Published in final edited form as:

Newborn (Clarksville). 2024 ; 3(1): 19–37. doi:10.5005/jp-journals-11002-0085.

Milk Fat Globules: 2024 Updates

Akhil Maheshwari[1,2], **Harshvardhan Mantry**[3], **Nitasha Bagga**[2,4], **Adrianna Frydrysiak-Brzozowska**[2,5], **Jargalsaikhan Badarch**[2,6], **Md Mozibur Rahman**[2,7]

[1]Department of Pediatrics, Louisiana State University, Shreveport, Louisiana, United States of America

[2]Global Newborn Society, Clarksville Maryland, United States of America

[3]Department of Physics, University of Illinois at Urbana-Champaign, Champaign, Illinois, United States of America

[4]Neonatology, Rainbow Children's Hospital and Birthright, Hyderabad, Telangana, India

[5]The Mazovian University in Płock, Collegium Medicum, Faculty of Health Sciences, Płock, Poland

[6]Department of Obstetrics, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia

[7]Neonatology, Institute of Child and Mother Health, Dhaka, Bangladesh

Abstract

Milk fat globules (MFGs) are a remarkable example of nature's ingenuity. Human milk (HM) carries contains 3–5% fat, 0.8–0.9% protein, 6.9–7.2% carbohydrate calculated as lactose, and 0.2% mineral constituents. Most of these nutrients are carried in these MFGs, which are composed of an energy-rich triacylglycerol (TAG) core surrounded by a triple membrane structure. The membrane contains polar lipids, specialized proteins, glycoproteins, and cholesterol. Each of these bioactive components serves important nutritional, immunological, neurological, and digestive functions. These MFGs are designed to release energy rapidly in the upper gastrointestinal tract and then persist for some time in the gut lumen so that the protective bioactive molecules are conveyed to the colon. These properties may shape the microbial colonization and innate immune properties of the developing gastrointestinal tract. Milk fat globules in milk from humans and ruminants may resemble in structure but there are considerable differences in size, profile, composition, and specific constituents. There are possibilities to not only enhance the

Corresponding Author: Akhil Maheshwari, Department of Pediatrics, Louisiana State University, Shreveport, Louisiana, United States of America, Akhil@globalnewbornsociety.org.

Authors Contribution

AM wrote the manuscript; HM, MMR, NB, AFB, JB added key components. All the authors critically reviewed and approved the manuscript prior to submission.

Conflict of interest: Dr Akhil Maheshwari is associated as the Editor-in-Chief of this journal and this manuscript was subjected to this journal's standard review procedures, with this peer review handled independently of the Editor-in-Chief and his research group.

nutritional composition in a goal-oriented fashion to correct specific deficiencies in the infant but also to use these fat globules as a nutraceutical in infants who require specific treatments. To mention a few, there might be possibilities in enhancing neurodevelopment, in defense against gastrointestinal and respiratory tract infections, improving insulin sensitivity, treating chronic inflammation, and altering plasma lipids. This review provides an overview of the composition, structure, and biological activities of the various components of the MFGs. We have assimilated research findings from our own laboratory with an extensive review of the literature utilizing key terms in multiple databases including PubMed, EMBASE, and Science Direct. To avoid bias in the identification of studies, keywords were short-listed a priori from anecdotal experience and PubMed's Medical Subject Heading (MeSH) thesaurus.

Keywords

1,4-β-N-acetylmuraminidase; Absorbable sphingosine; Acetyl-CoA carboxylase 1; Acyl-CoA synthetase; Acyl-CoA synthetase long chain family member 3; Acyl-CoA synthetase long chain family member 5; Adipophilin; Adipose differentiation-related proteins; ADPF; Alpha-1-antitrypsin; Annexin; Apocrine-like glands; Apolipoprotein A1; Apolipoprotein A-IV; Apolipoprotein C-III; Apolipoprotein E; Apolipoproteins; Arachidonic acid; Arginine-glycine-aspartate (RGD); *Bacteroidetes* ; Bayley Scales of Infant and Toddler Development II; *Bifidobacterium* ; Bile salt-stimulated lipase; Bone marrow stromal antigen 2; C16-ceramide; C18:0; C24-ceramide; Casein micelles; Cathelicidins; C-C motif chemokine ligand 2; CD9 antigen; Ceramidase; Ceramide; Ceramide-1-phosphate; Cerebrosides; *Chlorella vulgaris* ; Cholesterol; Choline; Chordin-like protein 2; Clusterin; Complement C3; Conjugated linoleic acid; *Coriobacteriaceae* ; De Brouckère mean diameter; Dermcidin; *Desulfovibrionaceae* ; Diacylglycerol acyltransferase 1; Disialylated gangliosides; Docosahexaenoic acid; Elongase; Endoplasmic reticulum *Enterobacteriaceae*; *Enterococcaceae* ; *Erysipelotrichaceae* ; Exosomes; FA-binding protein; Factor V/VIII domain containing; Fagan test of infant intelligence; Fatty acid desaturase; Fatty acid synthase; Fatty acid-binding protein; *Firmicutes* ; Folate receptor alpha; Food matrix; Free-play sustained attention test of Colombo; Gamma-glutamyltranspeptidase 1; Gangliosides; GD3; Gelsolin; Glutathione peroxidase 3; Glycam1; Glycan adhesion factors; Glycerol-3-phosphate acyltransferase 4; Glycobiome; Glycogen synthase kinase-3 β; Glycoproteins; Glycosphingolipids; Glycerol-3-phosphate acyltransferase 4; Glycogen synthase kinase-3 beta; Glycosylation-dependent cell adhesion molecule 1; Glycosylation-dependent cell adhesion molecule-1; GM3 Heat shock protein beta-1; Hormone-sensitive lipase; Human leukocyte antigen II; IgA α-chain; Insulin-like growth factor binding protein 2; Isobutyric acid; Isovaleric acid; Kyoto Encyclopedia of Genes and Genomes; Lactadherin; Lactating mammary gland packages; *Lactobacillus* ; *Lactobacillus rhamnosus* GG; Lactoferrin; Lactophorin; Lactosome; Lanosterol synthase; Large-size MFG; Linoleic; Lipid rafts; Lipid-ordered microdomains; Lipoprotein lipase; Long-chain FA-CoA ligase; Lysophosphatidic acid acyltransferase; Lysozyme; Lysozyme C; MARCKS-related proteinapolipoprotein D; Mastitis; Matrilin-3; MFG epidermal growth factor 8; MFGE8 Microfiltration; Milk fat globule EGF; Milk fat globules; Monocyte differentiation CD14; Mucin 4; Mucins; *Mycoplasma agalactiae* ; NAD(P) dependent steroid dehydrogenase-like; *Nannochloropsis gaditana* ; Neutral glycosphingolipids; Nonspecific lipid transfer protein; Number-weighted mean; O-lined glycan; Pasteurization; Per-Arnt-Sim (PAS) domain 6/7; Perilipin; Perilipin 2; Peroxisomal acyl-coenzyme A oxidase 3; Peroxisomal bifunctional enzyme; Peroxisomal multifunctional enzyme type 2; Phosphatidylcholine; Phosphatidylethanolamine; Phosphatidylinositol; Phosphatidylserine; Phospholipids; PL/TAG; Plasmalogens; Polar lipids; *Porphyromonadaceae* ; Proactivator polypeptide; *Proteobacteria* ; Proteose peptone component 3; *Rikenellaceae* ; *Salmonella enteritides* ; Sauter mean diameter; Small MFGs; Soluble

N-ethylmaleimide–sensitive fusion attachment protein receptor; Sphingoids; Sphingolipid; Sphingolipids; Sphingomyelin; Sphingomyelin phosphodiesterase; Sphingophospholipids; Sphingosine; Sphingosine-1-phosphate; *Spirulina platensis* ; *Staphylococcus aureus* ; Triacylglycerol core; Tail-interacting protein-47; Tenascin; Toll-like receptor 2; Triacylglycerol; Triassic period; Ubiquitin; V/VIII like domains; Visual evoked potential latencies; Xanthine oxidoreductase; α-amylase; α-linolenic acid; αvβ3 integrin receptors; αvβ5 integrin receptors; β-casein; ζ-potential; ω-3 polyunsaturated fatty acids; ω-3 PUFA; ω-6 PUFA

Introduction

Human milk is optimized for specific nutritional yield and bioactivities; there are complex lipids and proteins that serve important nutritional, digestive, immunological, and neurological functions.[1-4] Nearly 50% of the energy intake of HM-fed young infants comes from fat, which is equivalent to about 25 gm/day for about 6 months after birth.[5-7] The lactating mammary gland packages lipids in MFGs composed of a triacylglycerol (TAG) core with a surrounding triple membrane structure.[8-18] These globules contain polar lipids, specialized proteins, glycoproteins, and cholesterol.[16,19,20] These vesicles release energy rapidly in the upper gastrointestinal tract and then persist for some time in the gut lumen so that the protective bioactive molecules are conveyed to the colon.[1] These properties may shape the microbial colonization and innate immune properties of the developing gastrointestinal tract.[20] Milk fat globules in milk from humans and ruminants show some consistent structural features, but there are also notable differences in size, profile, composition, and specific constituents.[16]

In the last decade, several research groups have studied the organization and composition of MFGs.[3,4,14,20] Milk fat globules in human and ruminant milk share the basic structure but show differences in the concentration and proportions of polar lipids and the profile of proteins.[16] Some beneficial components of the MFG membrane such as lactadherin, ω-3 polyunsaturated fatty acids (FAs), and phospholipids are enriched in specific size fractions of the MFG pool.[16,21-23] The size and composition of MFGs change with the stage of lactation, ethnicity, and maternal diet.[1,16,24] This review summarizes our current understanding of MFGs and related bioactive components. Our primary focus is on HM but when needed, we have included information from studies of other ruminants.

Milk fat globules in HM vary in size, physical properties, fractions, and composition.[16] This information can be potentially important for: (a) our understanding of the actual bioavailability of measurable nutrients in HM;[20] (b) improving our understanding of the need for specific nutrients, vis-à-vis the constituents of MFGs, to design better strategies for treatment of intra- and/or extra-uterine growth restriction;[25] (c) to determine the impact of high concentrations of MFGs in low-volume, calorie-dense feedings that would not pose risks due to hyperosmolality in very premature or critically-ill infants;[20] (d) a la fractionation of blood meant for transfusion into specific components such as packed cells, plasma, and other components, identification and isolation of specific MFG subclasses and soluble whey nutrients to improve targeted, efficient use of banked HM;[11] (e) designing strategies to prevent contamination in different fractions;[26] (f) once the temporal deterioration of various fractions is understood, designing specific storage strategies;[27] (g)

designing strategies for optimal transportation of milk fractions in warmer periequatorial and tropical regions;[28] (h) reducing the risk of transmission of infectious diseases;[29] (i) retrieval of some "contaminated" milk components by specifically designed pasteurization, filtration, and/or other procedures;[30] and finally, (j) the possibility of engineering artificial MFGs for enteral delivery of nutrients, vaccines, and/or medications in critically-ill infants.[31] Such products could also help in 'humanizing' MFGs derived from other mammals that have important biological differences, or for complete de novo manufacturing for specifically-designed therapeutic vehicles.[27,32] These products could also be useful for infant nutrition in societies with religious/social restrictions on the use of banked HM.[33]

Milk Fat Globules (MFGs)

Dimensions—Milk Fat Globules are typically sized between 0.1 and 15 μm in diameter.[34] The globules contain a nonpolar TAG core covered by a surface-active membrane; these structural characteristics stabilize these globules in the emulsion and prevent enzymatic degradation and coalescence.[1] Milk lipid droplets measuring <0.5 μm are first seen in the rough ER and are then coated with phospholipid monolayers before translocation to the apical plasma membrane.[35] Many of these smaller droplets fuse together to gain size and then get additional lipid coatings from some components of the plasma membrane.[31] In HM, most MFGs measure about 4.2–5.1 μm in diameter;[16] the size (range distribution and average) of these globules is determined by factors such as including genetics, maternal diet, duration of lactation, and may also be altered to fit the nutritional requirements of the infant.[11]

The size of MFGs can be described by diameter, volume, number, and surface area.[16] The mean diameters of MFGs are commonly expressed as a number-weighted mean (d_n, $D_{1,0}$), volume-weighted mean (d_{vm}, $D_{4,3}$), or as the surface-weighted mean (d_{vs}, $D_{3,2}$).[16] The surface-weighted mean (Sauter mean diameter) emphasizes the globule surface area, as it is an important determinant of phenomena such as the release of components from surfaces, 2-dimensional surface reactions, and surface dissolution.[36,37] The size distribution by volume varies as smaller globules have a higher specific surface area: volume ratio compared to larger globules. The volume-weighed mean (De Brouckère mean diameter, d_{vm}, $D_{4,3}$) reflects the contribution of each particle in the distribution related to the volume.[38,39] Colostrum contains some of the largest MGFs. The size then decreases somewhat in transitional milk, and then it increases again by 7–10 days after birth.[17] Milk fat globules have been typically classified in three 3 volumetric subgroups (Fig. 1):

1. Small MFGs (<1 μm in diameter), which comprise nearly 80% of all globules and form a very large lipid interface surface, which is the key parameter in determining the susceptibility of MFG membrane to digestive hydrolysis and reactivity.[16] These particles contain unsaturated FAs, and may have additional functional effects on lactation beyond carrying and emulsifying the TAG core;[20]
2. Medium-size MFGs; these carry most (nearly 95%) of the volume of the lipid phase;[15]
3. Large-size MFG (≥8 μm in diameter), which typically form from fusion of medium-size MFGs.[15,16]

Milk fat globule size varies during different stages of lactation.[16] These variations likely optimize maternal energy costs to supply nutritional and bioactive components. Milk fat globules in human colostrum (≤4 days after delivery) are typically larger than those in transitional and mature milk.[16] Large-sized globules seen during early lactation could well be related to the immaturity of mammary glands, related to the inability to produce membrane phospholipids at the same pace at TAGs.[20] In contrast to the diameter, the average surface area of MFG increased from 1.1 ± 0 to 5.4 ± 0.7 m^2/g during the transition of colostrum to mature milk but the volume-weighted mean decreased.[40] Even though some variation is seen in the size of MFGs during lactation, the average diameters of mature MFGs remain relatively stable at around 4.5 μm.[16] The size changes are directly correlated to changing lipid and protein constituents.[16]

Composition

Human milk contains 2.8–3.8 gm/100 gm fat and phospholipids but these concentrations differ by species.[3,41] Ovine milk contains 6.5–9 gm/100 gm, caprine 3.5–5.6 gm/100 gm), and bovine 3.4–6.0 gm/100 gm. The most important lipids in MFGs are TAGs (98%), polar lipids, and cholesterol;[42] the TAG fraction is the predominant source of energy.[11]

The fat content of milk changes with the maternal environment, diet, and physiological state.[43] Colostrum contains more total phospholipids, stearic acid, long-chain polyunsaturated FAs, and gangliosides than transitional and mature milk.[44] FAs with 4–14 carbons can be synthesized *de novo* in the mammary gland, whereas the 16-carbon (16C) FAs are usually derived from maternal diet, circulation, or her own body fat stores.[3] As milk matures, increased production of 12–14C medium-chain FAs is reflected as decreased average length of the FA chains. Long-chain FA content remains similar throughout lactation. Triacylglycerol levels increase in the first few weeks after birth, but those of cholesterol and cholesterol esters gradually decrease. Sphingomyelin levels remain stable.

There is a gradual shift from the disialylated gangliosides (GDs), particularly GD3 (disialosylganglioside; Neu5Aca2-8Neu5Aca2-3Galb1-4Glc-Cer) in colostrum to GM3 (monosialodihexosylganglioside; alpha-Neu5Ac-(2->3)-beta-D-Gal-(1->4)-beta-D-Glc-(1<->1′)-Cer(d18:1/18:0) in mature milk.[45] Colostrum gangliosides contain more long chain- and less medium-chain FAs than those in mature milk. To recall, gangliosides are classified according to the number of sialic-acid residues on the molecular backbone (M = mono- or 1; D = di- or 2, as GM or GD), the number of residues attached to the sugar moiety, and the biosynthetic pathway from which these are derived.[46] Similarly, there are more monounsaturated FAs than long-chain FAs.[20] In the following sections, we present current data for fat/protein fractions in MFGs.

Fats in MFGs—Small MFGs contain more dietary FAs than the larger ones, although the proportion of *de novo* synthesized FAs may not be different.[17] Shorter (4–16C) FAs incorporated into these globules are typically obtained from de novo synthesis, whereas the longer ones (≥16C) are derived from TAGs in diet or adipose tissue.[17,47] During early lactation, low phospholipid/TAG ratios in the membranes and longer FAs (C17:0, C18:0, and C20:0) can enhance large globule formation.[17] Low PE levels and high PC: PE ratios

can make MFGs less amenable to fusion because of lower interfacial surface tension.[48] Mucin–1 and –15 and FAs such as C10:1, C11, C12, C12:1, C13:0, CD14:0, and C15:0 also increase the formation of small MFGs.[16] For each unit of fat, small MFGs present a larger total surface area for lipase action, and consequently, get digested faster.[49] There are also differences in the interaction of globules of different sizes with specific enzymes.[16]

Milk fat globule size is determined primarily by the phospholipid/TAG ratio, FA composition, and cholesterol content.[20] Smaller globules may contain more phospholipids.[9] Higher intracellular PE content can promote droplet fusion to produce larger MFGs.[18] In bovine milk, the type of esterified FAs in the lipids in the MFG core and membrane may also alter the size of the globules; longer long- and medium-chain FAs may lead to smaller MFGs.[20] Small and medium-sized MFGs may also show different digestion and fat release patterns.[20] GM (monosialylated GAs) and GD (disialylated GAs) are the two major GA species in HM, and GD_3 (Neu5Aca2-8Neu5Aca2-3Galb1-4Glc-Cer) is the most abundant GD in HM.[11]

In cattle, a higher intake of saturated lipids (50% palmitic acid) resulted in higher fat content in milk with larger MFGs.[50] In contrast, high polyunsaturated FA intake reduced MFG size with proportionately more polar lipids and unsaturated FAs. Linoleic (LA) (C18:2c9,12), α-linolenic (ALA) (C18:3c9,12,15) and palmitic (C16:0) acids accounted for approximately 88% of total FAs.[51] Dietary supplements of conjugated linoleic acid (*cis*-11, trans-9 and trans-10, cis-12 isomers) also suppressed milk fat synthesis with decreased MFG diameter.[51]

Membrane phospholipids represent only 0.5-1% of the total fat in milk but contain 15–20% of the total LC-PUFAs in milk.[20] In contrast, membrane SLs are highly saturated and maintain the lipid rafts, which might facilitate the delivery of sphingosine and ceramides to the distal gastrointestinal tract.[20] Glycosphingolipids, such as cerebrosides and gangliosides, are present in relatively low concentrations. Cerebrosides are neutral glycosphingolipids containing uncharged sugars.[52] In bovine milk, lipid metabolism may regulate the production of small or large MFGs. Small MFGs (average 3.29 μm) contained higher concentrations of unsaturated FAs compared to larger globules (average 4.92 μm). These findings could have been related to higher uptake of long-chain FA from the blood circulation in smaller MFGs.[17]

Colostrum contains more medium- and poly-unsaturated FAs than mature milk.[53] C18:1 ω-9 seems to be the most abundant FA in colostrum and milk, followed by C16:0, C18:0, and C18:2 ω-6. C18:1 ω-9 concentrations decreased, whereas C:16 increased from colostrum to milk. In general, smaller MFGs (<L3 μm) contain more medium-chain FAs (C10:1, C11, C12, C12:1, C13) such as myristic (C14:0) and pentadecanoic acid (C15:0) and a larger fraction of total unsaturated FAs. Large MFGs contain more long-chain saturated FAs (C17:0, C18:0, and C20:0).

Proteins in MFGs—Proteins are an important determinant of MFG size; the relative content of xanthine oxidase/xanthine oxidoreductase (XO/XOR) and butyrophilin (BTN)-1 is an important variable.[12] During early lactation, α-amylase, MARCKS (myristoylated alanine-rich C-kinase substrate)-related apolipoprotein D, apolipoprotein E, ubiquitin,

bone marrow stromal antigen 2, chordin-like protein 2, gamma-glutamyltranspeptidase 1, long-chain FA-CoA ligase 4, α-1-antitrypsin, insulin-like growth factor binding protein (IGFBP)-2 and matrilin-3 show higher expression. In later stages, annexin, complement C3, CD9 antigen, nonspecific lipid transfer protein, FA-binding protein, folate receptor alpha, glutathione peroxidase 3, gelsolin, heat shock protein beta-1, lysozyme C, proactivator polypeptide, BTN and XOR are more highly expressed.[16]

Milk fat globule size is an important predictor of protein signatures.[54,55] Small MFGs typically contain proteins needed for lipid metabolisms such as acyl-CoA synthetase long-chain family member 5 (ACSL5), acyl-CoA synthetase long-chain family member 3 (ACSL3), and glycogen synthase kinase-3 beta (GSK3B), lanosterol synthase (LSS), acetyl-CoA carboxylase 1 (ACC), NAD(P) dependent steroid dehydrogenase-like (NSDHL), and glycerol-3-phosphate acyltransferase 4 (GPAT4). Larger globules contain more lactadherin (gene Milk fat globule EGF, factor V/VIII domain containing; MFGE8), adipophilin (adipose differentiation-related protein, also known as perilipin 2, adipophilin; gene ADPF), and the ratio of glycam1 (glycosylation-dependent cell adhesion molecule 1)/lactophorin (gene Proteose peptone component 3; PP3) than smaller ones. Size might also alter surface polarity, and consequently, the FA and phospholipid composition. Differences in protein composition might be a secondary change throughout lactation.

Impact of processing of milk: The effect of the processing of HM on MFG structure needs further investigation. Some studies suggest that MFG FAs and TAGs may not be affected by homogenization and thermal processing, whereas others, working with bovine milk, have shown conflicting data.[28,56-58] Freezing of HM may result in the loss of some fat and of the MFG structure.[1] Some FAs formed during cold storage of HM may be cytotoxic.[1] Small vs large MFGs may be differentially affected by digestion due to variations in lipid profiles.[59] In bovine milk, smaller MFGs contain less long-chain but more medium-chain FAs and myristic acid in the core.[47] Gastric lipases preferentially hydrolyze short- and medium-chain FAs, and so the size of fat globules will affect the digestion rate and release of these FAs.[60] The profile and concentrations of phospholipids may also affect lipid absorption and metabolism.[61]

Homogenized MFGs can be digested more rapidly than native globules, although more details are needed on changes in surface area.[8] Homogenization can promote the binding of milk proteins to the MFG membrane, which avidly binds pepsin and pancreatic lipase.[11] However, MFG membrane proteins such as BTN, pulmonary adenoma susceptibility (PAS)-6/7, and mucins are resistant to proteases.[62]

Microfiltration is a useful way to purify MFGs with specific sizes.[27,63] Hansen et al.[64] have isolated MFG membranes containing 7% w/w polar lipids and 30% w/w proteins. They used ceramic diamicrofiltration of raw whole milk to first separate fat globules from casein micelles and whey proteins, and then started the procedures for MFG membrane extraction. Pasteurization (72°C, 15 seconds) prior to or after microfiltration had no impact on filtration efficiency or on MFG membrane yield and composition.[17]

Human milk also contains other extracellular lipid-enclosed structures such as exosomes and casein micelles (100–200 nm in diameter).[16,65] These subgroups can be dissociated using EDTA during milk processing, and so the presence of exosomes may confound the measurements of MFG size based on light-scattering analysis. There are also some smaller 25–30 nm lactosome particles, which do not contain a TAG core.[66] The lactosomes do not show major morphological changes over time. These particles contain osteopontin and β_2-microglobulin, which are involved in early innate and adaptive immune responses. However, there are no gangliosides, which would have enabled interaction with many bacterial toxins. In the context of nutrition, these particles contain α-lactalbumin, which is a component of lactose synthase, and phospholipids. However, the absence of a TAG core excludes the possibility of a major role in energy delivery. As currently understood, lactosomes do not share secretory pathways with MFGs.

Endocrine Regulation of MFG Size

Hormones such as prolactin and oxytocin can alter MFG size.[67] In bovine milk, progesterone is also an important regulator of MFG size.[68] Lipid droplets smaller than 3 μm may be more abundant in the luteal phase with higher progesterone levels than in the follicular phase.[66] The effect of progesterone was mediated via long-chain FAs in the mammary epithelium. The details of these findings still need to be elucidated.

High plasma insulin concentrations can lower the TAG: phospholipids ratios in the MFG membrane and raise the concentrations of monounsaturated FAs in the MFG core.[16] Negative systemic energy balance during the early stages of lactation can also alter MFG size.[69] MFGs produced during this period may contain more oleic acid (C18:1 *cis*-9), less palmitic acid (C16:0), an overall reduction in *de novo* synthesis of FAs, and a reduction in the size of MFGs.[16]

Ontogeny and Phylogeny of MFGs

Lactation appeared as a reproductive feature prior to the origin of mammals.[70,71] Mammals gradually accrued from synapsid ancestors, and the mammary gland may have evolved from apocrine-like glands, which were associated with hair follicles and provided moisture and antimicrobials to parchment-shelled eggs.[72-74] Some therapsids and the mammalia-formes began producing a nutrient-rich milk-like secretion during the Triassic period 220-280 million years ago (Fig. 2).[75,76] Genes encoding for MFG membrane proteins in milk are highly conserved, particularly those with nutritional or immunological attributes.[20,70] This mechanism is important for milk-fat secretion. Milk fat globule seem to have evolved by co-opting membrane (BTN), cytosol (XOR), and intracellular lipid droplet (adipophilin) proteins into new/expanded functions.[77-80]

Biophysical Forces Acting on MFGs

Liquid surfaces incur a significant energy cost as a large number of intermolecular bonds need to be broken to create a surface.[81] Consequently, the MFG droplets evolve into spheres over time to minimize the surface area.[82] Furthermore, smaller droplets aggregate into larger ones; a process that has been named as flocculation.[83] If we consider a fat globule with a radius r, the surface energy (U) of the droplet could be calculated as the product of its total

surface area ($4\pi r^2$) and the surface tension (γ_f); $U = 4\pi r^2 \times \gamma_f$.[84] If the square of the radius length determines the surface energy, a sphere would accord the least surface area of all 3-dimensional structures, and hence, carry the least surface energy. If two smaller droplets were to coalesce into a single larger droplet, the radius of this larger particle would be $R = 2^{1/3} \times r$. The total surface energy of the initial smaller droplet would be $U_1 = 8\ \gamma_f \times \pi r^2$, but that of a larger droplet would be lesser ($U_2 = \gamma_f \times 4\pi r^2 = 2^{2/3} \times 4\pi r^2$). As every natural system seeks the lowest energy state, such aggregations would be favored.[85]

Another observation of MFGs needs discussion. These globules show a continuous jiggling motion, which might stabilize these particles.[86] Current understanding ascribes this phenomenon to the Brownian motion of water molecules but this might be an oversimplification; a number of disparate forces might actually be involved here.[87-89] Further work is needed to understand these factors because deep freezing, the current standard practice for preserving maternal milk for later use, and pasteurization, used for sterilization, may alter the stability of MFGs.

In a highly-idealized model, the MFGs would be subjected to the following forces (Fig. 3):

- Gravitational weight, $m \times g$, a product of m, the mass of the particle, and g, the local acceleration due to gravity;[90]
- Buoyant force exerted by the solvent, the Archimedes principle, which would be water in the aqueous base of milk.[89] This would be computed as a product of the density of water, ρ_w, V as the volume of volume of the particle, and g as the local acceleration due to gravity; $F = \rho_w \times V \times g$;
- Drag force, a viscous force described by Stokes law.[91,92] This would depend on the speed (v) and the radius of the particle (r), and the constant of viscosity (η); $D = 6\pi \times \eta \times r \times v$;
- Random forces due to the thermal fluctuation, which would augment the Brownian motion of solvent molecules.[93] These movements might, paradoxically, stabilize the MFGs;
- Electrostatic forces between the charged phospholipid surface layers, the polar solvent, and other globules;[94]
- van der Waals forces between the organic part of the particles.[95,96]

Further work is also needed to understand Brownian motion[97] of MFGs. Despite the relatively large size of MFGs, these might still simulate ideal gas/Brownian particles.[98] The velocity scale can be viewed as $\sqrt{(k_B \times T/m)}$, where k_B would be the Boltzmann constant, T the temperature, and m the mass of the Brownian particle.[99-101] In this equation, the characteristic time scale of motion or the time needed by a particle to re-attain the state of rest after a collision would be $m/(6\pi \times \eta \times r) \propto r^2$. If this relationship stands correct for MFGs, smaller particles should attain a state of rest faster than larger ones and are therefore likely to be more stable. Smaller particles may also show longer intervals between two successive collisions. However, there are numerous assumptions in these models and further work is needed for this line of investigation.

Finally, there also have been discussions on developing mathematical models to understand the behavior of MFGs as single units. A relationship between membrane tension and gravity could help understand the spreading of membrane-enclosed vesicles, and consequently, membrane tension-related biological processes. Wang et al.[90] showed that equilibrium differential equations could be one plausible way to relate forces such as gravity, internal pressure, and membrane tension: (a) the deformed geometry in the vesicle models could be represented by a pseudo-ellipsoidal or pseudo-spherical cap under the action of gravity;[102] the pseudo-ellipsoidal cap may be more plausible from a mathematical point of view; (b) the membrane tension may decrease with distance from the basal surfaces; (c) the inclination between a tangent and a radial line might correlate with the locally-defined principle radius; (d) gravity could be an important variable in the spreading of vesicles as it might influence the distribution of membrane tension. These efforts may help in predicting the effects of gravity on the deformation of vesicles.

Milk Fat Globule Membrane

Structural Characteristics of the MFG Membrane—Milk Fat Globules are covered with a continuous, 10–50 nm (usually 15–20 nm) membrane derived from the cytoplasm following secretion into the alveolus.[16,17] It accounts for about 6% of the globular mass, and is constituted of about 60% proteins and 40% lipids. It stabilizes the globules in the emulsion.[16,17]

There is some evidence to show that the MFG membrane may include two distinct lipid phases. The first may be comprised of relatively less dense, liquid-disordered regions with unsaturated glycerophospholipids, proteins, glycoproteins, glycolipids, and some SM.[20] The second may show a distinct biphasic separation of SM and cholesterol into densely-packed, liquid-ordered domains called the ‘rafts’ (Fig. 4).[103,104] In these microdomains, SM interacts asymmetrically with large amounts of cholesterol in circular assemblies. Sphingomyelin and glycerophospholipids differ in head group structure, hydrocarbon tail length, and degree of unsaturation.[20] There may well be other types of microdomains enriched in other glycerophospholipids.[48]

Traditional models of the MFG suggest a 3-layered membrane covering of the TAG-rich core (Fig. 5).[11,18,20,34,62,63,105,106] The innermost is a surface-active, ER-derived layer comprised of polar glycerophospholipids such as PE, PI, and PS; proteins; SM; and gangliosides. The middle is relatively dense and rich in proteins. The outer two layers are rich in polar lipids, which are translocated across the apical plasma membrane of mammary epithelial cells. Many phospholipids and SM are located on the external aspect of the membrane. Some of the glycoproteins and glycolipids are also loosely attached to the surface, where the carbohydrate domains project into the surrounding aqueous phase. These have antimicrobial, anti-inflammatory, and prebiotic functions in the gut. In addition, there are many transmembrane proteins and cholesterol molecules. The distribution, size, and profile of polar lipids and proteins in the MFG membrane vary with diet and among species.

Some of the cholesterol in the globular membrane might interact with other glycerophospholipids in the liquid-ordered domain and protrude from the liquid-disordered

domain. Sphingomyelin and glycerophospholipids differ in structural characteristics in terms of head group structure, hydrocarbon tail length, and degree of saturation.[11] Sphingomyelin (SMs) show asymmetric molecular structures and high hydrogen-bonding potential, which influence the stability of MFGs.[107]

Lipids in the MFG membrane: Milk fat globule membranes typically contain TAGs (56–62%), polar lipids (26–46%), and some minor constituents such as diacylglycerols, free fatty acids (FFA), and sterols.[108] This hydrophobic core is covered by a protein-rich hydrophilic phospholipid membrane, which contains about 40% lipids and 60% proteins.[108] During passage through the ER and the Golgi to the apical plasma membrane, these droplets fuse together and grow. Whey proteins, casein, and lactose are added to the Golgi apparatus. The budding lipid droplets get covered with an electron-clouded inner face of the plasma membrane, which eventually forms the lipid bilayer of the MFG membrane.[109] The extrusion from mammary epithelial cells is mediated by FA transporters, such as the FA binding protein (FABP), acyl-CoA binding protein, and FA translocator CD36.[110,111] Gangliosides play a key regulatory role in this process.[20]

Polar lipids: Milk fat globule membranes contain many polar lipids, which vary by the size of MFGs, maternal ethnicity and geographical origin, and her diet.[17] The most important polar lipids include sphingolipids such as SM, and glycerophospholipids such as PE, PC, PS, and PI (Fig. 6). Sphingomyelin is present in higher quantity in HM than in milk from other mammals.[49] PE, PC and SM are the most abundant phospholipids in the MFG membrane; each constitutes about 30% each of the total phospholipid content in the MFG membrane. PS and PI contribute 5–10% each.

Compared to whole milk and large MFG fractions, the small MFG fractions are relatively enriched in polar lipids.[105] The phospholipid: TAG ratios in the membrane can also affect MFG formation. Low phospholipid/TAG ratios enhance large MFG formation.[20] High PE concentrations can promote droplet fusion by lowering the interfacial surface tension, but high PC: PE ratios can inhibit droplet fusion. Overall, HM contains less PE than in ruminants.[11] Mucins 1 and 15, high PL/TAG ratios, and certain FAs (C10:1, C11, C12, C12:1, C13:0, CD14:0, and C15:0) can increase small MFG formation. Similarly, C17:0, C18:0, and C20:0 FAs can also augment this process.[16,17,112]

Postpartum changes in the phospholipid content of milk need further study.[113] The total phospholipid concentrations and MFG size show considerable variability during the neonatal period.[3,114,115] On the day of delivery, most globules might be ≥10 μm in size and then become smaller rapidly to ≤1 μm by day 4. This correlates with increased total phospholipid and fat concentrations from colostrum to mature milk. However, PC, PI, PE, and PS concentrations may not always show a consistent pattern during the transition from colostrum to mature milk. Overall, the membranes of small globules contain lower concentrations of PC and SM.[20]

Milk phospholipids have been shown to improve cognition, neuroplasticity, and myelinization in both animal models and clinical studies.[116] In term infants, MFG membrane supplementation begun in the 1st week seems safe.[117] We still need to study

preterm infants, who may possibly show greater benefit because of lower stores. HM and MFG membrane components may possibly protect against necrotizing enterocolitis, stunted brain growth, and brain injury, retinopathy of prematurity, and infections.[10,118] The impact on eczema is uncertain.[118]

Fatty acids (FAs): Human MFG membranes contain more unsaturated FAs than the core TAGs.[20] Small MFGs may contain more unsaturated FAs because of the proportionately higher content of membrane material than in large globules. C16:0 is the most abundant FA in colostrum and early milk, and the levels decrease progressively with time. The opposite trend was seen in C18:0, ω-3 PUFAs, and ω-6 PUFA levels.[119] Unlike HM, infant formulae contain plant oil-based lipids that lack some of these components.[120] Milk fat globule membranes contain about 15% of the long chain-polyunsaturated FAs (LC-PUFAs), most of which are bound to phospholipids.[20] Compared to the total lipid compartment, phospholipids contain relatively more stearic acid but less oleic acid.[52] Except for PC, palmitic acid also constitutes a smaller proportion of milk phospholipids. There is a high arachidonic acid (ARA; 12%) content in PE and PI. Docosahexaenoic acid (DHA) contributes up to 5% and 3% in PE and PS, respectively.[121] In contrast, the SM content (0.4%) resembles that of total lipids.[43] Although the bioavailability of TAG and phospholipid-bound LC-PUFA might not differ, the metabolic disposition including the incorporation into the brain might be affected.[122] Thus, even though the nutritional importance of the MFG membrane lipids may not be affected by the LC-PUFA content, these are important as a source of specific lipids and a role in the development of various organs.

Gangliosides: Both endogenous and dietary GM3 and GD3 gangliosides activate the mucosal immune system by increasing the production of cytokines and IgA, and lymphocyte function.[10,123,124] In contrast, these gangliosides inhibit dendritic cells and could possibly promote tolerance against non-aggressive antigens.[125] Overall, HM gangliosides are believed to promote infant gut maturation with effects on neuronal growth, migration, maturation, neuritogenesis, synaptogenesis, and myelination.[126] HM contains high levels of gangliosides right from birth, and could improve cognitive development in infants aged 0–6 months.[45,127]

The MFG membrane is an exclusive carrier of gangliosides, particularly GD3 to the neonatal gut.[128] Gangliosides get incorporated into the intestinal mucosa and alter membrane fluidity and enterocyte function.[129] These are integral components in cell membranes, and the oligosaccharide residues that extend from the cell surface promote cell-cell communication. Dietary gangliosides can increase ether phospholipids and the uptake of LC-PUFAs.[130]

Sphingomyelin (SM): Sphingomyelin accounts for 25% of the total milk polar lipids and is complexed with cholesterol in a mass ratio of 3:1.[131] HM-fed infants obtain about 150 mg SM per day.[11] Sphingolipids are built on a backbone of sphingoids, a set of aliphatic amino alcohols.[132] In the MFG membrane, SM is important as it is the most important constituent of sphingolipids along with glucosyl- and lactosylceramides.[49,114]

In neonates, orally-ingested intact SM is not absorbed but it may still accelerate gut maturation.[115] The alkaline sphingomyelinase and ceramidase expressed on gut epithelium convert sphingomyelin to absorbable sphingosine, which can then be converted to sphingosine-1-phosphate. Sphingosine can inhibit protein kinase c, and induce cell cycle arrest and apoptosis.[133] In the intestine, sphingosine-1-phosphate may activate inflammation, angiogenesis, vascular permeability, and organ development.[134] The SM: cholesterol ratio in the MFG membrane can alter its structure with changes in temperature during cooling, storage, heating, and digestion.[135]

Sphingomyelin concentrations in HM remain largely unchanged over time in the postnatal period.[136] Its metabolites, including ceramide, sphingosine, ceramide-1-phosphate, and sphingosine-1-phosphate are important in inflammation and cell differentiation/apoptosis.[137] The clinical effects of SM are still being studied; in a pilot study, 24 very-low-birth-weight infants were randomized to receive HM with added standard milk (SM 13% of all phospholipids) or SM-fortified milk (SM 20% of all phospholipids).[138] Neurodevelopmental follow-up between 6-18 months after birth showed that SM-fortification improved behavior rating on Bayley Scales of Infant and Toddler Development II, the Fagan test of infant intelligence, visual evoked potential latencies, and free-play sustained attention test of Colombo.[138]

Choline: Choline is a highly methylated component of membrane constituents, PC, SM, and choline plasmalogens; and of the neurotransmitter acetylcholine.[139] It may be measurable as its unesterified form, or as phosphocholine, glycerophosphocholine, PC, and/or SM. PC and SM in the MFG membranes contribute about 10% to the total choline intake of infants.

The fetus and the neonate have high levels of choline in the blood and tissues as it is important in neurodevelopment.[140] Low choline status in early pregnancy increased the risk of neural tube defects and poor cognitive development. It is also important for neurogenesis and synaptogenesis; dietary supplementation can potentially promote cognitive functions and overall infant development. In one study of bovine colostrum and milk, the concentration of 26 MFG membrane proteins increased and 19 decreased at 7 days of lactation.[16] The concentrations of mucin-1 and –15 increased 7-fold, ADPF 3.4-fold, BTN 3.2-fold, and XDH 2.6-fold. The concentrations of acyl-CoA synthetase, lanosterol synthase, lysophosphatidic acid acyltransferase, and FA-binding protein, associated with lipid transport synthesis and secretion, rose 2.6-5.1 folds higher. In contrast, apolipoproteins A1, C-III, E, and A-IV were 2.6- 4.3-fold less concentrated in milk than in MFG membranes isolated from colostrum. Despite higher fat contents in colostrum, there may be early development shifts in milk fat transport.

Effect of gastrointestinal microflora: Bacterial infections can alter immune-related proteins of the MFG membrane. Mastitis due to *Mycoplasma agalactiae* can initiate an immune response with induction of host defense, inflammation, and oxidative stress, and suppression of milk fat metabolism and secretion.[141] Similarly, neutrophils produce more extracellular traps and a stronger bactericidal response when exposed to *Staphylococcus aureus*; the related antimicrobial peptides are present in the MFG membrane fraction.[142]

The effects of dietary supplementation with microalgae to increase PUFAs on the bovine milk FA profile and the number and diameter of MFGs have also been investigated. Dietary supplementation with *Chlorella* reduced the number of 1–3 μm globules compared to other diets.[16] Dietary composition did not consistently change all MFG size fractions. Changes in capric (C10:0), lauric (C12:0), myristic (C14:0), and oleic acid (C18:1) concentrations were not predictable. The structure of human MFG and the specific positional distribution of FAs may explain differences in the gut microbiota between infants who are fed with HM vs formula. Human milk contains β-16:0 (palmitic acid esterified on the *sn-2* position) in contrast to vegetable-sourced palmitic acid, which is esterified in the *sn*-1 or–3 positions.[143] One study showed that supplementing formula with β-16:0 increased fecal abundance of Lactobacilli and Bifidobacteria in infants after 6 weeks of feeding compared to a control formula containing vegetable-sourced 16:0.[144] The mechanisms behind these observations need to be determined.

HM-fed infants normally show a predominance of *Firmicutes.* However, a combination of milk fat and MFG membrane fragments altered fecal microbial composition in piglets by increasing *Proteobacteria* and *Bacteroidetes* at the expense of *Firmicutes*.[145] This may partly be explained by an increased intestinal content of immune modulatory peptides and milk lipid-derived metabolites. Another study in germ-free mice showed that an infant formula high in medium-chain FAs emulsified with bovine MFG membranes enriched the bacterial families *Bacteriodaceae, Desulfovibrionaceae, Rikenellaceae,* and *Porphyromonadaceae,* whereas formulas made with LCFAs emulsified with soy lecithin increased the abundance of *Enterobacteriaceae, Erysipelotrichaceae, Coriobacteriaceae,* and *Enterococcaceae.*[20] These effects may correlate with the chain length and the degree of desaturation of the fatty acids. MFG lipids can influence the relative distribution of various protein digestion products that enter the colon by altering the rate of hydrolysis of proteins in the small intestine.[20] Further work is needed.

Proteins in the MFG membrane: Proteins constitute 25–70% of the total MFG membrane (w/w) with a high proportion of glycoproteins and enzymes.[131] In HM, MFG membrane proteins comprise about 1–4% w/w of the total milk proteins.[63] These proteins regulate cellular processes and defense mechanisms in the maternal-infant pair. Proteomic studies have mapped up to 400 proteins in HM MFG membranes.[146] However, the protein extraction methods still need work.

Proteins such as BTN; a member of the immunoglobulin superfamily), xanthine dehydrogenase/oxidase (XDH/XO), adipophilin (ADPF; perilipin-2, PLIN2), and stomatin are involved in MFG formation.[16] High MFG epidermal growth factor 8 (MFGE8, lactadherin), ADPF, and glycosylation-dependent cell adhesion molecule-1 (GLYCAM1): proteose peptone component 3 (PP3) can also promote the formation of large MFGs. PP3 (lactophorin), is a small phosphoglycoprotein that is expressed in lactating mammary tissue. In addition, α-lactalbumin, lysozyme, β-casein, clusterin, lactoferrin, immunoglobulins such as the IgA α-chain, tenascin, apolipoproteins such as type A-I, and FA synthase have been identified.

These proteins exert a wide spectrum of bioactive properties. For instance, ADPH controls the trafficking of lipids towards the MFGs.[62] BTN is a member of the immunoglobulin superfamily, and it also connects the inner and the outer MFG membrane by binding XDH/XO and ADPH in a tripartite superstructure.[147] BTN1A1 is the main isoform in the human MFG membrane and regulates the lipid secretion in milk.[148] Mucin 1 (MUC1) is a glycoprotein that might bind pathogens as a decoy receptor.[149] XDH/XO is a redox enzyme that exerts its antimicrobial role by producing reactive oxygen and nitrogen species.[150] Lactadherin, also known as Per-Arnt-Sim (PAS) domain 6/7 or milk fat globule-epidermal growth factor-factor 8 (MFG-E8), is involved in the regulation of apoptosis and in innate immune responses.[151] FA-binding proteins (FABPs) typically transport long-chain FAs into the mammary epithelial cells.[152]

Many MFG membrane proteins regulate inflammatory reactions by altering cytokine expression, promoting apoptosis, and reducing oxidation.[153] Glycosylated compounds and the products of hydrolysis can protect against bacteria, viruses, and bacterial toxins.[154] Human MFG membrane contains human leukocyte antigen (HLA)-II, which is normally expressed on the surface of antigen-presenting cells.[20] This may promote antigen presentation to $CD4^+$ T-cells and promote immune responses/tolerance in the neonatal intestine.

Some MFG membrane proteins may be involved in T-cell maturation: (a) BTN in the MFG membranes suppresses the proliferation and activity of maternal and neonatal T-cells.[20] It also facilitates immune resolution following prolonged inflammation by clearing apoptotic cells; it binds phosphatidylserine on apoptotic cells via its C-terminal V/VIII-like domains, and its epidermal growth factor domain contains the arginine-glycine-aspartate (RGD) motif that interacts with $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptors to activate macrophages to clear the apoptotic débris;[20] (b) alkaline phosphatase in MFG membranes also has anti-inflammatory properties; it dephosphorylates pro-inflammatory molecules such as lipopolysaccharide (LPS), inhibiting TLR-mediated NF-κB signaling. Milk phospholipids and FAs, particularly LCFA, are strong stimulators of intestinal AP activity;[155] (c) osteopontin (OPN) in the MFG membrane activates the innate and adaptive immune systems of newborns. It works as an opsonin, binding directly to bacteria such as *Streptococcus agalactiae* and *S. aureus* to enhance clearance by macrophages. It also balances Th1 and Th2 immune responses.[20]

The mechanisms of MFG secretion from mammary epithelial cells are being investigated. A tripartite model proposes that interactions among the MFG membrane proteins, ADPF, XDH/XO, and BTN promote MFG secretion. These proteins presumably bind on the MFG surface and promote the adsorption of lipid droplets.[156] This complex leads to the deformation of the lipid droplets and the budding of more lipid droplets from the secretory system.

After release, MFGs may show some casein-balancing membrane loss. Caseins are known to be synthesized and secreted by the fusion of casein-containing vesicles with the apical plasma membrane and soluble *N*-ethylmaleimide–sensitive fusion attachment protein receptor (SNARE).[157] This model suggests even though BTN1, PLIN2, and XOR likely contribute to MFG budding, SNARE proteins connect the secretory vesicles together

and with the apical plasma membrane to promote the exocytosis of the budding MFGs. The combined release of MFGs and casein micelles from the mammary epithelium may contribute to the adsorption of casein micelles on the MFG membrane. Further studies are needed to explore these possibilities.

Glycosylated mucin 1 and 15, ADPF, BTN, and XDH are more concentrated in MFG membrane in the smaller the MFG fraction. Increased glycosylation helps MFG membrane proteins resist digestion in the upper gastrointestinal tract and retain these biological effects in the colon.[16] Mucin and lactadherin promote mucosal immunity in the stomach and upper intestine and protect against bacteria and viruses.[158]

Many proteomic studies have listed several casein and whey proteins in the MFG membrane.[159] Casein digestion may produce several bioactive peptides, which have been linked to gastrointestinal and immunological function, neurodevelopment, and even the effectiveness of antibiotics and probiotics.[160] Lactoferrin, a whey protein, is detectable both bound to the MFGM and in the aqueous phase of milk.[11] Analyses using the Kyoto Encyclopedia of Genes and Genomes (KEGG) have listed proteins involved in cell signaling, membrane transport, immune responses, and protein metabolism. These studies differ in exact genes and variants, but these differences could have emanated from the use of different databases for categorizing proteins.[161] Human MFGM contains important immune response mediators such as lactoferrin, immunoglobulins (alpha and gamma chain C region), monocyte differentiation CD14, clusterin, toll-like receptor 2, mucin 4, cathelicidins, and dermcidin, which have been identified as important in immune response.[161] Clusterin and lactadherin also play a role in cell damage and apoptosis, and consequently, in gut epithelial homeostasis.[162] Hormone-sensitive lipase, peroxisomal bifunctional enzyme, peroxisomal multifunctional enzyme type 2, peroxisomal acyl-coenzyme A oxidase 3, carboxyl ester lipase (GD3, BSSL), and sphingomyelin phosphodiesterase promote lipid digestion.[16]

Ribonucleic acid (RNA) in the MFG membrane: The cytoplasmic crescent of the MFG membrane holds cellular and microRNAs (miRNAs).[163] This transcriptome varies with a circadian rhythm and at different stages of lactation, to alter metabolic and immune regulation. Key genes involved in lactose synthesis and insulin signaling are closely regulated.[164]

MFG Core

The core of MFGs is comprised of TAGs, which constitute nearly 98% (w/w) of the total milk lipids.[16] In addition to being an important source of energy, MFGs serve as a vehicle for fat-soluble vitamins and carotenoids. Some of the other important fat constituents in these globules include phospholipids, glycolipids, and sphingomyelin, which originate from the ER. Once matured, these globules are secreted across the apical membrane of the mammary epithelial cells.

Milk fat contains over 400 different FAs, of which 15 constitute 90% of the total FA pool.[63] Compared to the MFG membrane, most TAGs in the MFG core consist of 18:1 (*n*–9) oleic (20–35%), 16:0 palmitic (18–23%), and 18:2 (*n*–6) linoleic (LA; 8–18%) acids.[20] Medium chain FAs (MCFAs) comprise 12% of total FAs, and < 1% are short-chain FAs.

TAGs contain nearly 85% of the milk LC-PUFAs such as 20:4 (*n*–6) ARA, 20:5 (*n*–3) eicosapentaenoic (EPA), and 22:6 (*n*–3) DHA. Even though the bioavailability of TAGs and phospholipid-bound LC-PUFAs might not differ, the metabolic disposition and incorporation into various organs differ. The 18:3 (*n*–3) α-linolenic acid (ALA) is less abundant, although there is a wide inter-individual variation. Small MFGs (1.6–3 μm) contain more medium-chain (C8-C12) but less long-chain saturated FAs such as stearic acid in their TAG core.

The location of FAs on the glycerol backbone is highly conserved within species.[165] Most saturated FAs are typically seen on the *sn*-2 position of TAGs. Palmitic acid constitutes 50–60% of all FAs at the *sn*-2 position (defined as β-16:0) in HM. The *sn*-1 and *sn*-3 positions are occupied primarily by unsaturated FAs such as oleic acid.[3]

Milk lipid fraction also contains minerals and lipophilic vitamins.[166] These components are embedded in both the MFG core and the MFGM. The fat fraction acts as a “natural carrier” for these compounds, increasing their bioaccessibility and bioavailability. Understanding the micronutrient content of the fat fraction could lead to their technological exploitation for the design of value-added products, in concert with the MFGM proteome (that acts as a scaffold for the binding of minerals and vitamins to MFGs) and the bioactive polar lipids.

Digestion of MFGs

Milk fat digestion in infants is a sequential, balanced process. The lipase triad of lingual, gastric, and pancreatic origin may show some variability but is active.[167,168] In the stomach, digestive hydrolysis is catalyzed by specific enzymes. As the pH drops below 5.5, the MFG membrane structure becomes less stable and leads to coagulation of the fat globules.[14]

In HM, TAGs carry FAs located in selected areas.[3,169] Nearly 70% of the *sn*-2 positions are esterified with a saturated FA; palmitic acid (C16:0) is a leading ligand (20–25%). The *sn*-1,3 positions are occupied by unsaturated FAs. FA region distribution affects the kinetics of digestion because gastric lipase shows stereospecificity for the *sn*-3. Medium chain-FAs (C8–C12) are more frequently located in the *sn*-3 position. FAs esterified at the *sn*-2 position of TAG are important because nearly 70% of the FAs absorbed as *sn*-2 monoacylglycerols are absorbed across the enterocytes and conserved in the original position during re-esterification into TAGs for secretion into the plasma as chylomicrons. The higher efficacy of absorption of palmitate at *sn*-2 compared to those at the *sn*-1,3 positions is well known. The absorption of FAs decreases as the FA chain length or unsaturation degree increases in HM.

Gastric lipases hydrolyze 5–40% of the dietary lipids in MFGs by acting on the *sn*-3 position of the TAGs.[170] This releases short- to medium-chain FAs, which play important metabolic roles such as in the acylation of proteins. Human gastric lipases can digest lipid droplets covered by polar lipids such as in the MFGs. The effects of gastric lipases are reinforced by endogenous milk lipases such as the BSSL and lipoprotein lipase.[171] In HM, lipases have easier access to the surface and subsequently to the interior of small MFGs, which can affect lipid digestion rates.[16]

Smaller MFGs adsorb more lipases and are digested faster than the larger ones but the composition and structure of the surrounding interfacial layer also influences the rates of lipid digestion.[11] The interfacial composition of an emulsion critically influences the activity of pancreatic lipase. Emulsifiers such as proteins, phospholipids, and surfactants interact with lipase to release FAs and promote/inhibit the adsorption of lipase on the emulsion surface.

Infants could have been at risk of not being able to utilize dietary fats in MFGs because of immature digestive processes. However, there are many compensating processes at work. First, there could have been difficulties for the lipases to penetrate the outer lipid coverings of the MFGs and reach the fats in the core.[172] Here, multiple gastric enzymatic reactions help detach these layers.[173] Next, there are mechanisms to compensate for the developmental inefficiencies such as the smaller size and motility of digestive organs, differences in gastric pH, low activity of digestive enzymes, and dietary patterns. The anionic phospholipids in HM promote lipase adsorption on the globules.[174] Furthermore, a bile salt-activated lipase (BSSL) in HM adds to the activities of gastric lipase, pancreatic-lipase-related protein 2 (PLRP2), and the phospholipase A_2 to compensate for insufficient bile salts and pancreatic triacylglycerol lipases (PTLs).[175-178] BSSL can digest medium and long TAGs, diacylglycerols, and phospholipids. It can also hydrolyze cholesterol esters, phospholipids, and ceramide.[179] Dietary SM is hydrolyzed by alkaline sphingomyelinase into phosphocholine and ceramide.[180] Neutral ceramidase hydrolyzes ceramide to FAs and sphingosine.[181] The sphingosine released in this process is adsorbed, phosphorylated to sphingosine-1-phosphate (S1P), and then converted to palmitic acid via the S1P-lyase present in the gut mucosa.[182] All these alternative processes can achieve about 95% efficiency of digestive lipolysis. Nearly 40–70% of dietary lipids are hydrolyzed to release long-chain PUFAs (C20:4 *n*–6, 20:5 *n*–3 and C22:6 *n*–3).[183] The *sn*-1,3 hydrolysis of milk fats by the PTLs releases *sn*-2 mono palmitin and the externally-positioned oleic acid.[184]

In HM, fat globules covered with MFG membrane are readily hydrolyzed when exposed to colipase and phospholipase A2, but not with pancreatic lipase.[185] Phospholipids could be protective against the digestive action of pancreatic lipase; the effects vary with the stage of digestion, the exact part of the gastrointestinal tract, and the type of phospholipid.[186] Phospholipids stabilized MFGs during storage by suppressing the adsorption of whey proteins on the globule surface.[187] SM also plays an important role in this process. The digestion of SM is less efficient in the upper intestine; these are partially cleaved to ceramide and sphingosine by alkaline sphingomyelinases.[182] This limited capacity of SM digestion favors the formation of SM-cholesterol complexes in the proximal parts of the intestine and exposes the lower small intestine and colon to SM and its bioactive metabolites.

In mouse pups, the MFG structure promotes metabolic programming.[188] Pups were fed from postnatal days 16 and 42 with either a control infant formula with submicronic fat droplets covered with milk proteins or with a novel formula with MFG-like larger fat droplets. Subsequently, all mice were fed a high-fat diet. Despite similar food intake, mice fed with the newer formula with MFG-like fat droplets showed better metabolic responses, lower body weight, less adipose tissue, and lower plasma insulin. These differential responses may be partly explained by different postprandial trafficking of FAs during infancy,

inducing different storage responses of the adipose tissues. However, a direct effect of MFG membrane in the concept formula is challenged by a recent study of human infants fed with a standard formula vs a formula enriched with MFG membrane fragments.[189] There were no differences in infant growth, weight gain, and body fat at 12 months and at 6.5 years.[189,190]

Diets containing MFG membranes likely promote cognitive development in infants. In one study, infants fed MFG membrane-enriched formula showed cognitive scores that were similar to those of breastfed infants but were significantly higher than those fed standard infant formula.[189] In another study, infants fed with an infant formula enriched with MFG membrane, LC-PUFAs, and synbiotics enriched infant formula-fed infants seem to show fewer behavioral problems up to 2.5 years compared to standard infant formula-fed infants.[191] A pilot randomized control trial showed that sphingomyelin-fortified milk improved the neurobehavioral development of very low birth weight infants during infancy.[138] The intervention groups performed significantly better at 18 months in the Bayley Scales of Infant and Toddler Development (BSID)-II, the Fagan test scores (evaluate visual recognition memory, habituation, and discrimination; relate these to intellectual functioning later in life), latency of visual evoked potentials, and sustained attention test scores. Further work is needed to elucidate the mechanisms by which MFG membrane components improve cognitive function.

The digestion of MFGs may be altered by the ζ-potential, the electric potential resulting from shear at the surface of these globules.[192] MFGs with large positive or negative ζ-potentials are electrostatically more stable; those with small ζ-potentials tend to coagulate or flocculate.[40] Smaller ζ-potential MFGs in HM are digested more easily, but flocculation of these smaller MFGs can reduce the surface area and access to digestive enzymes and bile salts.[40] These properties may be altered by the mineral composition of the milk, and the glycoprotein and glycolipid composition of the MFG membrane. The ζ-potentials also change over time after birth; the values for colostrum, transitional, and mature MFG were −5.60 ± 0.12, −6.72 ± 0.16, and −7.25 ± 0.61 mV, respectively.[16,67,193] Mature human MFGs have smaller ζ-potentials than in other mammals.[16]

The term ‘food matrix’ refers to the specific organization of foods that critically influence the release and absorption of nutrients during digestion in the gastrointestinal tract.[194] FA release from liquid matrix is quicker than from semi-solids. Similarly, the MFG structure, particularly the MFG membrane matrix, influences the rate of lipid digestion. In infant formula, lipid digestion was increased when intact proteins were replaced with hydrolyzed proteins.[195] The digestibility of fat is affected by its physical state, whether it is solid fat or liquid oil.[196] Whey protein-stabilized emulsions containing high levels of solid fat (hydrogenated soybean oil, melting point >37°C) release fewer FFAs during intestinal digestion *in vitro*.[196]

Biological Effects

The addition of MFG membranes to the infant diet has shown beneficial health effects in several preliminary studies. In addition to infant formula (0.5 gm/L) accelerated neurodevelopment and promoted cognitive function in healthy full-term infants with a low incidence of pathogen-associated adverse effects.[197] Healthy 6-month-old infants fed

infant formula fortified with milk polar lipids showed better hand-eye coordination and developmental quotients compared to controls fed standard infant formula.[127] However, there were no differences in cognitive development compared to healthy exclusively breastfed infants. In other studies, infants fed formula enriched with sphingomyelin (28–71 mg/mL) showed improved cerebral myelination.[198] SM metabolites such as cerebroside can cross the blood-brain barrier to promote myelination.[199] Noting the differences in term and preterm milk, SM could well play a role in promoting brain development in preterm or low-birth-weight infants.[200]

Milk fat globule membranes can promote the utilization of fats. Higher lipolysis and β-oxidation in early life prevents excessive weight gain later and lowers the risk of obesity.[11] In rats, supplementation with MFG membranes reduced adipogenesis and excessive weight gain by promoting brown fat formation in white adipose tissues.[13] Similar supplementation to high-fat diet-fed rats during pregnancy and lactation stimulated brown fat development in male offsprings.[201] MFG membrane supplementation during suckling reduced the risk of maternal high-fat diet-induced nonalcoholic fatty liver disease in mice, possibly due to less oxidative stress and restored mitochondrial function.[202] In another study, such intervention corrected the stunted skeletal growth of male offsprings at weaning and protected against abnormalities in bone microstructure and insulin resistance during adulthood.[203] Enhanced insulin-like growth factor-I activity may be a possible mechanism underlying these changes.

In formula-fed infants or rodent models, feedings supplemented with MFG membranes lead to notable changes in the fecal microbiome to simulate that of HM-fed infants.[204] In rodents, supplementation with MFG membranes increased the beta diversity of the cecal microbiome and improved spatial learning in the stress group.[205,206] The MFG membrane-mediated improved brain function may be related to the modulation of the gut microbiota via the gut-brain axis.[207] Similarly, in neonatal piglets, supplementation of infant formula with dairy MFG membranes and lipids influenced protein digestibility and microbiota composition.[208] In rats, MFG membrane supplementation promoted enterocyte proliferation and gut barrier function by inducing tight junction proteins.[11] In healthy term infants, MFG membrane components such as lactadherin (MFG-epidermal grow factor-8), sialic acid, and phospholipid promoted the growth of *Bifidobacterium* and suppressed the growth of *Veillonella, Escherichia* and *Shigella* spp.[209] These changes were relatively modest at 4 months, suggesting that age could be a confounder.

Administration of MFG membranes enhanced the intestinal barrier function in a rat model of short bowel syndrome.[210] Improved regulation of the NLRP6 inflammasome corrected gut dysbiosis.[210-212] Microbiota-modulated metabolites such as taurine, histamine, and spermine regulate the expression of the NLRP6 inflammasome, IL-18, and downstream antimicrobial peptide patterns in the intestine.[213] MFG membranes can also improve the viability of *Lactobacillus rhamnosus* GG from bile stress both *in vitro* and in murine models.[214] The promotion of probiotic survival by MFG membranes might provide a beneficial effect on gut health.[215]

Sphingomyelin, an important constituent of MFG membranes, affects lipid metabolism. When long-chain bases of SM were transported into cells via acyl-CoA synthetases,

the uptake of long-chain-FAs was inhibited.[216] In the intestine, SM and its metabolites modulated inflammatory signaling.[137] Sphingosine-1-phosphate (S1P) improved endothelial cell survival and migration.[136,217] Furthermore, metabolites of dietary sphingolipids influenced gut microbiota with increased commensal bacteria.[218] The mechanisms of these observations need further study.

Milk fat globule membranes need to be observed for nutritional and safety consequences depending on the actual composition of these membrane fractions.[219] Lipid-rich MFG membrane supplementation (n = 70, age 14 days) for 14 weeks did not result in any major safety concerns such as weight gain, morbidity, and metabolic markers, whereas protein-rich MFG membrane enrichment (n = 72) was associated with a higher rate of atopic dermatitis than in controls (n = 57).[220] These results need to be interpreted cautiously because the evaluation of skin lesions was not standardized and was based on parental reports. Further studies are needed.

Undigested MFG membranes that reach the colon support the colonization of microbial communities.[204] MFG membrane phospholipids can enrich *Porphyromonadaceae,* which differs from the impact of soy lecithin on *Enterobacteriaceae* and *Enterococcaceae*.[20] In another study, rat pups fed a formula supplemented with bovine MFG membrane increased gut microbial species richness and evenness compared with those who were fed a formula containing vegetable fat.[221] At the phylum level, the microbiota of rat pups fed MFG membrane resembled those reared on dam's milk with similar levels of *Firmicutes* and *Proteobacteria.* Control pups on regular formula showed more *Proteobacteria.*[222] In human infants, MFG membranes show intrinsic antimicrobial activity; a double-blind RCT showed that infants fed an experimental formula supplemented with bovine MFG membranes during 2–6 months of age experienced fewer acute otitis media infections than controls, fewer days with fever, a reduction in fever incidence, and improved behavioral outcomes.[153] Polar lipids such as sphingophospholipids and gangliosides in the MFG membrane also exhibit antimicrobial activities. In preterm newborns, infants fed a formula supplemented with bovine gangliosides showed smaller *E. coli* fecal counts enriched *Bifidobacterium* compared to a standard formula.[223]

XOR in the MFG membrane generates reactive oxygen and nitrogen species and can inhibit the growth of *E. coli* and *Salmonella enteritides.*[20] α-Lactalbumin, a minor protein in the MFG membrane, is digested by pepsin, trypsin, and chymotrypsin in the intestine to generate bactericidal peptides that activate leukocytes.[224] Lysozyme, another MFG membrane protein, can protect against both Gram-positive and -negative bacteria due to the presence of 1,4-β-N-acetylmuraminidase which can degrade bacterial cell walls.[225] These effects of lysozyme are supported by lactoferrin, which carries iron and interacts with the lipid A moiety of LPS to damage the bacterial membrane.[20]

Milk fat globule membrane glycobiome is also potentially important in anti-bacterial defenses.[20] The MFG membrane contains glycoconjugates (glycolipids and glycoproteins) harboring both *N*-linked and *O*-lined glycan moieties.[20] The glycosylation patterns of these glycoconjugates in milk determine resistance to certain bacteria from binding specific mucosal receptors. The glycoproteins (MUC1, lactadherin) and gangliosides of the MFGM

have the ability to interfere with such attachment. Mucin can also inhibit *Salmonella enterica serovar Typhimurium SL1344,* S-fimbriated *E. coli,* and rotavirus.[226,227] Further work is needed.

In infants, MFG membrane can bind and carry ingested probiotics such as Lactobacilli and *Bifidobacteria* to the colon.[228] HM bacteria can also subserve this function during the early stages of gut development Select OTUs assigned to *Bifidobacterium,* such as *B. breve, B. bifidum,* and *B. longum* that were identified in mother-infant pairs could preferentially associate with the MFG membrane utilizing glycan adhesion factors that enabled binding with mucin.[20] Increasing information indicates that the lactic acid bacteria (LAB), which include the phylum Firmicutes, class Bacilli, and order Lactobacillales, are comprised of 6 families: *Aerococcaceae, Carnobacteriaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae,* and *Streptococcaceae.*[229] MFG membrane glycoproteins can survive gastric digestion and can show prebiotic effects to support the growth of colonic bacteria such as *Ruminococcus* and *Bifidobacteria* genera, most likely due to sialic acid residues.[230] The *Ruminococci* are important mutualist gut bacteria that serve to degrade and convert complex polysaccharides into a variety of nutrients for their hosts.[231]

Conclusion

The size and composition of MFGs may be influenced by maternal ethnicity, genetic factors, physiological state, diet, stage of lactation, and metabolic state.[17] In addition to these identified sources of diversity, there are still unexplained individual variations. Post-secretion modifications such as membrane vesiculation or MFG fusion may also affect MFG size and structure.[232] More data are needed to understand the *in vivo* digestibility of the MFG size fractions, and to clarify the release and activity of the MFG membrane.[233] Understanding the composition and dynamics of MFGs could help not only for personalized nutrition for chronic diseases, but also as an important channel for enteral delivery of medications as needed and at desired rates of release.[233] Wide use of nutraceuticals will become a closer reality.[233]

References

1. Ballard O, Morrow AL. Human milk composition: Nutrients and bioactive factors. Pediatr Clin North Am 2013;60(1):49–74. DOI: 10.1016/j.pcl.2012.10.002. [PubMed: 23178060]
2. Samuel TM, Zhou Q, Giuffrida F, et al. Nutritional and non-nutritional composition of human milk is modulated by maternal, infant, and methodological factors. Front Nutr 2020;7:576133. DOI: 10.3389/fnut.2020.576133. [PubMed: 33117843]
3. Maheshwari A. Fats in human milk: 2022 updates on chemical composition. Newborn (Clarksville) 2022;1(4):384–396. DOI: 10.5005/jp-journals-11002-0050.
4. Valverde R, Dinerstein NA, Vain N. Mother's own milk and donor milk. World Rev Nutr Diet 2021;122:212–224. DOI: 10.1159/000514733. [PubMed: 34352749]
5. Butte NF. Fat intake of children in relation to energy requirements. Am J Clin Nutr 2000;72(5 Suppl):1246S–1252S. DOI: 10.1093/ajcn/72.5.1246s. [PubMed: 11063466]
6. Young BE, Krebs NF. Complementary feeding: Critical considerations to optimize growth, nutrition, and feeding behavior. Curr Pediatr Rep 2013;1(4):247–256. DOI: 10.1007/s40124-013-0030-8. [PubMed: 25105082]
7. WHO U, USAID A, AED U. Indicators for assessing infant and young child feeding practices. Geneva: World Health Organization. 2008.

8. Argov N, Lemay DG, German JB. Milk fat globule structure & function; nanosciece comes to milk production. Trends Food Sci Technol 2008;19(12):10. DOI: 10.1016/j.tifs.2008.07.006.
9. Argov N, Wachsmann-Hogiu S, Freeman SL, et al. Size-dependent lipid content in human milk fat globules. J Agric Food Chem 2008;56(16):7446–7450. DOI: 10.1021/jf801026a. [PubMed: 18656925]
10. Cavaletto M, Givonetti A, Cattaneo C. The immunological role of milk fat globule membrane. Nutrients 2022;14(21):4574. DOI: 10.3390/nu14214574. [PubMed: 36364836]
11. Chai C, Oh S, Imm JY. Roles of milk fat globule membrane on fat digestion and infant nutrition. Food Sci Anim Resour 2022;42(3):351–371. DOI: 10.5851/kosfa.2022.e11. [PubMed: 35611078]
12. Huang QX, Yang J, Hu M, et al. Milk fat globule membrane proteins are involved in controlling the size of milk fat globules during conjugated linoleic acid-induced milk fat depression. J Dairy Sci 2022;105(11):9179–9190. DOI: 10.3168/jds.2022-22131. [PubMed: 36175227]
13. Li T, Gao J, Du M, et al. Milk fat globule membrane attenuates high-fat diet-induced obesity by inhibiting adipogenesis and increasing uncoupling protein 1 expression in white adipose tissue of mice. Nutrients 2018;10(3):331. DOI: 10.3390/nu10030331. [PubMed: 29522452]
14. Lopez C. Milk fat globules enveloped by their biological membrane: Unique colloidal assemblies with a specific composition and structure. Curr Opin Colloid Interface Sci 2011;16(5)391–404. DOI: 10.1016/j.cocis.2011.05.007.
15. Sun Y, Roos YH, Miao S. Changes in milk fat globules and membrane proteins prepared from pH-adjusted bovine raw milk. Foods 2022;11(24):4107. DOI: 10.3390/foods11244107. [PubMed: 36553849]
16. Thum C, Roy NC, Everett DW, et al. Variation in milk fat globule size and composition: A source of bioactives for human health. Crit Rev Food Sci Nutr 2023;63(1):87–113. DOI: 10.1080/10408398.2021.1944049. [PubMed: 34190660]
17. Thum C, Wall C, Day L, et al. Changes in human milk fat globule composition throughout lactation: A review. Front Nutr 2022; 9:835856. DOI: 10.3389/fnut.2022.835856. [PubMed: 35634409]
18. Walter L, Narayana VK, Fry R, et al. Milk fat globule size development in the mammary epithelial cell: A potential role for ether phosphatidylethanolamine. Sci Rep 2020;10(1):12299. DOI: 10.1038/s41598-020-69036-5. [PubMed: 32704146]
19. McManaman JL. Lipid transport in the lactating mammary gland. J Mammary Gland Biol Neoplasia 2014;19(1):35–42. DOI: 10.1007/s10911-014-9318-8. [PubMed: 24567110]
20. Lee H, Padhi E, Hasegawa Y, et al. Compositional dynamics of the milk fat globule and its role in infant development. Front Pediatr 2018;6:313. DOI: 10.3389/fped.2018.00313. [PubMed: 30460213]
21. Newburg DS, Peterson JA, Ruiz-Palacios GM, et al. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. Lancet 1998;351(9110):1160–1164. DOI: 10.1016/s0140-6736(97)10322-1. [PubMed: 9643686]
22. Hahn-Holbrook J, Fish A, Glynn LM. Human milk omega-3 fatty acid composition is associated with infant temperament. Nutrients 2019;11(12):2964. DOI: 10.3390/nu11122964. [PubMed: 31817237]
23. Ding D, He X, Agarry IE, et al. Profile of human milk phospholipids at different lactation stages with UPLC/Q-TOF-MS: Characterization, distribution, and differences. J Agric Food Chem 2023;71(16):6326–6337. DOI: 10.1021/acs.jafc.2c07512. [PubMed: 37040528]
24. Seki D, Errerd T, Hall LJ. The role of human milk fats in shaping neonatal development and the early life gut microbiota. Microbiome Res Rep 2023;2(2):8. DOI: 10.20517/mrr.2023.09. [PubMed: 38047278]
25. Boquien CY. Human milk: An ideal food for nutrition of preterm newborn. Front Pediatr 2018;6:295. DOI: 10.3389/fped.2018.00295. [PubMed: 30386758]
26. Mead MN. Contaminants in human milk: Weighing the risks against the benefits of breastfeeding. Environ Health Perspect 2008;116(10):A427–A434. [PubMed: 18941560]
27. Jukkola A, Partanen R, Rojas OJ, et al. Separation of milk fat globules via microfiltration: Effect of diafiltration media and opportunities for stream valorization. J Dairy Sci 2016;99(11):8644–8654. DOI: 10.3168/jds.2016-11422. [PubMed: 27638266]

28. Wiking L, Gregersen SB, Hansen SF, et al. Heat-induced changes in milk fat and milk fat globules and its derived effects on acid dairy gelation–A review. Int Dairy J 2022;127:105213. DOI: 10.1016/j.idairyj.2021.105213.
29. Raza GS, Herzig KH, Leppaluoto J. Invited review: Milk fat globule membrane-A possible panacea for neurodevelopment, infections, cardiometabolic diseases, and frailty. J Dairy Sci 2021;104(7):7345–7363. DOI: 10.3168/jds.2020-19649. [PubMed: 33896625]
30. Steffen F, Hansen BP, Rasmussen JT, et al. Placing pasteurisation before or after microfiltration impacts the protein composition of milk fat globule membrane material. Int Dairy J 2018;81:35–41. DOI: 10.1016/j.idairyj.2017.12.015.
31. Alshehab M, Reis MG, Day L, et al. Milk fat globules, a novel carrier for delivery of exogenous cholecalciferol. Food Res Int 2019;126:108579. DOI: 10.1016/j.foodres.2019.108579. [PubMed: 31732024]
32. Acevedo-Fani A, Dave A, Singh H. Nature-assembled structures for delivery of bioactive compounds and their potential in functional foods. Front Chem 2020;8:564021. DOI: 10.3389/fchem.2020.564021. [PubMed: 33102443]
33. Rahman MM, Khatun S, Kabir N, et al. Establishment of the first religiously-compliant human milk bank in bangladesh. Newborn (Clarksville) 2022;1(4):376–383. DOI: 10.5005/jp-journals-11002-0047.
34. Raz C, Paramonov MM, Shemesh M, et al. The milk fat globule size governs a physiological switch for biofilm formation by Bacillus subtilis. Front Nutr 2022;9:844587. DOI: 10.3389/fnut.2022.844587. [PubMed: 36034896]
35. Masedunskas A, Chen Y, Stussman R, et al. Kinetics of milk lipid droplet transport, growth, and secretion revealed by intravital imaging: Lipid droplet release is intermittently stimulated by oxytocin. Mol Biol Cell 2017;28(7):935–946. DOI: 10.1091/mbc.E16-11-0776. [PubMed: 28179456]
36. Di Marzo L, Cree P, Barbano DM. Prediction of fat globule particle size in homogenized milk using Fourier transform mid-infrared spectra. J Dairy Sci 2016;99(11):8549–8560. DOI: 10.3168/jds.2016-11284. [PubMed: 27638254]
37. Kowalczuk PB, Drzymala J. Physical meaning of the Sauter mean diameter of spherical particulate matter. Particulate Science and Technology 2016;34(6):645–647. DOI: 10.1080/02726351.2015.1099582.
38. Lyu F, Thomas M, Hendriks WH, et al. Size reduction in feed technology and methods for determining, expressing and predicting particle size: A review. Animal Feed Science and Technology 2020;261:114347. DOI: 10.1016/j.anifeedsci.2019.114347.
39. Pinto G, Baptista A, Silva F, et al. Study on the influence of the ball material on abrasive particles' dynamics in ball-cratering thin coatings wear tests. Materials (Basel) 2021;14(3):668. DOI: 10.3390/ma14030668. [PubMed: 33535544]
40. Michalski MC, Briard V, Michel F, et al. Size distribution of fat globules in human colostrum, breast milk, and infant formula. J Dairy Sci 2005;88(6):1927–1940. DOI: 10.3168/jds.S0022-0302(05)72868-X. [PubMed: 15905422]
41. Wei W, Li D, Jiang C, et al. Phospholipid composition and fat globule structure II: Comparison of mammalian milk from five different species. Food Chem 2022;388:132939. DOI: 10.1016/j.foodchem.2022.132939. [PubMed: 35447582]
42. Anto L, Warykas SW, Torres-Gonzalez M, et al. Milk polar lipids: Underappreciated lipids with emerging health benefits. Nutrients 2020;12(4):1001. DOI: 10.3390/nu12041001. [PubMed: 32260440]
43. Aumeistere L, Ciprovica I, Zavadska D, et al. Impact of maternal diet on human milk composition among lactating women in latvia. Medicina (Kaunas) 2019;55(5):173. DOI: 10.3390/medicina55050173. [PubMed: 31137596]
44. Verardo V, Gomez-Caravaca AM, Arraez-Roman D, et al. Recent advances in phospholipids from colostrum, milk and dairy by-products. Int J Mol Sci 2017;18(1):173. DOI: 10.3390/ijms18010173. [PubMed: 28106745]

45. Tan S, Chen C, Zhao A, et al. The dynamic changes of gangliosides in breast milk and the intake of gangliosides in maternal and infant diet in three cities of China. Int J Clin Exp Pathol 2020;13(11):2870–2888. [PubMed: 33284868]
46. Kolter T. Ganglioside biochemistry. ISRN Biochem 2012;2012:506160. DOI: 10.5402/2012/506160. [PubMed: 25969757]
47. Mansson HL. Fatty acids in bovine milk fat. Food Nutr Res 2008;52(1). DOI: 10.3402/fnr.v52i0.1821.
48. Contarini G, Povolo M. Phospholipids in milk fat: Composition, biological and technological significance, and analytical strategies. Int J Mol Sci 2013;14(2):2808–2831. DOI: 10.3390/ijms14022808. [PubMed: 23434649]
49. He X, McClorry S, Hernell O, et al. Digestion of human milk fat in healthy infants. Nutr Res 2020;83:15–29. DOI: 10.1016/j.nutres.2020.08.002. [PubMed: 32987285]
50. Linn JG. Factors affecting the composition of milk from dairy cows. In: Products NRCUCoTOtItNAoA (Eds). Designing Foods: Animal Product Options in the Marketplace. Washington (DC): National Academies Press (US); 1988.
51. Sanchez-Hernandez S, Esteban-Munoz A, Gimenez-Martinez R, et al. A comparison of changes in the fatty acid profile of human milk of spanish lactating women during the first month of lactation using gas chromatography-mass spectrometry. A comparison with infant formulas. Nutrients 2019;11(12):3055. DOI: 10.3390/nu11123055. [PubMed: 31847315]
52. Yang F, Chen G. The nutritional functions of dietary sphingomyelin and its applications in food. Front Nutr 2022;9:1002574. DOI: 10.3389/fnut.2022.1002574. [PubMed: 36337644]
53. Sinanoglou VJ, Cavouras D, Boutsikou T, et al. Factors affecting human colostrum fatty acid profile: A case study. PLoS One 2017;12(4):e0175817. DOI: 10.1371/journal.pone.0175817. [PubMed: 28410426]
54. Jia W, Zhang R, Zhu Z, et al. A high-throughput comparative proteomics of milk fat globule membrane reveals breed and lactation stages specific variation in protein abundance and functional differences between milk of saanen dairy goat and holstein bovine. Front Nutr 2021;8:680683. DOI: 10.3389/fnut.2021.680683. [PubMed: 34124126]
55. Schultz-Pernice I, Engelbrecht LK, Petricca S, et al. Morphological analysis of human milk membrane enclosed structures reveals diverse cells and cell-like milk fat globules. J Mammary Gland Biol Neoplasia 2020;25(4):397–408. DOI: 10.1007/s10911-020-09472-1. [PubMed: 33394266]
56. Mulder H, Walstra P. The milk fat globule: Commonwealth Agricultural Bureaux Farnham Royal; 1974. pp. 54–97.
57. Andrews AT, Anderson M, Goodenough PW. A study of the heat stabilities of a number of indigenous milk enzymes. Journal of Dairy Research 1987;54(2):237–246. DOI: 10.1017/S0022029900025371.
58. Hansen SF, Larsen LB, Wiking L. Thermal effects on IgM-milk fat globule-mediated agglutination. Journal of Dairy Research 2019;86(1):108–113. DOI: 10.1017/S0022029918000778. [PubMed: 30520391]
59. Burge K, Vieira F, Eckert J, et al. Lipid composition, digestion, and absorption differences among neonatal feeding strategies: Potential implications for intestinal inflammation in preterm infants. Nutrients 2021;13(2):550. DOI: 10.3390/nu13020550. [PubMed: 33567518]
60. Rogalska E, Ransac S, Verger R. Stereoselectivity of lipases. II. Stereoselective hydrolysis of triglycerides by gastric and pancreatic lipases. J Biol Chem 1990;265(33):20271–20276. [PubMed: 2243091]
61. Gimenez MS, Oliveros LB, Gomez NN. Nutritional deficiencies and phospholipid metabolism. Int J Mol Sci 2011;12(4):2408–2433. DOI: 10.3390/ijms12042408. [PubMed: 21731449]
62. Manoni M, Di Lorenzo C, Ottoboni M, et al. Comparative proteomics of milk fat globule membrane (MFGM) proteome across species and lactation stages and the potentials of MFGM fractions in infant formula preparation. Foods 2020;9(9):1251. DOI: 10.3390/foods9091251. [PubMed: 32906730]

63. Wang C, Qiao X, Gao Z, et al. Advancement on milk fat globule membrane: Separation, identification, and functional properties. Front Nutr 2021;8:807284. DOI: 10.3389/fnut.2021.807284. [PubMed: 35155526]
64. Hansen SF, Hogan SA, Tobin J, et al. Microfiltration of raw milk for production of high-purity milk fat globule membrane material. J Food Eng 2020;276:109887. DOI: 10.1016/j.jfoodeng.2019.109887.
65. Hu Y, Thaler J, Nieuwland R. Extracellular vesicles in human milk. Pharmaceuticals (Basel) 2021;14(10):1050. DOI: 10.3390/ph14101050. [PubMed: 34681274]
66. Argov-Argaman N, Smilowitz JT, Bricarello DA, et al. Lactosomes: Structural and compositional classification of unique nanometer-sized protein lipid particles of human milk. J Agric Food Chem 2010;58(21):11234–11242. DOI: 10.1021/jf102495s. [PubMed: 20925428]
67. Mizuno K, Nishida Y, Taki M, et al. Is increased fat content of hindmilk due to the size or the number of milk fat globules? Int Breastfeed J 2009;4:7. DOI: 10.1186/1746-4358-4-7. [PubMed: 19607695]
68. Argov-Argaman N, Raz C, Roth Z. Progesterone regulation of milk fat globule size is VLDL dependent. Front Endocrinol (Lausanne) 2020;11:596. DOI: 10.3389/fendo.2020.00596. [PubMed: 33013694]
69. Churakov M, Karlsson J, Edvardsson Rasmussen A, et al. Milk fatty acids as indicators of negative energy balance of dairy cows in early lactation. Animal 2021;15(7):100253. DOI: 10.1016/j.animal.2021.100253. [PubMed: 34090089]
70. Capuco AV, Akers RM. The origin and evolution of lactation. J Biol 2009;8(4):37. DOI: 10.1186/jbiol139. [PubMed: 19439024]
71. Oftedal OT. The evolution of milk secretion and its ancient origins. Animal 2012;6(3):355–368. DOI: 10.1017/S1751731111001935. [PubMed: 22436214]
72. Oftedal OT. The mammary gland and its origin during synapsid evolution. J Mammary Gland Biol Neoplasia 2002;7(3):225–252. DOI: 10.1023/a:1022896515287. [PubMed: 12751889]
73. Oftedal OT. The origin of lactation as a water source for parchment-shelled eggs. J Mammary Gland Biol Neoplasia 2002;7(3):253–266. DOI: 10.1023/a:1022848632125. [PubMed: 12751890]
74. Folk GE Jr, Semken HA Jr. The evolution of sweat glands. Int J Biometeorol 1991;35(3):180–186. DOI: 10.1007/BF01049065. [PubMed: 1778649]
75. Lefevre CM, Sharp JA, Nicholas KR. Evolution of lactation: Ancient origin and extreme adaptations of the lactation system. Annu Rev Genomics Hum Genet 2010;11:219–238. DOI: 10.1146/annurev-genom-082509-141806. [PubMed: 20565255]
76. Kemp TS. The origin and early radiation of the therapsid mammal-like reptiles: A palaeobiological hypothesis. J Evol Biol 2006;19(4):1231–1247. DOI: 10.1111/j.1420-9101.2005.01076.x. [PubMed: 16780524]
77. Pavlova T, Spacil Z, Vidova V, et al. Adipophilin and perilipin 3 positively correlate with total lipid content in human breast milk. Sci Rep 2020;10(1):360. DOI: 10.1038/s41598-019-57241-w. [PubMed: 31941931]
78. Chong BM, Reigan P, Mayle-Combs KD, et al. Determinants of adipophilin function in milk lipid formation and secretion. Trends Endocrinol Metab 2011;22(6):211–217. DOI: 10.1016/j.tem.2011.04.003. [PubMed: 21592818]
79. Haarsma L, Nelesen S, VanAndel E, et al. Simulating evolution of protein complexes through gene duplication and co-option. J Theor Biol 2016;399:22–32. DOI: 10.1016/j.jtbi.2016.03.028. [PubMed: 27038665]
80. Wells JN, Bergendahl LT, Marsh JA. Co-translational assembly of protein complexes. Biochem Soc Trans 2015;43(6):1221–1226. DOI: 10.1042/BST20150159. [PubMed: 26614664]
81. Verma HC. Concepts of physics part I. 4th edition. New Delhi, India: S. Chand & Company Ltd; 2008. pp. 284–286.
82. Lewis M. Food dimensions; size, shape and numbers. Sawston, United Kingdom: Woodhead Publishing; 2023. pp. 12–86.
83. Koroleva M, Tokarev A, Yurtov E. Modeling droplet aggregation and percolation clustering in emulsions. Arab J Chem 2019;12(8):4458–4465. DOI: 10.1016/j.arabjc.2016.07.001.

84. Leong K, Wang F. A molecular dynamics investigation of the surface tension of water nanodroplets and a new technique for local pressure determination through density correlation J Chem Phys 2018;148(14):144503. DOI: 10.1063/1.5004985. [PubMed: 29655336]
85. Colombo M, Wright C. First principles in the life sciences: The free-energy principle, organicism, and mechanism. Synthese 2021;198(14):3463–3488. DOI: 10.1007/s11229-018-01932-w.
86. Panchal BR, Truong T, Prakash S, et al. Effect of fat globule size on the churnability of dairy cream. Food Res Int 2017;99(Pt 1):229–338. DOI: 10.1016/j.foodres.2017.05.027. [PubMed: 28784479]
87. Davies-Jones R An expression for effective buoyancy in surroundings with horizontal density gradients. J Atmos Sci 2003;60(23):2922–2955. DOI: 10.1175/1520-0469(2003)060<2922:AEFEBI>2.0.CO;2.
88. Bannon P Theoretical foundations for models of moist convection. J Atmos Sci 2002;59(12):1967–1982. DOI: 10.1175/1520-0469(2002)059<1967:TFFMOM>2.0.CO;2.
89. Das P A non-archimedean approach to the equations of convection dynamics. J Atmos Sci 1979;36(11):2183–2190. DOI: 10.1175/1520-0469(1979)036<2183:ANAATT>2.0.CO;2.
90. Wang L, Chen W, Guo H. Response of membrane tension to gravity in an approximate cell model. Theor Biol Med Model 2019;16(1):19. DOI: 10.1186/s12976-019-0116-2. [PubMed: 31801614]
91. Dey S, Zeeshan Ali S, Padhi E. Terminal fall velocity: The legacy of Stokes from the perspective of fluvial hydraulics. Proc Math Phys Eng Sci 2019;475(2228):20190277. DOI: 10.1098/rspa.2019.0277. [PubMed: 31534429]
92. Cartwright JHE. Stokes' law, viscometry, and the Stokes falling sphere clock. Philos Trans A Math Phys Eng Sci 2020;378(2179):20200214. DOI: 10.1098/rsta.2020.0214. [PubMed: 32762440]
93. Gomez-Solano JR, July C, Mehl J, et al. Non-equilibrium work distribution for interacting colloidal particles under friction. New J Phys 2015;17:045026. DOI: 10.1088/1367-2630/17/4/045026.
94. Smoczynski M Role of phospholipid flux during milk secretion in the mammary gland. J Mammary Gland Biol Neoplasia 2017;22(2):117–129. DOI: 10.1007/s10911-017-9376-9. [PubMed: 28243823]
95. D'Incecco P, Ong L, Pellegrino L, et al. Effect of temperature on the microstructure of fat globules and the immunoglobulin-mediated interactions between fat and bacteria in natural raw milk creaming. J Dairy Sci 2018;101(4):2984–2997. DOI: 10.3168/jds.2017-13580. [PubMed: 29398025]
96. Wu C, Qian T, Sheng P. Droplet spreading driven by van der Waals force: a molecular dynamics study. J Phys Condens Matter 2010;22(32):325101. DOI: 10.1088/0953-8984/22/32/325101. [PubMed: 21386483]
97. Lahiri R, Arvind, Sain A. Brownian motion in a classical ideal gas: A microscopic approach to Langevin's equation. Pramana - J Phys 2004;62:1015–1028. DOI: 10.1007/BF02705249.
98. Rings D, Schachoff R, Selmke M, et al. Hot brownian motion. Phys Rev Lett 2010;105(9):090604. DOI: 10.1103/PhysRevLett.105.090604. [PubMed: 20868149]
99. Mokshin AV, Yulmetyev RM, Hänggi P. Diffusion processes and memory effects. New J Phys 2005;7:9. DOI: 10.1088/1367-2630/7/1/009.
100. Pitre L, Plimmer MD, Sparasci F, et al. Determinations of the Boltzmann constant. Comptes Rendus Physique 2019;20(1–2):129–139. DOI: 10.1016/j.crhy.2018.11.007.
101. de Oliveira MJ. Boltzmann stochastic thermodynamics. Phys Rev E 2019;99(5–1):052138. DOI: 10.1103/PhysRevE.99.052138. [PubMed: 31212577]
102. Sinha KP, Das S, Karyappa RB, et al. Electrohydrodynamics of vesicles and capsules. Langmuir 2020;36(18):4863–4886. DOI: 10.1021/acs.langmuir.9b03971. [PubMed: 32275824]
103. Wei T, Huang Y, Weng C, et al. Lipid rafts may affect the coalescence of milk fat globules through phase transition after thermal treatment. Food Chem 2023;399:133867. DOI: 10.1016/j.foodchem.2022.133867. [PubMed: 35994856]
104. Pan J, Chen M, Li N, et al. Bioactive functions of lipids in the milk fat globule membrane: A comprehensive review. Foods 2023;12(20):3755. DOI: 10.3390/foods12203755. [PubMed: 37893646]

105. Lu J, Argov-Argaman N, Anggrek J, et al. The protein and lipid composition of the membrane of milk fat globules depends on their size. J Dairy Sci 2016;99(6):4726–4738. DOI: 10.3168/jds.2015-10375. [PubMed: 26995123]
106. Wooding FB, Mather IH. Ultrastructural and immunocytochemical evidence for the reorganisation of the milk fat globule membrane after secretion. Cell Tissue Res 2017;367(2):283–295. DOI: 10.1007/s00441-016-2505-8. [PubMed: 27677271]
107. Yasuda T, Al Sazzad MA, Jantti NZ, et al. The influence of hydrogen bonding on sphingomyelin/colipid interactions in bilayer membranes. Biophys J 2016;110(2):431–440. DOI: 10.1016/j.bpj.2015.11.3515. [PubMed: 26789766]
108. Smoczyński M, Staniewski B, Kiełczewska K. Composition and structure of the bovine milk fat globule membrane—Some nutritional and technological implications. Food Reviews International 2012;28(2):188–202. DOI: 10.1080/87559129.2011.595024.
109. McManaman JL. Formation of milk lipids: A molecular perspective. Clin Lipidol 2009;4(3):391–401. DOI: 10.2217/clp.09.15. [PubMed: 26084294]
110. Spitsberg VL, Matitashvili E, Gorewit RC. Association and coexpression of fatty-acid-binding protein and glycoprotein CD36 in the bovine mammary gland. Eur J Biochem 1995;230(3):872–878. DOI: 10.1111/j.1432-1033.1995.tb20630.x. [PubMed: 7541353]
111. Yang Y, Spitzer E, Kenney N, et al. Members of the fatty acid binding protein family are differentiation factors for the mammary gland. J Cell Biol 1994;127(4):1097–1109. DOI: 10.1083/jcb.127.4.1097. [PubMed: 7962070]
112. Reinhardt TA, Lippolis JD. Developmental changes in the milk fat globule membrane proteome during the transition from colostrum to milk. J Dairy Sci 2008;91(6):2307–2318. DOI: 10.3168/jds.2007-0952. [PubMed: 18487653]
113. Yang MT, Lan QY, Liang X, et al. Lactational changes of phospholipids content and composition in chinese breast milk. Nutrients 2022;14(8):1539. DOI: 10.3390/nu14081539. [PubMed: 35458100]
114. Ma L, Fong BY, MacGibbon AKH, et al. Qualitative and quantitative study of glycosphingolipids in human milk and bovine milk using high performance liquid chromatography-data-dependent acquisition-mass spectrometry. Molecules 2020;25(17):4024. DOI: 10.3390/molecules25174024. [PubMed: 32899251]
115. Nilsson A, Duan RD. Absorption and lipoprotein transport of sphingomyelin. J Lipid Res 2006;47(1):154–171. DOI: 10.1194/jlr.M500357-JLR200. [PubMed: 16251722]
116. Nieto-Ruiz A, Garcia-Santos JA, Verdejo-Roman J, et al. Infant formula supplemented with milk fat globule membrane, long-chain polyunsaturated fatty acids, and synbiotics is associated with neurocognitive function and brain structure of healthy children aged 6 years: The COGNIS study. Front Nutr 2022;9:820224. DOI: 10.3389/fnut.2022.820224. [PubMed: 35356726]
117. Jiang B, Xia Y, Zhou L, et al. Safety and tolerance assessment of milk fat globule membrane-enriched infant formulas in healthy term Chinese infants: A randomised multicenter controlled trial. BMC Pediatr 2022;22(1):465. DOI: 10.1186/s12887-022-03507-8. [PubMed: 35918695]
118. Ambrozej D, Dumycz K, Dziechciarz P, et al. Milk fat globule membrane supplementation in children: Systematic review with meta-analysis. Nutrients 2021;13(3):714. DOI: 10.3390/nu13030714. [PubMed: 33668227]
119. Khor GL, Tan SS, Stoutjesdijk E, et al. Temporal changes in breast milk fatty acids contents: A case study of malay breastfeeding women. Nutrients 2020;13(1):101. DOI: 10.3390/nu13010101. [PubMed: 33396781]
120. Delplanque B, Gibson R, Koletzko B, et al. Lipid quality in infant nutrition: Current knowledge and future opportunities. J Pediatr Gastroenterol Nutr 2015;61(1):8–17. DOI: 10.1097/MPG.0000000000000818. [PubMed: 25883056]
121. Hadley KB, Ryan AS, Forsyth S, et al. The essentiality of arachidonic acid in infant development. Nutrients 2016;8(4):216. DOI: 10.3390/nu8040216. [PubMed: 27077882]
122. Li J, Pora BLR, Dong K, et al. Health benefits of docosahexaenoic acid and its bioavailability: A review. Food Sci Nutr 2021;9(9):5229–5243. DOI: 10.1002/fsn3.2299. [PubMed: 34532031]
123. Rueda R The role of dietary gangliosides on immunity and the prevention of infection. Br J Nutr 2007;98 Suppl 1:S68–S73. DOI: 10.1017/S0007114507832946. [PubMed: 17922964]

124. Schnaar RL, Gerardy-Schahn R, Hildebrandt H. Sialic acids in the brain: Gangliosides and polysialic acid in nervous system development, stability, disease, and regeneration. Physiol Rev 2014;94(2):461–518. DOI: 10.1152/physrev.00033.2013. [PubMed: 24692354]

125. Bronnum H, Seested T, Hellgren LI, et al. Milk-derived GM(3) and GD(3) differentially inhibit dendritic cell maturation and effector functionalities. Scand J Immunol 2005;61(6):551–557. DOI: 10.1111/j.1365-3083.2005.01566.x. [PubMed: 15963050]

126. Palmano K, Rowan A, Guillermo R, et al. The role of gangliosides in neurodevelopment. Nutrients 2015;7(5):3891–3913. DOI: 10.3390/nu7053891. [PubMed: 26007338]

127. Gurnida DA, Rowan AM, Idjradinata P, et al. Association of complex lipids containing gangliosides with cognitive development of 6-month-old infants. Early Hum Dev 2012;88(8):595–601. DOI: 10.1016/j.earlhumdev.2012.01.003. [PubMed: 22289412]

128. Venkat M, Chia LW, Lambers TT. Milk polar lipids composition and functionality: A systematic review. Crit Rev Food Sci Nutr 2024;64(1):31–75. DOI: 10.1080/10408398.2022.2104211. [PubMed: 35997253]

129. Okuda T Dietary control of ganglioside expression in mammalian tissues. Int J Mol Sci 2019;21(1):177. DOI: 10.3390/ijms21010177. [PubMed: 31887977]

130. Park EJ, Suh M, Thomson AB, et al. Dietary gangliosides increase the content and molecular percentage of ether phospholipids containing 20:4n-6 and 22:6n-3 in weanling rat intestine. J Nutr Biochem 2006;17(5):337–344. DOI: 10.1016/j.jnutbio.2005.08.005. [PubMed: 16216486]

131. Dewettinck K, Rombaut R, Thienpont N, et al. Nutritional and technological aspects of milk fat globule membrane material. Int Dairy J 2008;18(5):436–457. DOI: 10.1016/j.idairyj.2007.10.014.

132. Gomez-Larrauri A, Presa N, Dominguez-Herrera A, et al. Role of bioactive sphingolipids in physiology and pathology. Essays Biochem 2020;64(3):579–589. DOI: 10.1042/EBC20190091. [PubMed: 32579188]

133. Lee M, Lee SY, Bae YS. Functional roles of sphingolipids in immunity and their implication in disease. Exp Mol Med 2023;55(6):1110–1130. DOI: 10.1038/s12276-023-01018-9. [PubMed: 37258585]

134. Obinata H, Hla T. Sphingosine 1-phosphate and inflammation. Int Immunol 2019;31(9):617–625. DOI: 10.1093/intimm/dxz037. [PubMed: 31049553]

135. Et-Thakafy O, Guyomarc'h F, Lopez C. Lipid domains in the milk fat globule membrane: Dynamics investigated in situ in milk in relation to temperature and time. Food Chem 2017;220:352–361. DOI: 10.1016/j.foodchem.2016.10.017. [PubMed: 27855911]

136. Dei Cas M, Paroni R, Signorelli P, et al. Human breast milk as source of sphingolipids for newborns: Comparison with infant formulas and commercial cow's milk. J Transl Med 2020;18(1):481. DOI: 10.1186/s12967-020-02641-0. [PubMed: 33317546]

137. Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. Nature 2014;510(7503):58–67. DOI: 10.1038/nature13475. [PubMed: 24899305]

138. Tanaka K, Hosozawa M, Kudo N, et al. The pilot study: Sphingomyelin-fortified milk has a positive association with the neurobehavioural development of very low birth weight infants during infancy, randomized control trial. Brain Dev 2013;35(1):45–52. DOI: 10.1016/j.braindev.2012.03.004. [PubMed: 22633446]

139. Tayebati SK, Amenta F. Choline-containing phospholipids: Relevance to brain functional pathways. Clin Chem Lab Med 2013;51(3):513–521. DOI: 10.1515/cclm-2012-0559. [PubMed: 23314552]

140. Derbyshire E, Obeid R. Choline, neurological development and brain function: A systematic review focusing on the first 1000 days. Nutrients 2020;12(6):1731. DOI: 10.3390/nu12061731. [PubMed: 32531929]

141. Addis MF, Pisanu S, Ghisaura S, et al. Proteomics and pathway analyses of the milk fat globule in sheep naturally infected by Mycoplasma agalactiae provide indications of the in vivo response of the mammary epithelium to bacterial infection. Infect Immun. 2011;79(9):3833–3845. DOI: 10.1128/IAI.00040-11. [PubMed: 21690237]

142. Reinhardt TA, Sacco RE, Nonnecke BJ, et al. Bovine milk proteome: Quantitative changes in normal milk exosomes, milk fat globule membranes and whey proteomes

resulting from Staphylococcus aureus mastitis. J Proteomics 2013;82:141–154. DOI: 10.1016/j.jprot.2013.02.013. [PubMed: 23459212]

143. Hewelt-Belka W, Garwolinska D, Mlynarczyk M, et al. Comparative lipidomic study of human milk from different lactation stages and milk formulas. Nutrients 2020;12(7):2165. DOI: 10.3390/nu12072165. [PubMed: 32708300]
144. Yaron S, Shachar D, Abramas L, et al. Effect of high beta-palmitate content in infant formula on the intestinal microbiota of term infants. J Pediatr Gastroenterol Nutr 2013;56(4):376–381. DOI: 10.1097/MPG.0b013e31827e1ee2. [PubMed: 23201699]
145. Le Huerou-Luron I, Bouzerzour K, Ferret-Bernard S, et al. A mixture of milk and vegetable lipids in infant formula changes gut digestion, mucosal immunity and microbiota composition in neonatal piglets. Eur J Nutr 2018;57(2):463–476. DOI: 10.1007/s00394-016-1329-3. [PubMed: 27744547]
146. Liao Y, Alvarado R, Phinney B, et al. Proteomic characterization of human milk fat globule membrane proteins during a 12 month lactation period. J Proteome Res 2011;10(8):3530–3541. DOI: 10.1021/pr200149t. [PubMed: 21714549]
147. Afrache H, Gouret P, Ainouche S, et al. The butyrophilin (BTN) gene family: From milk fat to the regulation of the immune response. Immunogenetics 2012;64(11):781–794. DOI: 10.1007/s00251-012-0619-z. [PubMed: 23000944]
148. Ogg SL, Weldon AK, Dobbie L, et al. Expression of butyrophilin (Btn1a1) in lactating mammary gland is essential for the regulated secretion of milk-lipid droplets. Proc Natl Acad Sci USA 2004;101(27):10084–10089. DOI: 10.1073/pnas.0402930101. [PubMed: 15226505]
149. Chen W, Zhang Z, Zhang S, et al. MUC1: Structure, function, and clinic application in epithelial cancers. Int J Mol Sci 2021;22(12):6567. DOI: 10.3390/ijms22126567. [PubMed: 34207342]
150. Martin HM, Hancock JT, Salisbury V, et al. Role of xanthine oxidoreductase as an antimicrobial agent. Infect Immun 2004;72(9):4933–4939. DOI: 10.1128/IAI.72.9.4933-4939.2004. [PubMed: 15321984]
151. Yi YS. Functional role of milk fat globule-epidermal growth factor VIII in macrophage-mediated inflammatory responses and inflammatory/autoimmune diseases. Mediators Inflamm 2016;2016:5628486. DOI: 10.1155/2016/5628486. [PubMed: 27429513]
152. Xu H, Diolintzi A, Storch J. Fatty acid-binding proteins: Functional understanding and diagnostic implications. Curr Opin Clin Nutr Metab Care 2019;22(6):407–412. DOI: 10.1097/MCO.0000000000000600. [PubMed: 31503024]
153. Demmelmair H, Prell C, Timby N, et al. Benefits of lactoferrin, osteopontin and milk fat globule membranes for infants. Nutrients 2017;9(8):817. DOI: 10.3390/nu9080817. [PubMed: 28788066]
154. Liu B, Newburg DS. Human milk glycoproteins protect infants against human pathogens. Breastfeed Med 2013;8(4):354–362. DOI: 10.1089/bfm.2013.0016. [PubMed: 23697737]
155. Singh SB, Carroll-Portillo A, Coffman C, et al. Intestinal alkaline phosphatase exerts anti-inflammatory effects against lipopolysaccharide by inducing autophagy. Sci Rep 2020;10(1):3107. DOI: 10.1038/s41598-020-59474-6. [PubMed: 32080230]
156. Kotozaki Y, Satoh M, Nasu T, et al. Human plasma xanthine oxidoreductase activity in cardiovascular disease: Evidence from a population-based study. Biomedicines 2023;11(3):754. DOI: 10.3390/biomedicines11030754. [PubMed: 36979733]
157. Honvo-Houeto E, Henry C, Chat S, et al. The endoplasmic reticulum and casein-containing vesicles contribute to milk fat globule membrane. Mol Biol Cell 2016;27(19):2946–2964. DOI: 10.1091/mbc.E16-06-0364. [PubMed: 27535430]
158. Wedekind SIS, Shenker NS. Antiviral properties of human milk. Microorganisms 2021;9(4):715. DOI: 10.3390/microorganisms9040715. [PubMed: 33807146]
159. Martin Carli JF, Dzieciatkowska M, Hernandez TL, et al. Comparative proteomic analysis of human milk fat globules and paired membranes and mouse milk fat globules identifies core cellular systems contributing to mammary lipid trafficking and secretion. Front Mol Biosci 2023;10:1259047. DOI: 10.3389/fmolb.2023.1259047. [PubMed: 38169886]
160. Almeida CC, Mendonca Pereira BF, Leandro KC, et al. Bioactive compounds in infant formula and their effects on infant nutrition and health: A systematic literature review. Int J Food Sci 2021;2021:8850080. DOI: 10.1155/2021/8850080. [PubMed: 34095293]

161. Zhang Y, Zhang X, Mi L, et al. Comparative proteomic analysis of proteins in breast milk during different lactation periods. Nutrients 2022;14(17):3648. DOI: 10.3390/nu14173648. [PubMed: 36079904]
162. Janiszewska E, Kmieciak A, Kacperczyk M, et al. The influence of clusterin glycosylation variability on selected pathophysiological processes in the human body. Oxid Med Cell Longev 2022;2022: 7657876. DOI: 10.1155/2022/7657876. [PubMed: 36071866]
163. Melnik BC, Schmitz G. Milk's role as an epigenetic regulator in health and disease. Diseases 2017;5(1):12. DOI: 10.3390/diseases5010012. [PubMed: 28933365]
164. Carrillo-Lozano E, Sebastian-Valles F, Knott-Torcal C. Circulating microRNAs in breast milk and their potential impact on the infant. Nutrients 2020;12(10):3066. DOI: 10.3390/nu12103066. [PubMed: 33049923]
165. Innis SM, Dyer R. Dietary triacylglycerols with palmitic acid (16:0) in the 2-position increase 16:0 in the 2-position of plasma and chylomicron triacylglycerols, but reduce phospholipid arachidonic and docosahexaenoic acids, and alter cholesteryl ester metabolism in formula-Fed piglets. J Nutr 1997;127(7):1311–1319. DOI: 10.1093/jn/127.7.1311. [PubMed: 9202085]
166. Baldi A, Pinotti L. Lipophilic microconstituents of milk. Adv Exp Med Biol 2008;606:109–125. DOI: 10.1007/978-0-387-74087-4_3. [PubMed: 18183926]
167. Hamosh M The role of lingual lipase in neonatal fat digestion. Ciba Found Symp 1979(70):69–98. DOI: 10.1002/9780470720530.ch5.
168. Smith LJ, Kaminsky S, D'Souza SW. Neonatal fat digestion and lingual lipase. Acta Paediatr Scand 1986;75(6):913–918. DOI: 10.1111/j.1651-2227.1986.tb10316.x. [PubMed: 3105239]
169. Lopez-Lopez A, Lopez-Sabater MC, Campoy-Folgoso C, et al. Fatty acid and sn-2 fatty acid composition in human milk from Granada (Spain) and in infant formulas. Eur J Clin Nutr 2002;56(12):1242–1254. DOI: 10.1038/sj.ejcn.1601470. [PubMed: 12494309]
170. Hamosh M, Scanlon JW, Ganot D, et al. Fat digestion in the newborn. Characterization of lipase in gastric aspirates of premature and term infants. J Clin Invest 1981;67(3):838–846. DOI: 10.1172/jci110101. [PubMed: 7204558]
171. Freed LM, Berkow SE, Hamosh P, et al. Lipases in human milk: Effect of gestational age and length of lactation on enzyme activity. J Am Coll Nutr 1989;8(2):143–150. DOI: 10.1080/07315724.1989.10720289. [PubMed: 2708730]
172. Ye A, Cui J, Singh H. Proteolysis of milk fat globule membrane proteins during in vitro gastric digestion of milk. J Dairy Sci 2011;94(6):2762–2770. DOI: 10.3168/jds.2010-4099. [PubMed: 21605745]
173. Holton TA, Vijayakumar V, Dallas DC, et al. Following the digestion of milk proteins from mother to baby. J Proteome Res 2014;13(12):5777–5783. DOI: 10.1021/pr5006907. [PubMed: 25385259]
174. Bourlieu C, Mahdoueni W, Paboeuf G, et al. Physico-chemical behaviors of human and bovine milk membrane extracts and their influence on gastric lipase adsorption. Biochimie 2020;169:95–105. DOI: 10.1016/j.biochi.2019.12.003. [PubMed: 31866313]
175. Li X, Lindquist S, Lowe M, et al. Bile salt-stimulated lipase and pancreatic lipase-related protein 2 are the dominating lipases in neonatal fat digestion in mice and rats. Pediatr Res 2007;62(5):537–541. DOI: 10.1203/PDR.0b013e3181559e75. [PubMed: 17805199]
176. Xiao X, Mukherjee A, Ross LE, et al. Pancreatic lipase-related protein-2 (PLRP2) can contribute to dietary fat digestion in human newborns. J Biol Chem 2011;286(30):26353–26363. DOI: 10.1074/jbc.M111.249813. [PubMed: 21652702]
177. Nilsson A, Duan RD, Ohlsson L. Digestion and absorption of milk phospholipids in newborns and adults. Front Nutr 2021;8:724006. DOI: 10.3389/fnut.2021.724006. [PubMed: 34490332]
178. Lindquist S, Hernell O. Lipid digestion and absorption in early life: An update. Curr Opin Clin Nutr Metab Care 2010;13(3):314–320. DOI: 10.1097/MCO.0b013e328337bbf0. [PubMed: 20179589]
179. Hernell O, Blackberg L. Human milk bile salt-stimulated lipase: Functional and molecular aspects. J Pediatr 1994;125(5 Pt 2):S56–S61. DOI: 10.1016/s0022-3476(06)80737-7. [PubMed: 7965454]

180. Duan RD. Alkaline sphingomyelinase: An old enzyme with novel implications. Biochim Biophys Acta 2006;1761(3):281–291. DOI: 10.1016/j.bbalip.2006.03.007. [PubMed: 16631405]
181. Duan RD, Cheng Y, Jonsson BA, et al. Human meconium contains significant amounts of alkaline sphingomyelinase, neutral ceramidase, and sphingolipid metabolites. Pediatr Res 2007;61(1):61–66. DOI: 10.1203/01.pdr.0000250534.92934.c2. [PubMed: 17211142]
182. Nilsson A Role of sphingolipids in infant gut health and immunity. J Pediatr 2016;173(Suppl):S53–S69. DOI: 10.1016/j.jpeds.2016.02.076. [PubMed: 27234412]
183. Ahmed TB, Eggesbo M, Criswell R, et al. Total fatty acid and polar lipid species composition of human milk. Nutrients 2021;14(1):158. DOI: 10.3390/nu14010158. [PubMed: 35011034]
184. Innis SM, Dyer R, Quinlan P, et al. Palmitic acid is absorbed as sn-2 monopalmitin from milk and formula with rearranged triacylglycerols and results in increased plasma triglyceride sn-2 and cholesteryl ester palmitate in piglets. J Nutr 1995;125(1):73–81. DOI: 10.1093/jn/125.1.73. [PubMed: 7815179]
185. Abrahamse E, Minekus M, van Aken GA, et al. Development of the digestive system-experimental challenges and approaches of infant lipid digestion. Food Dig 2012;3(1–3):63–77. DOI: 10.1007/s13228-012-0025-x. [PubMed: 23293684]
186. Blackberg L, Hernell O, Olivecrona T. Hydrolysis of human milk fat globules by pancreatic lipase: Role of colipase, phospholipase A2, and bile salts. J Clin Invest 1981;67(6):1748–1752. DOI: 10.1172/jci110213. [PubMed: 7240418]
187. Lu N, Wang J, Chen Z, et al. The effect of adding phospholipids before homogenization on the properties of milk fat globules. Food Sci Tech 2021;146:111659. DOI: 10.1016/j.lwt.2021.111659.
188. Oosting A, Kegler D, Wopereis HJ, et al. Size and phospholipid coating of lipid droplets in the diet of young mice modify body fat accumulation in adulthood. Pediatr Res 2012;72(4):362–369. DOI: 10.1038/pr.2012.101. [PubMed: 22850409]
189. Timby N, Domellof E, Hernell O, et al. Neurodevelopment, nutrition, and growth until 12 mo of age in infants fed a low-energy, low-protein formula supplemented with bovine milk fat globule membranes: A randomized controlled trial. Am J Clin Nutr 2014;99(4):860–868. DOI: 10.3945/ajcn.113.064295. [PubMed: 24500150]
190. Timby N, Adamsson M, Domellof E, et al. Neurodevelopment and growth until 6.5 years of infants who consumed a low-energy, low-protein formula supplemented with bovine milk fat globule membranes: A randomized controlled trial. Am J Clin Nutr 2021;113(3):586–592. DOI: 10.1093/ajcn/nqaa354. [PubMed: 33564853]
191. Nieto-Ruiz A, Dieguez E, Sepulveda-Valbuena N, et al. The effects of an infant formula enriched with milk fat globule membrane, long-chain polyunsaturated fatty acids and synbiotics on child behavior up to 2.5 years old: The COGNIS study. Nutrients 2020;12(12). DOI: 10.3390/nu12123825.
192. Michalski M, Michel F, Sainmont D, et al. Apparent ζ-potential as a tool to assess mechanical damages to the milk fat globule membrane, colloids and surfaces B: Biointerfaces. J Col Surfb 2002;23(1):23–30. DOI: 10.1016/S0927-7765(01)00203-X.
193. Cebo C, Lopez C, Henry C, et al. Goat alpha(s1)-casein genotype affects milk fat globule physicochemical properties and the composition of the milk fat globule membrane. J Dairy Sci 2012;95(11):6215–6229. DOI: 10.3168/jds.2011-5233. [PubMed: 22921619]
194. Aguilera JM. The food matrix: Implications in processing, nutrition and health. Crit Rev Food Sci Nutr 2019;59(22):3612–3629. DOI: 10.1080/10408398.2018.1502743. [PubMed: 30040431]
195. Vandenplas Y, Ksiazyk J, Luna MS, et al. Partial hydrolyzed protein as a protein source for infant feeding: Do or don't? Nutrients 2022;14(9):1720. DOI: 10.3390/nu14091720. [PubMed: 35565688]
196. Guo Q, Bellissimo N, Rousseau D. The physical state of emulsified edible oil modulates its in vitro digestion. J Agric Food Chem 2017;65(41):9120–9127. DOI: 10.1021/acs.jafc.7b03368. [PubMed: 28949127]
197. Li F, Wu SS, Berseth CL, et al. Improved neurodevelopmental outcomes associated with bovine milk fat globule membrane and lactoferrin in infant formula: A randomized, controlled trial. J Pediatr 2019;215:24–31.e8. DOI: 10.1016/j.jpeds.2019.08.030. [PubMed: 31668885]

198. Schneider N, Hauser J, Oliveira M, et al. Sphingomyelin in brain and cognitive development: Preliminary data. eNeuro 2019;6(4). DOI: 10.1523/ENEURO.0421-18.2019.

199. Zheng L, Fleith M, Giuffrida F, et al. Dietary polar lipids and cognitive development: A narrative review. Adv Nutr 2019;10(6):1163–1176. DOI: 10.1093/advances/nmz051. [PubMed: 31147721]

200. Bitman J, Wood DL, Mehta NR, et al. Comparison of the phospholipid composition of breast milk from mothers of term and preterm infants during lactation. Am J Clin Nutr 1984;40(5):1103–1119. DOI: 10.1093/ajcn/40.5.1103. [PubMed: 6496390]

201. Li T, Gong H, Yuan Q, et al. Supplementation of polar lipids-enriched milk fat globule membrane in high-fat diet-fed rates during pregnancy and lactation promotes brown/beige adipocyte development and prevents obesity in male offspring. FASEB J 2020;34(3):4619–4634. DOI: 10.1096/fj.201901867RRR. [PubMed: 32020679]

202. Zhang Q, Ye L, Xin F, et al. Milk fat globule membrane supplementation during suckling ameliorates maternal high fat diet-induced hepatic steatosis in adult male offspring of mice. J Nutr 2021;151(6):1487–1496. DOI: 10.1093/jn/nxab026. [PubMed: 33693864]

203. Han L, Du M, Ren F, et al. Milk polar lipids supplementation to obese rats during pregnancy and lactation benefited skeletal outcomes of male offspring. Mol Nutr Food Res 2021;65(15):e2001208. DOI: 10.1002/mnfr.202001208. [PubMed: 34008920]

204. He X, Parenti M, Grip T, et al. Fecal microbiome and metabolome of infants fed bovine MFGM supplemented formula or standard formula with breast-fed infants as reference: A randomized controlled trial. Sci Rep 2019;9(1):11589. DOI: 10.1038/s41598-019-47953-4. [PubMed: 31406230]

205. O'Mahony SM, McVey Neufeld KA, Waworuntu RV, et al. The enduring effects of early-life stress on the microbiota-gut-brain axis are buffered by dietary supplementation with milk fat globule membrane and a prebiotic blend. Eur J Neurosci 2020;51(4):1042–1058. DOI: 10.1111/ejn.14514. [PubMed: 31339598]

206. Kosmerl E, Rocha-Mendoza D, Ortega-Anaya J, et al. Improving human health with milk fat globule membrane, lactic acid bacteria, and bifidobacteria. Microorganisms 2021;9(2). DOI: 10.3390/microorganisms9020341.

207. Charton E, Bourgeois A, Bellanger A, et al. Infant nutrition affects the microbiota-gut-brain axis: Comparison of human milk vs. infant formula feeding in the piglet model. Front Nutr 2022;9:976042. DOI: 10.3389/fnut.2022.976042. [PubMed: 36211510]

208. Wu Y, Zhang X, Han D, et al. Early life administration of milk fat globule membrane promoted SCFA-producing bacteria colonization, intestinal barriers and growth performance of neonatal piglets. Anim Nutr 2021;7(2):346–355. DOI: 10.1016/j.aninu.2020.07.012. [PubMed: 34258422]

209. Zhao J, Yi W, Liu B, et al. MFGM components promote gut Bifidobacterium growth in infant and in vitro. Eur J Nutr 2022;61(1):277–288. DOI: 10.1007/s00394-021-02638-5. [PubMed: 34324046]

210. Yu Z, Huang S, Li Y, et al. Milk fat globule membrane alleviates short bowel syndrome-associated liver injury in rats through inhibiting autophagy and NLRP3 inflammasome activation. Front Nutr 2022;9:758762. DOI: 10.3389/fnut.2022.758762. [PubMed: 35308293]

211. Zheng D, Kern L, Elinav E. The NLRP6 inflammasome. Immunology 2021;162(3):281–289. DOI: 10.1111/imm.13293. [PubMed: 33314083]

212. Belizario JE, Faintuch J. Microbiome and gut dysbiosis. Exp Suppl 2018;109:459–476. DOI: 10.1007/978-3-319-74932-7_13. [PubMed: 30535609]

213. Levy M, Thaiss CA, Zeevi D, et al. Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. Cell 2015;163(6):1428–1443. DOI: 10.1016/j.cell.2015.10.048. [PubMed: 26638072]

214. Zhang L, Chichlowski M, Gross G, et al. Milk fat globule membrane protects lactobacillus rhamnosus gg from bile stress by regulating exopolysaccharide production and biofilm formation. J Agric Food Chem 2020;68(24):6646–6655. DOI: 10.1021/acs.jafc.0c02267. [PubMed: 32396007]

215. Jiang C, Cheong LZ, Zhang X, et al. Dietary sphingomyelin metabolism and roles in gut health and cognitive development. Adv Nutr 2022;13(2):474–491. DOI: 10.1093/advances/nmab117. [PubMed: 34549256]

216. Narita T, Naganuma T, Sase Y, et al. Long-chain bases of sphingolipids are transported into cells via the acyl-CoA synthetases. Sci Rep 2016;6:25469. DOI: 10.1038/srep25469. [PubMed: 27136724]
217. Xiong Y, Hla T. S1P control of endothelial integrity. Curr Top Microbiol Immunol 2014;378:85–105. DOI: 10.1007/978-3-319-05879-5_4. [PubMed: 24728594]
218. Rohrhofer J, Zwirzitz B, Selberherr E, et al. The impact of dietary sphingolipids on intestinal microbiota and gastrointestinal immune homeostasis. Front Immunol 2021;12:635704. DOI: 10.3389/fimmu.2021.635704. [PubMed: 34054805]
219. Fontecha J, Brink L, Wu S, et al. Sources, production, and clinical treatments of milk fat globule membrane for infant nutrition and well-being. Nutrients 2020;12(6). DOI: 10.3390/nu12061607.
220. Billeaud C, Puccio G, Saliba E, et al. Safety and tolerance evaluation of milk fat globule membrane-enriched infant formulas: A randomized controlled multicenter non-inferiority trial in healthy term infants. Clin Med Insights Pediatr 2014;8:51–60. DOI: 10.4137/CMPed.S16962. [PubMed: 25452707]
221. Bhinder G, Allaire JM, Garcia C, et al. Milk fat globule membrane supplementation in formula modulates the neonatal gut microbiome and normalizes intestinal development. Sci Rep 2017;7:45274. DOI: 10.1038/srep45274. [PubMed: 28349941]
222. Suarez-Martinez C, Santaella-Pascual M, Yague-Guirao G, et al. Infant gut microbiota colonization: Influence of prenatal and postnatal factors, focusing on diet. Front Microbiol 2023;14:1236254. DOI: 10.3389/fmicb.2023.1236254. [PubMed: 37675422]
223. Rueda R, Sabatel JL, Maldonado J, et al. Addition of gangliosides to an adapted milk formula modifies levels of fecal Escherichia coli in preterm newborn infants. J Pediatr 1998;133(1):90–94. DOI: 10.1016/s0022-3476(98)70184-2. [PubMed: 9672517]
224. Pellegrini A, Thomas U, Bramaz N, et al. Isolation and identification of three bactericidal domains in the bovine alpha-lactalbumin molecule. Biochim Biophys Acta 1999;1426(3):439–448. DOI: 10.1016/s0304-4165(98)00165-2. [PubMed: 10076060]
225. Nakimbugwe D, Masschalck B, Deckers D, et al. Cell wall substrate specificity of six different lysozymes and lysozyme inhibitory activity of bacterial extracts. FEMS Microbiol Lett 2006;259(1):41–46. DOI: 10.1111/j.1574-6968.2006.00240.x. [PubMed: 16684100]
226. Liu B, Yu Z, Chen C, et al. Human milk mucin 1 and mucin 4 inhibit Salmonella enterica serovar typhimurium invasion of human intestinal epithelial cells in vitro. J Nutr 2012;142(8):1504–1509. DOI: 10.3945/jn.111.155614. [PubMed: 22718031]
227. Yolken RH, Peterson JA, Vonderfecht SL, et al. Human milk mucin inhibits rotavirus replication and prevents experimental gastroenteritis. J Clin Invest 1992;90(5):1984–1991. DOI: 10.1172/JCI116078. [PubMed: 1331178]
228. Bezirtzoglou E, Stavropoulou E. Immunology and probiotic impact of the newborn and young children intestinal microflora. Anaerobe 2011;17(6):369–374. DOI: 10.1016/j.anaerobe.2011.03.010. [PubMed: 21515397]
229. Bintsis T Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. AIMS Microbiol 2018;4(4):665–684. DOI: 10.3934/microbiol.2018.4.665. [PubMed: 31294241]
230. Pacheco AR, Barile D, Underwood MA, et al. The impact of the milk glycobiome on the neonate gut microbiota. Annu Rev Anim Biosci 2015;3:419–445. DOI: 10.1146/annurev-animal-022114-111112. [PubMed: 25387230]
231. La Reau AJ, Suen G. The Ruminococci: Key symbionts of the gut ecosystem. J Microbiol 2018;56(3):199–208. DOI: 10.1007/s12275-018-8024-4. [PubMed: 29492877]
232. Martinez-Sanchez V, Visitacion Calvo M, Viera I, et al. Mechanisms for the interaction of the milk fat globule membrane with the plasma membrane of gut epithelial cells. Food Res Int 2023;173(Pt 1):113330. DOI: 10.1016/j.foodres.2023.113330. [PubMed: 37803640]
233. O'Brien K, Breyne K, Ughetto S, et al. RNA delivery by extracellular vesicles in mammalian cells and its applications. Nat Rev Mol Cell Biol 2020;21(10):585–606. DOI: 10.1038/s41580-020-0251-y. [PubMed: 32457507]

Key Points

- Human milk (HM) contains 3–5% fat, 0.8–0.9% protein, 6.9–7.2% carbohydrate calculated as lactose, and 0.2% mineral constituents. Most of these nutrients are carried in milk fat globules (MFGs).
- The MFGs are composed of an energy-rich triacylglycerol core surrounded by a membrane structure. The membrane contains polar lipids, specialized proteins, glycoproteins, and cholesterol. Each of these bioactive components serves important nutritional, immunological, neurological, and digestive functions.
- Milk fat globules are designed to release energy rapidly in the upper gastrointestinal tract and then persist for some time in the gut lumen so that the protective bioactive molecules are conveyed to the colon. These properties may shape the microbial colonization and innate immune properties of the developing gastrointestinal tract.
- Understanding the composition and dynamics of MFGs could help enhance the nutritional composition of milk in a goal-oriented fashion.
- There is a possibility that MFGs could emerge as a nutraceutical for enteral delivery of medications in infants and older patients.

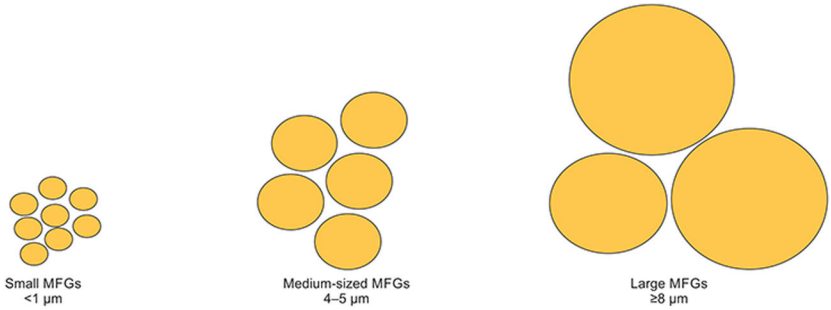

Fig. 1:

Milk fat globules are typically sized between 0.1 and 15 μm in diameter. There are 3 important volumetric subgroups, including the small (<1 μm diameter), medium (4–5 μm), and large (≥8 μm)

Eon	Era	Period	Millions of years ago
Phanerozoic	Cenozoic	Quanternary	1.6
		Tertiary	66
	Mesozoic	Cretaceous	138
		Jurassic	205
		Triassic	240
	Paleozoic	Permian	290
		Pennsylvanian	330
		Mississippian	360
		Devonian	410
		Silurian	435
		Ordovician	500
		Cambrian	570
Proterozoic			
Archean			
Hadean			

Fig. 2:

Evolution of milk: Some therapsids and Mammalia-formes began producing a milk-like secretion during the Triassic period 220–280 million years ago (indicated by the red line)

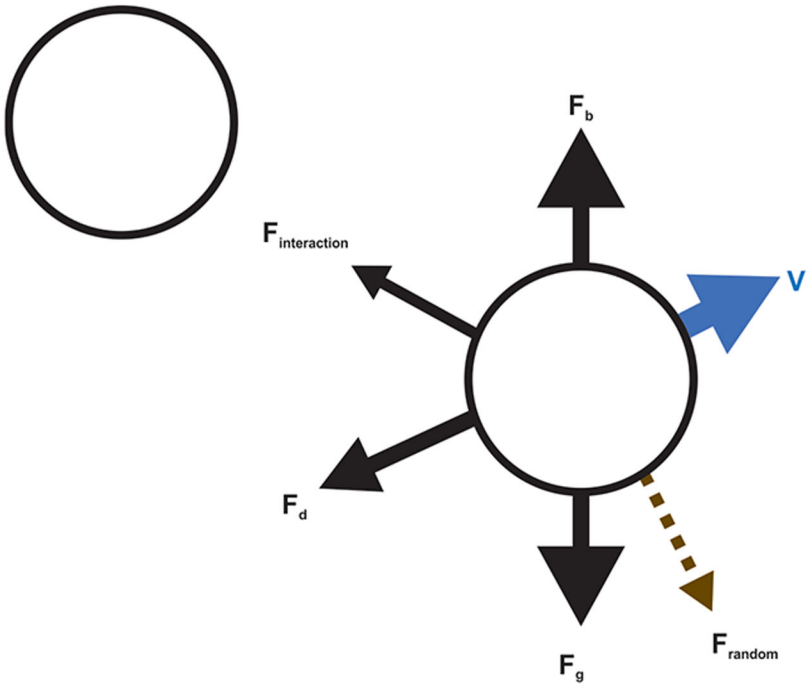

Fig. 3:

Forces acting on MFGs likely include (clockwise): (A) Gravitational weight (F_g); (B) Drag force (F_b); (C) $F_{interaction}$, a summated effect of the van der Waals and the electrostatic forces. These interactions could be attractive or repulsive depending on the intrinsic characteristics of the globules and the separating distance. As depicted above, the $F_{interaction}$ forces should be visualized along a line joining the centers of the two spheres; (D) Buoyant force (F_b); and (E) Random forces (F_{random}). V refers to the velocity of the Brownian particle

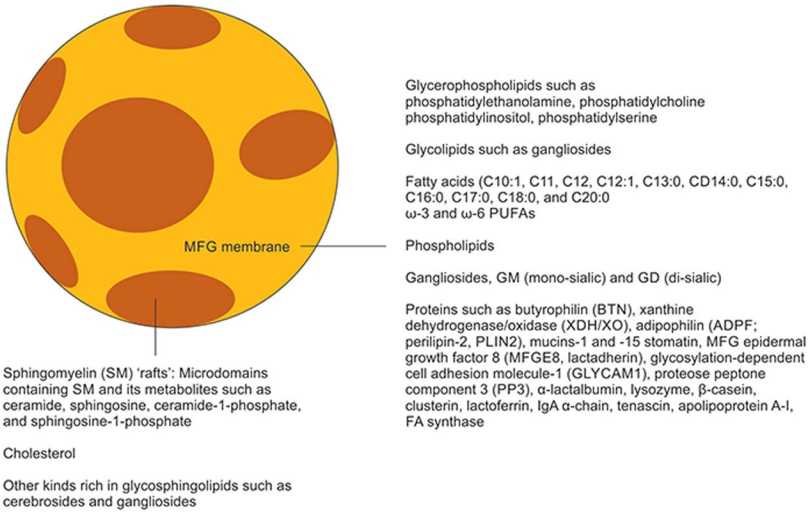

Fig. 4:

The MFG membrane may include two distinct lipid phases. The first set may be comprised of relatively less dense, liquid-disordered regions with unsaturated glycerophospholipids, proteins, glycoproteins, glycolipids, and some SM. In the second, SM and cholesterol form densely-packed, liquid-ordered domains, the 'rafts'

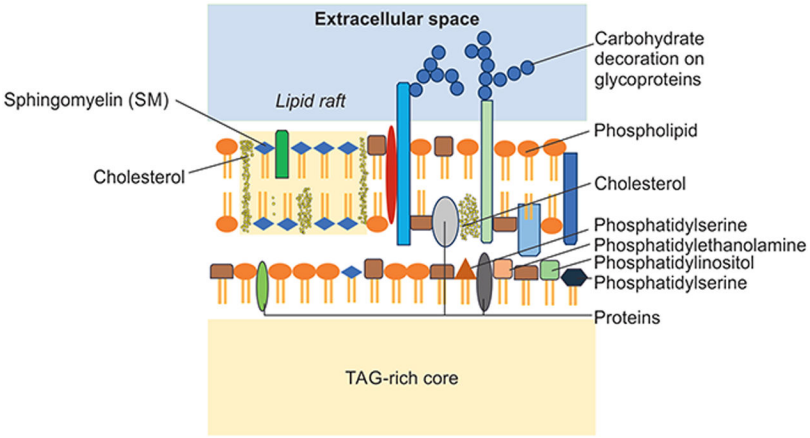

Fig. 5:

The MFG membrane is a triple-layered membrane, where the external two layers are derived from the apical membrane. The proteins extending from the surface are heavily decorated with carbohydrates. Most of the MFG membrane shows a liquid-ordered phase with SM in close association with cholesterol. Some microdomains, the lipid rafts, are relatively rigid and play an important role in cell signaling. The internal layer is a loosely-packed glycerophospholipid matrix containing unsaturated glycerophospholipids such as phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine. This layer is derived from the endoplasmic reticulum and typically contains two unsaturated FAs bound to a glycerol backbone

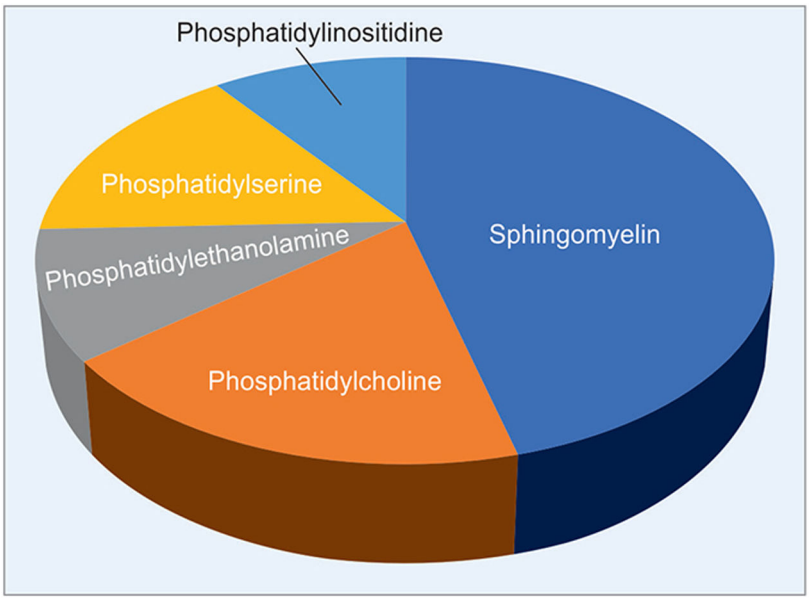

Fig. 6:

Milk fat globules contain polar lipids. The most important constituents include sphingolipids such as sphingomyelin, and glycerophospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, and phosphatidylinositidine. Sphingomyelin is present in higher quantities in HM than in milk from other mammals