevelopment (Chen et al., 2015; Zheng et al., 2020). At 18 mo, little to no difference was observed for the cognitive development between both groups, but there were some differences between groups in some elements of language assessment. There were no differences in temperament at 12 or 18 mo between the control and experimental group. Timby et al. (2014) also observed similar outcomes for the impact of the MFGM-10 ingredient in infant formulas on cognitive development in neonates. A total of 240 infants were recruited, including 80 breast-fed infants as a reference group (Timby et al., 2014). The 160 infants were assigned either the low-protein, low-energy MFGM-supplemented formula or a standard formula up to 6 mo of age. It was found that those fed the MFGM-supplemented formula had higher cognitive scores than those in the control group, which were more similar to the breast-fed group. The breast-fed infants had better verbal domain scores compared with both formula-fed groups.

Several other clinical studies have investigated the effect of other MFGM ingredients on neurodevelopmental outcomes in infant cohorts. An experimental group of

infants were given a formula containing MFGM, fructo-oligosaccharides, *B. infantis*, *Lb. rhamnosus*, PUFA, and nucleotides (Nieto-Ruíz et al., 2019). The control group received a standard infant formula, and a breast-fed reference group was included in the study design. In contrast to the other studies on MFGM supplementation, no significant differences were found in the formula-fed infants or their neurological development, which was measured by general movements at different time points of growth. Perhaps a different approach should have been taken to measure the neurological development of the infants (i.e., the Bayley-III scales used in other cognitive development studies; Timby et al., 2014). However, the visual function of the experimental formula-fed infants was similar to that of the breast-fed infants in the reference group. This clinical trial was part of the COGNIS study; several other measurements of growth and development (i.e., weight and height, were measured over 2.5 yr). In a separate aspect of the study, child behavior was measured (Nieto-Ruíz et al., 2020). There was no difference in behavior between groups at 18 mo. At 2.5 yr, the children fed standard formula were frequently classed as borderline on internalizing, total problems, and attention-deficit hyperactivity disorder compared with the breast-fed infants. The experimental group presented fewer clinical pathological affective problems compared with the standard formula–fed group. Of the infants fed the experimental formula containing MFGM, there was a higher percentage of children that had normal behavior, similar to that of the breast-fed infants. There were few affective problems in the infants fed the experimental formula than those fed standard formulas and breast-fed infants at 2.5 yr.

More recently, Schipper et al. (2023) studied the impact of dairy lipids derived from MFGM in infant formula on cognitive development and compared with a standard formula-fed and breast-fed infants. It was found that α-linolenic acids levels were higher in erythrocytes of both formula-fed groups compared with breast-fed infants. The levels of eicosapentaenoic acid were higher in erythrocytes of the experimental group than standard formula-fed infants and closer to levels in the breast-fed infants. The dimensional change card sort test scores were higher in the experiment group compared with the control group, and the Flanker inhibitory control and attention test levels of infants fed experimental formula were also comparable to those of breast-fed infants. A more recent study by Chen et al. (2024) comprehensively reviewed cognitive development of infants fed infant formula containing 1,3-dioleolyl-2-palmitate (**OPO**) and MFGM, which mimics the lipid composition in breast-milk. After 4 mo, gross motor skills and development quotient scores were similar in the MFGM and OPO group to breast-fed infants, but the scores were not statistically significantly different from standard formula or breast-fed infants. However, there was no difference in motor skills between groups after 6 mo. A relatively small study size was used for the experimental groups (n = 17 for MFGM and OPO formula and n = 12 for the control group given standard formula) compared with the 50 infants in the breast-fed reference group.

Separately, a study where a cream-derived MFGM ingredient was added to infant formula showed the positive impact of this ingredient on neurodevelopment in infants (Xia et al., 2021). The composite social-emotional and general adaptive behavior scores were higher in MFGM-enriched formula-fed infants than standard formula-fed group. The cognitive, language, and motor scores were also higher in MFGM group than the standard formula-fed group. Furthermore, there was a significant difference in cognitive score between standard formula and breast-fed infants, but not in MFGM formula-fed infants at 6 mo. Short-term memory was also higher in MFGM formula-fed infants than standard formula-fed infants at 12 mo. This result could suggest that specific fatty acids released from the MFGM ingredient during lipolysis have a role in the development of the hippocampus, a region of the brain that is critical for memory function. The MFGM is a rich source of dairy lipids that contain both saturated and unsaturated fatty acids. Gangliosides, SM, and PS in particular are lipids that are associated with neurodevelopment and brain function, as reviewed by others (Kim et al., 2014; Guillermo et al., 2015; Pan et al., 2023). After their release and absorption during digestion, they could be transported and utilized in certain pathways associated with brain development and health. Phosphatidylserine is an important component of the brain with docosahexaenoic acid (**DHA**); phosphatidylcholine and phosphatidylethanolamine are precursors to the synthesis of PS, so the dietary intake of these lipids could therefore affect development of various aspects of the brain, including the hippocampus, which is associated with memory (Kimura and Kim, 2013; Kimura and Kimura, 2021). A small amount of DHA could also be released when PS is digested to contribute to hippocampal development (Kim et al., 2014). Interestingly, there was an increase in serum gangliosides in breast-fed infants and MFGM formula-fed infants at 4 mo. A follow-up study of children aged 6.5 yr by Timby et al. (2021) observed no difference in children given the MFGM-enriched formula compared with those that were breast-fed in cognitive tests, growth parameters, or plasma concentrations of lipids, insulin, or glucose. Supplementation with MFGM had no overall effect on neurodevelopment at 6.5 yr. There were only slightly higher IQ scores in children that were breast-fed compared with those that were formula-fed. Combined the results of these clinical studies on neurodevelopment and cognition suggest that

MFGM has a role in accelerating neurodevelopment in early stages of life in formula-fed infants that is closer to the neurodevelopment of breast-fed infants. This finding may be attributed to the presence of SM, a key phospholipid that is involved in the myelination process in the brain (Schneider et al., 2019).

The results above are similar to studies by Schneider et al. (2023) and Deoni et al. (2018), where the intervention groups received a formula with a blend of nutrients, including a WPC 80% (**WPC-80**) ingredient co-enriched with MFGM components and α-LA, to study the impact of myelination in the brain and improve overall cognitive outcomes. Compared with the control group, there was an obvious difference in myelination in different regions of the brain between groups shown by neuroimaging. In contrast to the study by Timby et al. (2021), the study by Schneider et al. (2023) was up to 2 yr of age where differences were still observed for the infants fed a blend of nutrients including MFGM from an enriched WPC-80 ingredient. A possible explanation for this difference is that at different stages of growth, there are compositional changes in breastmilk components that are required for the brain's developmental process (Chen and Ma, 2023). Schneider et al. (2023) correlated different nutrients to myelination at different stages (i.e., arachidonic acid intake at 12 and 24 mo and docosahexaenoic acid intake at 24 mo). This proposed mechanism is analogous to the changes in composition observed in breastmilk throughout lactation; the lactose, fat, and protein ratios change to cater to the nutritional needs of the neonate for growth and development at different life stages (Chen and Ma, 2023). The myelin water fraction of the brain had a positive correlation to the improved motor scores observed at 3, 6, and 12 mo. There were some positive correlations found for cognitive development and gray matter volume, but this had a negative correlation to the myelin water fraction. The relationship between anger levels and the myelin water fraction in the whole brain and gray matter were negatively correlated but were not statistically significant. They observed an improvement in relation to sleep and social ability; night awakenings were less frequent in the infants given the experimental formula, and less social fearfulness was observed in this group over 24-mo trial visits. The improvement in sleep may have been attributed to the α-LA-enriched WPC-80 ingredient (Layman et al., 2018). Although this clinical study found no statistically significant difference in neurodevelopment for cognitive, language, and motor scores or overall social-emotional development, infant behavior, and performance tasks, there were still some positive correlations and differences between myelination and cognitive outcomes data at each time-interval between groups. This suggests that that the infants fed the MFGM-enriched formula had improved myelination in different regions of the brain that had an impact on their overall cognitive development up to 2 yr of age.

These clinical studies validate some of the bioactive mechanisms by which MFGM could promote neurodevelopment in the early stages of life. Although the studies reviewed above included MFGM or its components in the experimental infant formulas, the combination of other nutritive components should also be considered, for example, the formulas with added probiotics and PUFA. It is known that PUFA also support cognitive function in healthy individuals and that probiotics support gut health (Bazinet and Layé, 2014; Barkhidarian et al., 2021). Some of the benefits observed may be derived from intakes of such nutritive factors. However, these effects may also be indicative of the interaction between MFGM fragments and the matrix. Furthermore, the lipid fraction of MFGM ingredients is often associated with cognitive development, and more research is required on the identification of the specific lipids or fatty acids derived from MFGM that support this bioactivity. It has already been shown in vitro that cells could alter their lipid composition when subjected to lipids and that this effect can also be matrix-dependent (Kosmerl et al., 2023b; Martínez-Sánchez et al., 2023). The mechanism behind this selective alteration should be investigated further to understand the cells' behavior in terms of which fatty acids they favor at absorption. Based on the clinical evidence, it may be concluded that MFGM contributes, to some extent, to the neurodevelopment of formula-fed infants in a way that is similar to that of breast-fed infants.

The Impact of MFGM on Gut Microbiome, Health, and Metabolism. Another area of growing interest in MFGM research is the impact of these ingredients on the gut microbiome. Recent research suggests that there is a significant link between the gut and brain; the mechanisms of this phenomenon are still ongoing (Yang et al., 2016; Laue et al., 2022). There is speculation that the colonization of the infant's gut commences in utero, as studies have found various phylum present in the placenta postpartum (Aagaard et al., 2014). The placenta provides all nutrients and stores waste from the fetus during pregnancy. The infant's gut microbiota can depend on the mode of delivery, i.e., Cesarean section or vaginal; the intestinal colonization will reflect the maternal vaginal flora (*Lactobacillus*) or epidermal (*Staphylococcus*). In addition, fewer *Bacteroides* and *Bifidobacterium* are present in the gut of infants delivered via C-section. The feeding pattern and antibiotic exposure can also influence the gut microbiota of the infant. It is known that breast-fed infants and formula-fed infants have a difference in their microflora diversity (shown in Table 4). Regardless of feeding type, breastfeeding and parental feeding both influence the colonization patterns in the

neonate. Breastmilk provides the infant with all essential macronutrients, including antimicrobial proteins, oligosaccharides, fatty acids, and secretory IgA. However, breastmilk is not sterile; it also provides a source of live bacteria within the milk and from the contact and transfer of the flora present on the mother's skin during nursing (Kordy et al., 2020). Human milk oligosaccharides (**HMO**) stimulate the growth of *Bifidobacterium* and prevent attack by enteropathogens (Garrido et al., 2016). However, as infant formulas are becoming more humanized, similar components are being added, such as HMO, MFGM, and probiotics (*B. infantis*, *Lactobacillus reuteri*); however, some of these components may be structurally different due to processing and origin. It has been found that formula-fed infants have a more diverse range of bacterial species in their gut compared with those that are breast fed (Yang et al., 2016). The addition of MFGM to infant formulas may establish a gut microbiome composition like that of breast-fed infants.

Infants have an undeveloped digestive tract compared with adults, which has implications for the digestion of breastmilk and infant formula (Indrio et al., 2022). Similarly, the gut function and immune system of the infant is also undeveloped; preterm infants are at higher risk of leaky gut, as the neonate is more susceptible to infection by pathogenic bacteria (Sanidad and Zeng, 2020). It is suggested that the gut microbiome can be linked to the development of the immune system and, possibly, the brain (Indrio et al., 2022).

In vitro studies have shown that MFGM-enriched ingredients promote the growth of probiotic bacteria and therefore, suggest there is some modulatory effect from MFGM. Some studies have investigated changes of the flora within the gut of infants fed MFGM compared with breast-fed infants (Lee et al., 2018a; He et al., 2019b; Chichlowski et al., 2021). Minor differences in the abundance of fecal bifidobacteria between both formula-fed groups was seen by He et al. (2019b). As expected, the microbiome was indistinguishable between groups when complimentary feeding started. Similar results were observed by Chichlowski et al. (2021) and Lee et al. (2021); there were subtle differences in the stool microbiome of infants fed infant formula containing MFGM. The latter study found that the MFGM-enriched formula altered the microbiota-associated metabolites and microbial diversity. It was found that there were differences in abundance of species at d 120 between the experiment and control group compared with the baseline (Chichlowski et al., 2021). *Bacteroides plebeius* and *Bacteroides uniformis* were more abundant in the infants fed MFGM and lactoferrin formula, whereas *Bifidobacterium adolescentis* and *Ruminococcus* sp. were more abundant in the control group. Furthermore, the addition of MFGM promoted growth of *Lactobacillus* compared with bifidobacteria in the gut of infants that received this formula (Cerdó et al., 2022); this growth may have been attributed to the presence of prebiotics added to the formula in combination with PUFA and MFGM. The species richness was lower in formula-fed infants than those that were breast fed at 6 mo. Contrary to the above results, an MFGM-supplemented formula with OPO showed that gut microbiota composition was similar between the MFGM and OPO group and breast-fed infants with the dominant genus being *Bifidobacterium* (Chen et al., 2024). However, after 1 mo, the abundance differed between the standard formula group and the MFGM group; this difference was not significant between the breast-fed group and the MFGM and OPO group. The above results could support that MFGM supplementation could have a bifidogenic effect, as it has shown some modulatory effect in the stool microbiome.

It seems that MFGM affects the metabolites produced via the microbiota more than inducing compositional differences in fecal microbiota (He et al., 2019a,b; Chichlowski et al., 2021; Lee et al., 2021). In a study analyzing the fecal metabolome of infants fed formulas containing MFGM, it was found that the fecal metabolites were associated with the amount of water present in the infant's stool, and less water was found in the stool of the formula-fed group (He et al., 2019b). It was also found that there were more amino acid degradation metabolites than sugar monomers/carbohydrate fermentation by-products in the formula-fed group. Lower levels of certain amino acids, 2-hydroxyisovalerate, cadaverine, and 4-hydroxyphenyllactate, were also observed in the MFGM formula group compared with standard formula group. However, supplementation did not change the fecal metabolome to the extent of HMO; this finding may be due to other ingredients present within the formula. These results are similar to those of He et al. (2019a) and Lee et al. (2021); the breast-fed infants had a preference for fat metabolism, but formula-fed infants favored protein metabolism. Interestingly, the study by Lee et al. (2021) found that supplementation with MFGM suppressed protein degradation pathways and levels of insulinogenic amino acids and facilitated fatty acid oxidation and ketogenesis. This metabolism in infants fed the MFGM-enriched formula was more like that of breast-fed infants; this result suggests that perhaps the interfacial composition of the emulsions formed during processing of the infant formula influences the metabolic pathways during digestion in the infant. The study by Chichlowski et al. (2021) also observed trends in the stool metabolites; lactate was found in the stools of infants fed the MFGM and lactoferrin supplemented formula, which can be found in high levels in breast-fed infants. This could be evidence for the presence of *Bifidobacterium* and *Bacteroides*, among others, which are producers of lactate.

One likely reason for the preference in protein degradation pathways in the formula-fed infants is due to the presence of milk proteins present on the lipid droplet surface. In human milk, the surface load of the fat globule is predominantly MFGM components, which are mostly phospholipids and MFGM proteins; this composition could favor the fatty acid metabolic pathways, as lipase can adsorb to the surface of fat globules with ease during digestion. The order of addition for MFGM and protein ingredients during infant formula processing should be considered by infant formula manufacturers; in vitro digestion studies have showed that the rate of lipolysis differs based on the lipid droplet coating when the emulsion is formed. However, the authors of the study did not describe the processing of the experimental formula, so it could be difficult to support this conclusion in a human study without these details (Lee et al., 2021).

Another possible reason for the difference in metabolism could be due to cells in favor of fatty acid uptake from MFGM components as opposed to vegetable-derived fatty acids in a food matrix, as shown by Kosmerl et al. (2023b) and Martínez-Sánchez et al. (2023). The epithelial cells may be more sensitive to the uptake of dairy-derived fatty acids from triglycerides, and these are more easily digested in the form of short-chain fatty acids or medium-chain fatty acids (Gómez-Cortés et al., 2018). Long-chain fatty acids released during digestion require assistance to be absorbed by villi during gastroduodenal digestion. The rate of lipolysis may control the subsequent breakdown of other nutrients such as proteins during gastroduodenal digestion, and the interaction between MFGM and other lipids should be considered to improve the metabolism of infant formula. More recent infant formula designs have included both dairy- and vegetable-derived lipids to mimic the fatty acid profile of human milk (Liu et al., 2022).

In terms of the effect of MFGM-enriched infant formula on gut health and immunity, it was found that both the incidence of diarrhea and respiratory-associated adverse events were reduced in infants fed this formula type with lactoferrin (Li et al., 2019). Based on the results from in vivo and in vitro studies, this result could be due to the presence of MFGM proteins upregulating the expression of tight junction proteins, which prevent a leaky gut (Jiang et al., 2022b). As a bioactive protein, lactoferrin has also been shown to reduce diarrheal episodes in vivo and in human trials (Ochoa et al., 2013; Li et al., 2014). A study by Jiang et al. (2022a) obtained similar results to those of Li et al. (2019); the MFGM formula was well tolerated among the infants who were fed this formula, and there was a similar incidence of diarrhea to that of breast-fed infants. Few clinical studies have investigated the immunomodulatory impact of MFGM-enriched formulas (Li et al., 2021). The MFGM formula-fed group had lower concentrations of cytokines, IL-2, and IL-17A, than those in the standard formula-fed group. The cytokine concentrations in the MFGM formula group were comparable to the breast-fed group. These results are in agreement with those of Lee et al. (2018b); IL-2 levels were lower in the MFGM group compared with the control group of infants. This result suggests that MFGM may have immunomodulatory capacity. However, more study on this mechanism is required as this study had limitations to its design; there was no blood sampling at the baseline to compare the cytokine response before and after the intervention (Li et al., 2021).

Table 4. Difference in microflora species by feeding type in infants; adapted from Gritz and Bhandari (2015)

Breast-fed	Formula-fed
Bifidobacteria	Bifidobacteria species
Enterobacteria species	*Escherichia coli*
	Clostridium difficile
	Bacteroides species
	Prevotella species
	Lactobacillus species

The role of MFGM in modulating the gut microbiota has been shown to be minimal in clinical studies, with some promising results in the study with the formula containing both MFGM and OPO oil blend (Chen et al., 2024). However, there were limitations to this study, such as sample size for the experimental group compared with the breast-fed group. This study is another example of the need to investigate MFGM within complex food matrixes such as those with human milk fat analogs. In vitro digestion studies have provided evidence that the rate of lipolysis differs depending on the way in which the formulas are processed (Pan et al., 2024a; 2024b; Sun et al., 2024). Similarly, the lipid matrix in which MFGM is formulated should also be studied closely. Few clinical studies have demonstrated the impact of MFGM on anti-inflammatory and immune development. These studies may support the idea that MFGM supports the immune system of vulnerable infants with an undeveloped immune system, especially preterm infants. These clinical studies also suggest that MFGM can alter metabolic pathways during its digestion in an infant formula matrix. The specific component or composition of the MFGM fraction responsible for this modulatory effect should be investigated further. This investigation may provide a better understanding of the metabolism of infant formula to achieve a metabolism that is closer to human milk. Nevertheless, Table 5 provides a comprehensive overview of the numerous clinical trials conducted to evaluate the diverse health benefits of MFGM-enriched infant formulas across various infant populations. These studies have explored a wide spectrum of potential advantages associated with this nutritional enhancement.

Table 5. Summary of clinical studies on MFGM-enriched infant formula in infant cohorts[1]

MFGM source	Bioactivity	Study design/treatment	Analysis/objective	Results	Reference
MFGM-10	Neurodevelopment	Experiment group: Stage 1 and 2 infant formulas with 5 g/L MFGM + 0.6 g/L Lf Control group: Standard infant formula no MFGM/lactoferrin	Measured motor, language, and cognitive development, attention, and incidence of diarrhea and respiratory-associated adverse events through 545 d of age	Neurodevelopmental scores were higher for infants fed MFGM + Lf formula than control group at 12 mo. Lower incidence of diarrhea and respiratory-associated adverse events in the trial group.	Li et al., 2019
Unspecified	Neurodevelopment	Experiment group: MFGM, FOS, *B. infantis*, *Lb. rhamnosus*, PUFA, and nucleotides Control group: standard infant formulas without above nutrients Reference group: breast-fed infants	Measured visual function and neurological development by general movements test	No difference in neurological development; similar visual function to breast-fed infants.	Nieto-Ruíz et al., 2019
Unspecified	Neurodevelopment	Experiment group: MFGM, FOS, *B. infantis*, *Lb. rhamnosus*, PUFA, and nucleotides Control group: standard infant formulas without above nutrients Reference group: breast-fed infants	Measured child behavior	No difference at 18 mo; at 2.5 yr standard formula children were classed frequently as borderline on internalizing, total problems, and attention-deficit hyperactivity disorder than breast-fed infants. Experimental group presented less clinical pathological affective problems compared with the standard-fed group. A higher percentage of children fed the experimental formula had normal behavior that was similar to BF infants.	Nieto-Ruíz et al., 2020
MFGM-10	Gut microbiome	Experimental group: formula with MFGM + Lf Control group: standard formula	Measured stool metabolite	Subtle differences in stool microbiome and metabolome of infants fed formula with MFGM + Lf.	Chichlowski et al., 2021
Unspecified	Ingested tolerance	Experimental group: MFGM-enriched formula Control group: standard formula Reference group: breast-fed infants	Tolerance and safety events and anthropometric measurements	Formula was safe and well tolerated in healthy term infants. Less diarrhea in infants fed MF, incidence similar to breast-fed infants.	Jiang et al., 2022a
MFGM-10	Immunity	Experimental group 1: *Lb paracasei* F19 formula Experimental group 2: MFGM formula Control group: standard formula Reference group: breast-fed infants	Seven cytokines analyzed, anti-diphtheria IgG, and anti-polio virus IgG concentrations before and after second and third immunizations	MFGM group had lower IL-2 and IL-17A concentrations than standard formula. The cytokine concentrations were comparable to breast-fed group, suggest MFGM has immunomodulatory capacity.	Li et al., 2021

Continued

Table 5 (Continued). Summary of clinical studies on MFGM-enriched infant formula in infant cohorts[1]

MFGM source	Bioactivity	Study design/treatment	Analysis/objective	Results	Reference
MFGM-10	Gut microbiome	Experimental group: MFGM-supplemented formula Control group: standard formula Reference group: breast-fed infants	Characterization of fecal microbiome and metabolome, gut microbiome	Minor difference in the abundance of fecal bifidobacteria between both formula-fed groups Fecal metabolite was associated with amount of water in stool, less water in formula-fed groups' stool; more amino acid degradation metabolites vs. sugar monomers/carbohydrate fermentation by-products in formula-fed group. The microbiome and metabolome were indistinguishable between groups when complimentary feeding started. Lower levels of certain amino acids, 2-hydroxyisovalerate, cadaverine, and 4-hydroxyphenyllactate were observed in the MFGM formula group compared with standard formula group. Supplementation did not change the fecal metabolome to extent of HMO; maybe due to other ingredients.	He et al., 2019b
MFGM-10	Metabolism and gut microbiome	Experimental group 1: MFGM-enriched formula Control group: standard formula Reference group: breast-fed infants	Serum metabolome measured; fecal microbiota analyzed	Supplementation of MFGM suppressed protein degradation pathways and levels of insulinogenic amino acids. Facilitated fatty acid oxidation and ketogenesis. MFGM did not induce compositional difference in fecal microbiota but altered the microbiota-associated metabolites and microbial diversity.	Lee et al., 2021
SureStart MFGM Lipid 100	Neurodevelopment	Experimental group: MFGM-enriched formula stage 1 and 2 Control group: standard formula stage 1 and 2 Reference group: breast-fed infants	Neurodevelopment via Bayley-III scales	Composite social-emotional and general adaptive behavior score were higher in MFGM-enriched formula-fed infants than standard formula. Cognitive, language, and motor were higher in MFGM group than standard formula. Significant difference in cognitive score between standard formula and breast-fed infants, but not MFGM formula fed infants at 6 mo. Short-term memory was higher in MFGM formula-fed infants than standard formula-fed infants at 12 mo. Increase in serum gangliosides in breast-fed infants and MFGM formula fed infants at 4 mo.	Xia et al., 2021

Continued

Table 5 (Continued). Summary of clinical studies on MFGM-enriched infant formula in infant cohorts[1]

MFGM source	Bioactivity	Study design/treatment	Analysis/objective	Results	Reference
Unspecified	Neurodevelopment and gut microbiome	Experimental group: OPO + MFGM formula Control group: Standard formula without MFGM or OPO References group: breast-fed infants	Gut microbiota of fecal samples, Children Neuropsychological and Behavior Scale-Revision 2016 (CNBS-R2016), Bone mineral density	Growth patterns of MFGM + OPO formula similar to breast-fed infants. After 4 mo, gross motor skills and development quotient scores were similar in MFGM + OPO. group to breast-fed infants but not statistically significant to standard formula or breast-fed infants No difference in motor skills between groups after 6 mo. Higher bone mineral density in the MFGM + OPO group but no statistically significant difference between all groups. The gut microbiota composition was similar between the MFGM + OPO group and breast-fed infants. The dominant genus was *Bifidobacterium;* however, after 1 mo the abundance differed between the standard formula group and the MFGM group but not significantly between the reference group and MFGM + OPO.	Chen et al., 2024
MFGM-10	Neurodevelopment and gut microbiome	Experimental group: synbiotics-, PUFA-, and MFGM-supplemented formula Control group: standard formula Reference group: breast-fed infants	Characterization of microbial enterotypes, BSID-III at 12 mo, PLON-R at 4 yr	Cognitive, motor, and language were lower in infants with a slow gut microbial maturation. The experimental formula had more *Lactobacillus* growth than *Bifidobacterium* in the gut. Language and expressive scores were significantly different in infants with fast gut microbial maturation compared with breast-fed infants.	Cerdó et al., 2022
MFGM-10	Neurodevelopment and growth	Experimental group: MFGM-supplemented formula Control group: standard formula Reference group: breast-fed infants	Bayley-III	The cognitive score was 4.0 higher in the MFGM group than the standard formula group. Formula-fed babies had higher plasma insulin concentrations than breast-fed infants at 4 and 6 mo.	Timby et al., 2014
Unspecified	Cognitive development	Experimental group: MFGM/MPL droplet formula Control group: standard formula Reference group: breast-fed infants	Neurocognitive function and erythrocyte fatty acid composition	ALA levels were higher in both formula group compared with breast-fed infants, eicosapentaenoic acid was high in concept group than standard formula fed infants and closer to breast-fed infants. DCCS scores were higher in experiment group compared with control. The FICA levels of infants fed experimental formula were comparable to breast-fed infants.	Schipper et al., 2023

Continued

Table 5 (Continued). Summary of clinical studies on MFGM-enriched infant formula in infant cohorts[1]

MFGM source	Bioactivity	Study design/treatment	Analysis/objective	Results	Reference
MFGM-10	Metabolism	Experimental group: MFGM isolate-supplemented formula Control group: standard formula Reference group: breast-fed infants	Amino acid metabolism, fatty acid oxidation (metabolomics)	Breast-fed infants have preference for fat metabolism, but formula-fed infants favored protein metabolism.	He et al., 2019a
MFGM-10	Neurodevelopment and growth	Experimental group: MFGM-supplemented formula Control group: standard formula Reference group: breast-fed infants	Cognitive and executive functions, plasma lipids	No difference in cognitive tests, growth parameters, or plasma concentrations of lipids, insulin, or glucose. Overall, no effect on neurodevelopment at 6.5 yr.	Timby et al., 2021
α-MFGM WPC	Cognitive development and growth	Experimental group: folic acid, iron, vitamin B12, ARA, DHA, phospholipid- and α-LA-enriched formula Control group: formula with Lower enrichment than experimental group Reference group: breast-fed infants	Cognitive and behavioral assessment, physical growth, brain imaging	Increased myelination in infants fed experimental formula. The sleep and sociability improved in experiment group compared with control group. Improved motor development in the experimental group at 12 mo. Less social fearfulness in infants fed experimental formula. Reduced anger levels up to 24 mo.	Schneider et al., 2023

[1]Lf = lactoferrin; BF = breast-fed; BSID-III; Bayley Scales of Infant and Toddler Development, third edition; PLON-R = Prueba de Lenguaje Oral de Navarra–Revisada; DCCS = dimensional change card sort; FICA = flanker inhibitory control and attention test; OPO = 1,3-dioleolyl-2-palmitate.

TAKE-HOME MESSAGES

Some of the possible mechanisms for the bioactive effects of MFGM that have been reported are outlined in this review. It is clear that processing of milk and MFGM ingredients has implications for the individual MFGM proteins, which could enhance or hinder the effects of these proteins in biological studies. However, a knowledge gap still exists in understanding the effects of processing, MFGM source, and composition on biological function. Further study is required on the matrix in which MFGM is present (e.g., yogurt, infant formula, milk, or supplements) to understand the possible interactions between MFGM constituents and other components (e.g., milk proteins and carbohydrates). Losses of phospholipids during processing and the implications for the biological, physicochemical, and functional aspects of the MFGM need to be studied in detail. Further studies on physicochemical changes occurring among the MFGM components due to processing of milk products and during isolation of MFGM, and the effects of those interactions on its biological activity, could offer deeper insights into the mechanisms of the components. However, it is clear that the combination and synergy of functional milk phospholipids and MFGM proteins are responsible for the biological activities regardless of structural characteristics, as these are disrupted with processing. This would be useful in designing strategies for targeted isolation of MFGM or its components with minimized losses and maximized economic value. Furthermore, the differences in macronutrient composition between MFGM ingredients should be standardized to foster innovation of novel nutritional products. The phospholipid profile of MFGM sources has been identified as the cornerstone for the range of health benefits associated with consumption. However, despite the concentrations of individual phospholipids being varied across MFGM sources, their presence in the same ratio is key to their function as bioactive compounds.

For some clinical trials, it was difficult to conclude if MFGM alone resulted in the effects observed when other materials, such as lactoferrin and PUFA, were present in the experimental formulas. In addition, not all in vitro studies digested the MFGM matrix before studying biological activities; this factor should be a consideration for future studies, as it would give a more realistic scenario of what would happen in a human system. Although there is strong evidence and well-studied mechanisms to support the effect of the MFGM on gut and immune health, in vitro models are required to study cognitive function and development mechanisms. These mechanisms include protection of beneficial bacteria through the gastrointestinal tract and encouragement of colonization to prevent pathogen and

virus adhesion and reduce the likelihood of gut dysbiosis, distress, and permeability. Animal studies also supported the ability of MFGM ability to reduce intestinal permeability, promote neurogenesis, and regulate immune maturation, all via respective signaling pathways which promote the necessary protein production to support these benefits. Although there is support from in vivo studies to suggest some impact on brain function, the results from the clinical studies on infant cohorts are inconsistent; cell models could provide insights into potential mechanisms that could be factored into such studies to provide more robust evidence for the role of MFGM in neurodevelopment. The MFGM is a unique complex with many components that have demonstrated effects on brain, gut, and immune health and development, and has been shown to be well tolerated among infant cohorts. Overall, there is strong evidence that a variety of MFGM-derived materials have shown biological activity, which provides benefits to enhance human health.

NOTES

Funding for this project was provided by the JT Parker Endowment for Dairy Foods of the Food Science and Technology Department of The Ohio State University (Columbus, OH). No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: BF = breast-fed; BSID-III = Bayley Scales of Infant and Toddler Development, third edition; CLSM = confocal laser scanning microscopy; DCCS = dimensional change card sort; DHA = docosahexaenoic acid; FICA = flanker inhibitory control and attention test; HMO = human milk oligosaccharide; HPP = high-pressure processing; LAB = lactic acid bacteria; LC-MS/MS = liquid chromatography tandem MS; Lf = lactoferrin; LTLT = low temperature, long time; M1 = washing method 1: 3,000 × *g*, 5 min, cream + 3 washes; M2 = washing method 2: 3,750 × *g*, 15 min, one wash; M3 = washing method 3: 15,000 × *g*, 20 min, cream + 3 washes; MFGM = milk fat globule membrane; MFGM-10 = Lacprodan MFGM-10; OPO = 1,3-dioleolyl-2-palmitate; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PEF = pulsed electric field; PI = phosphatidylinositol; PL = phospholipid; PLON-R = Prueba de Lenguaje Oral de Navarra–Revisada; PS = phosphatidylserine; SFE = supercritical fluid extraction; SM = sphingomyelin; WPC = whey protein concentrate; WPC-80 = WPC 80%; WPPC = whey protein phospholipid concentrate.

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