Symposium review: The relevance of bovine milk phospholipids in human nutrition—Evidence of the effect on infant gut and brain development*

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ABSTRACT

This paper reflects the concepts reviewed during the presentation in the Joint MILK/Lactation Biology Symposium at the ADSA 2018 Annual Meeting. Our intention is to update the concepts and advances in the area of research regarding milk phospholipids or polar lipid fraction as part of a dairy ingredient used today in nutritional studies that focus on gut health as well as brain development of infants. Although processing advances have allowed the production of novel ingredients rich in milk fat globule membrane (MFGM) components, mostly monitored by phospholipid concentration and presence of membrane proteins, there is wide variability in their composition and structure. Furthermore, we aimed to include in the phospholipid fraction of milk nanovesicles designated as milk exosomes, which are secreted into milk by different secretion mechanisms than those of the fat globules but are also made up of a unique mixture of polar lipids. We consider imperative the study of polar lipid-derived structures from milk regarding composition and structure to gain insights into their biological effect in human health. Nevertheless, and tolerating the differences in composition and concentration of their components, studies supplementing the diet of infants with polar lipids (i.e., MFGM components) have shown significant advances in several areas of human health and well-being. Here we present a summary of the important components of MFGM and milk exosomes as well as an overview of the effects on gut health and brain and cognitive development when added to the diet of infants.

Key words: milk fat globule membrane, phospholipid, exosome, brain development, gut health

INTRODUCTION

Milk has always been considered a very interesting mammalian fluid due to its molecular, physicochemical, and structural complexity. To date, many components of both human and bovine milk still pose questions regarding their structure, function, role, and biological significance to human health. One of those components is phospholipids, which are a complex mixture of more than 30 molecular species of phosphatidylcholine (**PC**), phosphatidylethanolamine (**PE**), phosphatidylserine (**PS**), phosphatidylinositol (**PI**), and sphingophospholipids that have been clearly identified within the bovine milk fat globule membrane (**MFGM**; Contarini and Povo, 2013). Phospholipids are found in 2 fractions of milk: MFGM, which accounts most of the phospholipids in milk constituting the trilayer membrane surrounding each fat globule (Gallier et al., 2010, 2014; Lopez et al., 2010), and nanovesicles, which are secreted into milk by the mammary gland cells and are implicated in cell-to-cell communication by means of their functionally active cargo (e.g., mRNA, microRNA, and different proteins; Zemleni et al., 2017).

This assortment of lipids organized in both the MFGM and nanovesicles is unique in milk and has been found to have a positive effect in many aspects of human health by various mechanisms. Two main beneficial health effects—benefit to the intestinal cells via leaky gut syndrome studies and several brain development and cognitive improvements—have been studied by more than one group each. This review presents a compact overview of interesting aspects of milk phospholipid fractions and their role in intestinal health and brain development as observed by the authors.

COMPOSITION OF MILK PHOSPHOLIPIDS

Phospholipids are ubiquitous molecules in membranes and vesicles, and milk seems to possess a unique mixture of them. They are classified as polar lipids and chemically are amphiphilic molecules that consist of a hydrophobic tail, given by fatty acids, and a hydrophilic head, given by a specific polar group. In general, 2 main

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Figure 1. Structure of glycerophospholipids found in milk and the predominant families based on the polar head group. Atomic color code: C = gray, H = white, O = red, N = blue, P = orange; double bonds are depicted in green.

classes of phospholipids are found in milk (Contarini and Povolo, 2013). The first is glycerophospholipids, which are formed by a glycerol molecule esterified by 2 fatty acids and a phosphoric acid molecule, which in turn is bound to additional polar groups (choline, serine, ethanolamine, or inositol). Figure 1 depicts the main groups or families of glycerophospholipids based on the chemical composition of their polar groups.

The second most important group of polar lipids found in milk is sphingolipids, which comprise a sphingosine backbone (2-amino-4-octadecene-1,3-diol) sometimes bound to a fatty acid (through an amide bond) and a polar head. The polar groups usually found in milk vary greatly and include carbohydrates (glucose, galactose, and lactose), which are referred to as glycosphingolipids (Figure 2; Contarini and Povolo, 2013). However, sphingomyelin is the dominant species. It comprises a phosphocholine head group; due to this, it is usually classified as a sphingophospholipid and included in the phospholipid fraction (Figure 2; Contarini and Povolo, 2013).

Sphingomyelin (**SM**) is a very important constituent of the phospholipid fraction of milk due to its high concentration in the MFGM and its implication in many beneficial effects on human health, such as neuronal development in infants and protection of neonates from bacterial infections (Bourlieu et al., 2015; Claumarchirant et al., 2016; Murthy et al., 2016). Last, gangliosides are another kind of sphingolipid that are recently getting attention owing to their potential bioactivities in brain function and the immune system, among others (Rueda, 2013). They possess highly complex polar head groups constituted by one or more sialic acid units in addition to oligosaccharides (Ma et al., 2015).

The concentration and composition (polar head and fatty acid tail) of glycerophospholipids and sphingolipids in bovine raw milk are greatly affected by different factors, including breed of the cow, stage of lactation, season of the year, nutrition and diet, and processing and treatment. Interestingly, the analytical methods of extraction and analysis add variation in determining the content of the polar lipid fraction (Bitman and

Figure 2. Structure of the most common sphingolipids found in milk based on the polar head group. The sphingosine backbone (right chain in each structure) is depicted here bound to a palmitic acid (left chain). Atomic color code: C = gray, H = white, O = red, N = blue, P = orange; double bonds are depicted in green.

Wood, 1990; Avalli and Contarini, 2005; Rombaut and Dewettnick, 2006; Graves et al., 2007; Rombaut et al., 2007; Lopez et al., 2008; Contarini and Povolo, 2013; Pimentel et al., 2018). Overall, the concentration of the polar lipid fraction ranges between 9 and 40 mg/100 g of raw milk (Table 1). The predominant species are PE, PC, and SM, accounting for approximately 80% of

total polar lipids. An interesting aspect to highlight is the concentration ratio of PC and SM. It can be seen from Table 1 that they keep a 1.0 to 1.1 ratio with the exception of the data reported by Kielbowicz et al. (2013), where the SM content is significantly lower than that of PC. These results could be the product of using charge aerosol detection coupled with liquid

Table 1. Composition of the polar lipid fraction (mg/100 g of milk) of raw milk

Polar lipid fraction	Phospholipid species ¹ (mg/100 mg of polar lipid fraction)					PC/SM ratio	Reference
	PE	PC	SM	PS	PI		
20.4	31.0	28.0	28.7	8.1	4.1	1.0	Bitman and Wood, 1990
10.5	26.8	22.0	21.6	16.1	13.6	1.0	Lopez et al., 2008
23.0	34.2	25.4	23.6	2.8	6.2	1.1	Christie, 1987
29.0	42.0	19.2	18.0	6.7	4.8	1.1	Rombaut et al., 2005
40.0	31.5	26.0	23.8	8.8	4.9	1.1	Rombaut et al., 2006
33.6	33.2	27.4	25.1	9.3	5.2	1.1	Rombaut et al., 2006
24.5	46.4	21.1	19.8	7.4	5.3	1.1	Rombaut et al., 2007
24.2	36.9	27.0	23.7	6.3	6.1	1.1	Le et al., 2011
35.0	31.1	37.3	28.0	3.1	0.6	1.3	Garcia et al., 2012
9.4	32.3	27.3	20.5	10.5	9.3	1.3	Avalli and Contarini, 2005
31.3	33.4	43.2	5.1	8.1	10.3	8.5	Kielbowicz et al., 2013

¹PE = phosphatidylethanolamine; PC = phosphatidylcholine; SM = sphingomyelin; PS = phosphatidylserine; PI = phosphatidylinositol.

chromatography, a recently developed method that provides greater sensitivity and better precision than techniques such as UV, fluorescence, refractive index, and evaporative light scattering detection (Hazotte et al., 2007; Kielbowicz et al., 2013).

Phosphatidylcholine represents up to 35% of the total phospholipids, and its function is to maintain the permeability of the membrane (Dewettinck et al., 2008; Jimenez-Flores et al., 2009) due to its flexible morphology. Depending on the water concentration and temperature, PC can associate into 1 of the 4 possible phases, including lamellar crystalline, lamellar β' , lamellar α , and oblique β' (Fedotova and Lencki, 2008). Phosphatidylethanolamine constitutes up to 30% of the total phospholipids in the MFGM and is mostly bound to UFA (68%) with high levels of C18:1 and C18:2 and less C14:0 and C16:0 (Sanchez-Juanes et al., 2009). Phosphatidylinositol and PS are present in lower concentrations in milk. They are anionic phospholipids that are distributed asymmetrically throughout the inner monolayer of MFGM (Deeth, 1997).

The classification of phospholipids and sphingolipids in milk described so far is based on the polar head group, but higher complexity derives from the wide variety of fatty acids bound to either the glycerol or sphingosine backbone. It includes chain lengths from C2 through C24, SFA, MUFA, and PUFA as well as n-3, n-6, and n-9. According to the data shown in Table 2, the most predominant species are C16 to C18, SFA and MUFA with low concentrations of C4 to C8, and PUFA (Lopez et al., 2008).

As can be inferred from the information presented here, the composition of the polar lipid fraction of milk is anything but simple. Moreover, the chemical composition of these lipids has a direct effect on the molecular structure and organization into the different vesicles

found in milk (namely the MFGM and nanovesicles). This has been studied for milk SM in various biomimetic models, where it has been proven that because of its structure (and in combination with cholesterol), it forms liquid ordered-phase domains in the external layer of the MFGM, laterally segregated from the rest of the phospholipids into lipid rafts, in analogy with mammalian plasma membranes (Lopez et al., 2010; Guyomarc'h et al., 2017). Furthermore, the fatty acid length bound (24:0 and 16:0) determines the height of the domain because it causes a mismatch, which in turn defines the internal organization and topography of the vesicle (Garner et al., 2007; Guyomarc'h et al., 2014, 2017). The heterogeneous packing of milk SM and its structural domain is now thought to have a biological implication in regulating different mechanisms in the MFGM, such as recognition and binding of bacteria, enzymes, and other proteins, or, alternatively, participate in the physical stability of phospholipid vesicles (Guyomarc'h et al., 2017).

Milk phospholipids are found as constituents in mainly 2 structures in milk: the MFGM, which are synthesized and secreted alongside with fat globules during lactation, and milk exosomes (MEX), which are nanovesicles secreted into milk by the mammary gland cells by mechanisms completely different than those of the fat globules.

A high number of studies have reported the composition, structure, function, biosynthesis, and biological function of the MFGM. It is a complex structure that surrounds the fat globules, imparting stability, protection, and selectivity toward enzymes, ligands, and receptors. The MFGM comprises a trilayer of heterogeneously distributed proteins and polar lipids (20–60% protein and 80–40% polar lipids) with a thickness between 10 and 50 nm (Keenan and Mather, 2006). The inner or primary monolayer is in direct contact with the triglycerides from the fat globule (Vanderghem et al., 2011) and comprises mostly PE, PS, and PI derived from the endoplasmic reticulum and the cytoplasm of the lactating cell during its biosynthesis (Deeth, 1997). The outer bilayer, which is derived from the apical plasma membrane of epithelial mammary cells, has lower concentrations of PE, PS, and PI and high concentrations of PC, SM, cholesterol, and gangliosides (Deeth, 1997; Lopez et al., 2008). An additional glycocalyx that varies during lactation is associated with this outer bilayer, which acts as a source of ligands and binding sites for bacteria and viruses (Spitsberg and Gorewit, 1997; Evers, 2004; Wilson et al., 2008). Interactions among MFGM constituents and other molecules occur mainly on this outer surface (Vanderghem et al., 2011). Detailed information and schematic representations of

Table 2. Fatty acid composition of glycerophospholipids and sphingolipids from milk

Fatty acid fraction	Abundance (% of polar lipid fraction)
Fatty acid by chain length	
C4–C8	2.1–2.3
C10–C15	11.6–17.3
C16–C18	74.5–79.1
C20–C24	5.9–7.2
Fatty acid by saturation	
SFA	55.2–67.0
UFA	33.0–44.8
MUFA	25.8–36.9
PUFA	7.2–7.9
n-6 PUFA	4.5–5.7
n-3 PUFA ¹	0.6–1.9

¹Including eicosapentaenoic acid and docosahexaenoic acid.

the structure of the MFGM have been already published (Gallier et al., 2010, 2014; Singh and Gallier, 2017).

In contrast with the vast number of publications regarding structure and biological effects of the MFGM, a new group of vesicular structures found in milk are getting more and more attention for their potential effects on human health. Milk exosomes are cell-released phospholipid vesicles ranging from 30 to 120 nm in diameter and with a density of 1.13 to 1.19 g/mL (Zhang et al., 2014) that enclose a variety of cargo, including mRNA, microRNA, long noncoding RNA, DNA, and proteins. In milk, both human and bovine, MEX have been shown to transfer their RNA-derived cargo to immune cells to potentially modulate immune cell function (Lakkaraju and Rodriguez-Boulan, 2008; Hata et al., 2010; Zemleni et al., 2017). In general, exosomes and nanovesicles are secreted by both eukaryotic and prokaryotic cells (via exocytosis of multivesicular bodies) into the environment for cell-to-cell communication, protection, and exchange of genetic information (Nieuwland and Sturk, 2010; Colombo et al., 2014; Kalra et al., 2016; Rani et al., 2017). They dock on to the surfaces of recipient cells, where they transmit signals from the cell surface and transfer their contents to produce functional responses (French et al., 2017). This mechanism, though critical, is a poorly understood process thought to be mediated mostly by the proteins and genetic material, which in turn, is dependent on the parent cell (French et al., 2017). However, it has recently become evident that the bioactivity of exosomes resides not only in the latter molecules but also in their lipidic content. In general, the lipids present in exosomes are similar to those found in cellular membranes: cholesterol, phospholipids, and sphingolipids. They are enriched in PC, SM, and PE (Subra et al., 2010). However, the specific concentration and their organization in lipid domains and rafts are completely determined by the parent cell (Skotland et al., 2017). Studies performed in human-derived exosomes from many types of cells (i.e., B-lymphocytes, reticulocytes, and mast and dendritic cells) indicate that phospholipids are asymmetrically distributed in the bilayer (Skotland et al., 2017). Sphingomyelin, other sphingolipids, and PC are expected to be in the outer layer, whereas the rest of the phospholipids are expected to be mainly in the inner layer of the membrane (van Meer et al., 2008). This distribution is prone to be modified by the presence of different proteins and enzymes, and, as a result, the affinity or selectivity of the exosomes might be affected (Clark, 2011; Hankins et al., 2015).

It is important to point out that the majority of studies regarding lipid composition and structure

of exosomes have been performed in human-derived nanovesicles. To date, little to no information about exosomal bovine lipids from milk has been reported. This opens a window of research opportunities because evidence suggests that dietary MEX are bioavailable and that they deliver their cargo to peripheral tissues in humans (Kusuma et al., 2016; Zemleni et al., 2017). Once the MEX reach the intestinal mucosa, 2 processes occur: (1) they are disassembled for subsequent transfer of cargo to endogenous exosomes, and (2) they are transported intact through the mucosa, reaching the liver, spleen, and kidneys for further distribution to tissues (Zemleni et al., 2017). The fate of the lipids and proteins of MEX would be hard to predict because the process they undergo will determine the metabolic pathway for both components, and this might result in different biological effects in humans. Currently, constituents of exosomes can be consulted in 3 available online databases: Exocarta (Mathivanan et al., 2012), which contains information on the protein, RNA, and lipid exclusively from exosomes of various origins, including human and bovine, and EVpedia (Kim et al., 2015) and Vesiclepedia (Kalra et al., 2012), which are very similar sites that possess databases from all extracellular vesicles, including exosomes.

One important aspect for consideration in using MFGM phospholipids, besides the experimental results of their biological activity, is related to their structure and composition. We have taken a preliminary and superficial look at some of the available commercial and experimental ingredients containing MFGM. Figure 3 is just an example of the very heterogeneous structures produced by different processes (not described due to commercial interests). From observation of these structures, it is evident that their incorporation in different formulations has to follow optimization procedures.

EVIDENCE OF THE ROLE OF MILK PHOSPHOLIPIDS IN INTESTINAL AND BRAIN DEVELOPMENT

Milk is an optimal source of nutrients for humans due to the wide variety of components secreted by the mammary cells: proteins, carbohydrates, and lipids. These constituents have been well studied, characterized, and thoroughly proven to have a beneficial role in human nutrition. Recent evidence suggests, however, the potential positive role of an additional lipid fraction that was overlooked in the past decades: phospholipids, sphingolipids, and sphingophospholipids. As reviewed above, this group of molecules can be found as the main constituents of vesicular structures in relatively low concentrations in milk and dairy products, synthesized by different mechanisms: MFGM and MEX. So far, clini-

Figure 3. Transmission electron microscopy images of 3 different commercial samples of ingredients containing milk fat globule membrane (MFGM). Samples A, B, and C are from undisclosed commercial ingredient sources. Although we can detect bovine milk origin, the main differences are the total amount of milk phospholipids per gram of powder and the organization into different microstructures.

cal and epidemiological data have provided evidence in animal models that the polar lipid fraction from MFGM affects infant gastrointestinal development. It has been proposed that one possible mechanism to benefit from milk polar lipids may occur via bacteria-lipid interaction, which in turn results in gut microbiome modification (Santiago-Rodriguez et al., 2016). The biochemical pathway that allows gut microbiota to bind lipids and express lipolytic and phospholipolytic enzymes to digest milk fat is currently being explored by our research group through physicochemical and molecular biology experiments. So far we have found that the structure (i.e., liposomes vs. emulsified lipids) and concentration in which the polar lipids are presented to some strains of bacteria such as *Lactobacillus* determine how the bacterial cells bind these molecules, the preference toward different phospholipid compositions, and the expression of phospholipolytic enzymes once they interact in vitro systems.

The protective and replenishing effects of MFGM components in the intestinal epithelium of infants altering the gut microbiome was also proven in rat pups (pup-in-a-cup rearing model) fed with 1.2 and 6 g/L MFGM for 10 d. Supplementation of the tested formula modified phylum microbiota in the colon, resembling that of the pups fed with mothers' milk instead of harboring a higher percentage of *Proteobacteria* and a lower percentage of *Firmicutes* like those fed the control diet, which lacked MFGM lipids (Bhinder et al., 2017). This outstanding work found that a diet deficient in MFGM produced shortfalls in intestinal development compared with mothers' milk, whereas addition of MFGM in the feeding formula restored intestinal growth, assessed by monitoring Paneth and goblet cell numbers as well as tight junction patterns. Moreover, Bhinder et al. (2017) reported a protective aftermath against inflammation caused by *Clostridium difficile* toxin.

In addition to this important effect on intestinal health in animal models, there is accumulating evidence that MFGM components influence brain development, with high differences compared with formula-fed newborns (Oshida et al., 2003; Gurnida et al., 2012; Tanaka et al., 2013). Polar lipid supplementation could narrow the gap in neuronal functions such as cognitive performance, behavioral development, and myelination-promoting markers between breastfed and formula-fed infants. Moreover, a recent study has shown that a diet enriched in MFGM components from buttermilk have a positive effect on cognitive decay of aging rats by amelioration of insulin resistance as detected by insulin signaling in the hippocampus and cerebral cortex (Tomé-Carneiro et al., 2018). All these findings even pose the idea that milk polar lipid supplementation has a beneficial effect on neural functions throughout life.

Here we discuss the outcomes of 3 clinical studies (double-blinded randomized controlled trials) in which the effect of milk polar lipid fraction was tested directly on the neuronal, cognitive, or behavioral development of infants without any negative outcome or side effect such as diarrhea or fever. The first study was conducted in Indonesia in 2010 (Gurnida et al., 2012) and was oriented to assess the effect of gangliosides on cognitive development. As reviewed in previous sections, gangliosides are complex sphingolipids that contain at least 1 molecule of sialic acid and diverse carbohydrates. In humans, they are found in the brain, making up approximately 10% of the total mass of brain lipids. They play an important role in this organ because they participate in the formation of synapses between neural cells and during neural transmission, facilitating the binding of transmitter molecules to the synaptic membranes (Rahmann, 1995). They also contribute to neural growth and are thought to act as substrates for neural layer formation, which generates higher cogni-

Table 3. Cognitive development mean scores on the Griffiths Mental Development Scale of infants (Griffiths, 1954; McAlinden, 1997) subjected to supplementation with milk gangliosides for 6 mo¹

Test (score)	Group ²			<i>P</i> -value
	Treatment	Control	Breastfed	
Locomotor IQ	120.0	117.2	113.7	0.225
Personal-social IQ	121.2	119.0	115.4	0.368
Hearing and speech IQ	120.3	116.7	115.1	0.114
Hand and eye coordination IQ	129.5	122.0	123.9	0.006
Performance IQ	131.1	122.2	127.8	<0.001
General IQ	125.4	120.6	120.0	0.041

¹Adapted and reproduced from Gurnida et al. (2012).

²The treatment group (n = 35) received 9 mg of milk gangliosides/100 g of formula, including 2 to 3 mg of GD3/100 g of formula. The control group (n = 35) received 6 mg of milk gangliosides/100 g of formula. The breastfed group (n = 40) received breast milk.

tive functions in the brain (Rösner, 2003). Previous to this study, it was proven in a piglet model (Wang et al., 2007) that sialic acid supplementation improved memory and learning. Gurnida et al. (2012) evaluated the supplementation of a mixture of milk phospholipids and gangliosides (fundamentally GM3 and GD3) in infant formula on the cognitive development functions of normal, healthy 2- to 8-wk-old infants (Gurnida et al., 2012). After receiving supplementation for 6 mo (24 wk), which is a critical period of brain growth and development, significantly increased cognitive scores were observed (Table 3) as measured by the Griffiths Mental Development Scale (Griffiths, 1954; McAlinden, 1997), which covers a general or total scale and 5 additional scales (all reported as IQ numeric values): locomotor, personal-social, hearing and speech, eye and hand coordination, and performance. The results showed that hand and eye coordination, performance, and general scores were significantly improved and similar to those of breastfed infants compared with the control group, which received a decreased concentration of gangliosides. This suggests that milk ganglioside supplementation may induce advantages in cognitive development, particularly in those aspects related to motor skills, compared with diets or formulas that lack these molecules.

The second study, conducted by Tanaka et al. (2013) in Japan and published a year later, explored the role of milk SM in the neurobehavioral development of infants with very low birth weight given the imperative necessity to optimize their growth and development via dietary sources. It is known that SM contributes to the myelination of the central nervous system in developing rats (Oshida et al., 2003) and alters in a positive way the levels of docosahexaenoic acid, the deficiency of which is implicated in decreased dendritic arborization and impaired expression of the genes that regulate neurogenesis, neurotransmission, and connectivity of neural cells (Hibbeln et al., 2007). The study aimed not

only to evaluate the fortification of the feeding formula with milk SM but also to assess the source of polar lipids because the control group was fed a mixture of phospholipids and sphingolipids isolated from egg lecithin (Tanaka et al., 2013).

The main results (Table 4) of the study strongly indicated that after at least 12 mo of feeding, the infants presented improved neurobehavioral scores, measured by different techniques such as the Bayley Scales of Infant Development (2nd ed.), which evaluates mental and psychomotor parameters (Bayley, 1993); the Fagan test for intelligence, which measures novelty rate preference as an indicator of intellectual development (Fagan, 1984); the visual evoked potential test (Hood et al., 2003), which tests the level of myelination and neurotransmission speed via the optic nerves; and the sustained attention test (Colombo et al., 2004) and memory test (Bauer, 1996), which are also indicators of neural development.

These results indicate that a slight increase in SM content in the diet might be positively associated with neurodevelopment in low-weight infants. Early-life nutrition supplemented with SM, among other nutrients, has an important role in promoting myelination and production of neurotransmitter molecules in the brain during early infancy and, consequently, cognitive outcomes (Deoni et al., 2018). It was also stated that milk SM promotes fluidity of neurons and acts in the prefrontal cortex of the brain, given the results from the visual evoked potential, attention, and memory tests (Tanaka et al., 2013). The source of SM and polar lipids was not discussed in the publication, but the authors observed that milk SM administered in the trial group was a safe form of artificial milk compared with egg SM. We believe that the composition and organization of phospholipids and sphingolipids in milk influence the function and resulting effect in human health because they are arranged in a completely different structure than they are in egg (Anton, 2013).

Table 4. Neurobehavioral mean scores of very-low-weight infants subjected to supplementation with milk sphingomyelin (SM) for 12 mo¹

Test	Group ²		P-value
	Treatment	Control	
Bayley Scales of Infant Development (2nd ed.)			
Mental development	97.8	91.1	NS
Psychomotor development	93.8	88.8	NS
Behavior rating scale			
Orientation	65.1	44.5	<0.01
Emotional	69.5	43.1	<0.01
Motor quality	80.9	48.0	<0.01
Total score	76.0	47.5	<0.01
Fagan test			
Novelty preference rate (%)	50.8	44.2	<0.01
Visual evoked potential test			
Latency (ms)	100.5	103.0	NS
Sustained attention test			
Distractive behaviors (no. in 4 min)	1.7	4.4	<0.05
Memory test			
Counts ³	0.8	0.3	NS

¹Reproduced and adapted from Tanaka et al. (2013).²The treatment group (n = 12) received 20 mg of milk SM/100 g of formula. The control group (n = 12) received 13 mg of egg SM/100 g of formula.³Count represents the number of times an infant finds a toy hidden in 1 of 2 cups.

The third study, published by Timby et al. (2014), aimed to test whether supplementation of MFGM components (sialic acid, phospholipids, and phosphosphingolipids) reduced differences in cognitive development and early growth between formula-fed and breastfed infants. The trial took place in Sweden in 2014, where the infants were fed a formula enriched with MFGM (70 mg/100 mL), a control formula with a basal concentration of polar lipids (30 mg/100 mL), or breast milk (Table 5) until 12 mo of age. After that period the infants were tested with the Bayley Scales of Infant and Toddler Development (3rd ed.; Bayley, 2006) using the cognitive, motor, and verbal subscale as an indicator of neural development.

One of the main outcomes was that infants fed an MFGM-supplemented diet obtained a significantly

higher cognitive score than the control group (105.8 compared with 101.8; $P = 0.008$) but, interestingly, not significantly different from the breastfed group (106.4; $P = 0.73$). The authors hypothesized that these findings could have been a direct result of either one of the many bioactive components of the MFGM proven to have an essential role in brain development or, more likely, a combination of many components such as SM, gangliosides, phospholipids, and even bioactive membrane proteins (Timby et al., 2014).

CONCLUSIONS

The information reviewed here highlights the enormous potential of the polar lipid fraction of milk to exert positive effects on the development of infants at

Table 5. Cognitive development mean scores on Bayley Scales of Infant and Toddler Development (Bayley, 2006) of infants subjected to supplementation with milk fat globule membrane (MFGM) for 12 mo¹

Test	Group ²			P-value		
	Treatment (a)	Control (b)	Breastfed (c)	a-b	a-c	b-c
Cognitive	105.8	101.8	106.4	<0.75	0.73	0.003
Motor	98.6	98.2	100.2	<0.82	0.25	0.16
Fine motor	9.69	9.77	10.24	<0.79	0.022	0.060
Gross motor	9.72	9.58	9.76	<0.93	0.92	0.59
Verbal	102.6	102.5	106.7	<0.94	0.022	0.014
Receptive	10.48	10.41	11.47	<0.87	0.023	0.012
Expressive	10.51	10.53	10.76	<0.94	0.36	0.39

¹Reproduced and adapted from Timby et al. (2014).²The treatment group (n = 71) received 70 mg of MFGM/100 mL of formula. The control group (n = 64) received 30 mg of polar lipids/100 mL of formula. The breastfed group (n = 70) received breast milk.

a neuronal level, the known benefits obtained in the regulation of immune responses, and the modulation of intestinal microbiome and protection from bacterial infection. Even though the number of studies is very low and the interventions are heterogeneous, with different concentrations given in different periods of time, this opens a wide possibility of applications and future research, especially with infant nutrition and development of formulations that molecularly have a closer resemblance to the golden standard: breast milk. Another important aspect to highlight is that all of these studies and trials used milk phospholipids, sphingolipids, and gangliosides contained in MFGM. In comparison, no reports to this date are focused on testing the effect of polar lipids from MEX; hence, the scientific base of knowledge is limited. We believe that it is imperative to shed light onto the connection between composition, structure, and function in milk vesicles, whether they are MFGM or MEX phospholipidic components, and to perform not only in vivo or in vitro studies but also chemical and physicochemical characterization, which can help us understand and discuss from a molecular standpoint the functions and bioactivities in living organisms.

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