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NUTRITION INSTITUTE

SCIENCE MONOGRAPH
Issue 1 | August 2023

FUNCTIONAL COMPONENTS OF HUMAN MILK

The Role of the Milk Fat Globule Membrane (MFGM) in Infant and Child Nutrition

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FOREWORD

Recent advances in the science and technology of DNA, RNA, and proteomics coupled with bioinformatic tools have allowed us to define the roles of several components in human milk and make infant formulas that are closer in composition to human milk. These components include pre- and probiotics, docosahexaenoic acid (DHA), arachidonic acid (ARA), lactoferrin, and milk fat globule membrane (MFGM).

This monograph represents an updated, comprehensive summary of our knowledge regarding MFGM. The structure and function of MFGM is coupled with preclinical and clinical human studies, which established the safety and tolerability of infant formulas with added MFGM. Extensive preclinical evidence elegantly demonstrates the role of the components of MFGM on neurodevelopment processes, gut function, and immunological profiling of anti-infective activities. These data are complemented by human studies in premature infants, term infants, and children on the efficacy of MFGM on neurodevelopmental aspects, gut health, and immunological parameters and thereby add significantly to our understanding of the role of MFGM on children's health. The addition of MFGM to infant formulas is one more important step in improvements to infant nutrition.

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It is well known that breastfeeding provides many benefits to the newborn infant, including promoting health and development. Research has identified numerous components of breast milk that are involved in these favorable outcomes. A goal of the infant formula industry is to achieve outcomes of formula-fed infants that are more similar to those of breastfed infants. This is achieved by adjusting the nutrient contents of formula, but also by adding components with well-recognized functionalities.

Back in the 1960s, however, mistaken interpretations of research performed at the time led the formula industry to remove milk fat from their products based on the notion that cholesterol, saturated fatty acids, and consequently milk fat were significant risk factors for cardiovascular disease later in life. The milk fat was therefore replaced by a mixture of vegetable oils attempting to mimic the fatty acid composition of human milk. What was not realized was that milk fat also contains the MFGM surrounding the fat globules. This complex membrane contains a multitude of components, many of which have been shown to provide functionalities and its omission may therefore have caused unintended biological consequences in infants fed formula.

Recently, bovine MFGM, which is in many aspects similar to MFGM from human milk, has been added to infant formula. This monograph describes in detail the components of the MFGM, their functions, and their possible physiological significance. It provides an extensive review of the preclinical research that has been performed on both the MFGM and its individual components, including sphingomyelin, gangliosides, phosphatidylserine, phosphatidylcholine and choline, sialic acid, and several protein constituents. Functionality associated with these components, including brain development and cognitive function, immunity, and gut health, are presented in detail and underlying mechanisms are discussed.

The monograph also thoroughly reviews the clinical studies to date that support the decision to include MFGM in infant formula. Overall, there is considerable support for favorable effects on cognition and neurodevelopment, defense against infections, and improved immune function. The possibility that these outcomes are mediated by alterations in metabolism and the gut microbiota is discussed. It is recognized that commercial sources of MFGM, as well as its components, vary among manufacturers and that these variations may have been the cause of differences observed in outcomes among clinical trials. Although addition of some components of MFGM have yielded outcomes similar to those observed for adding MFGM as a dairy fraction, it remains uncertain if addition of individual components will altogether result in outcomes found for MFGM. Thus, the addition of MFGM as a complete dairy fraction appears prudent with our present understanding.

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ABSTRACT

A mother's own milk is considered the gold standard in infant nutrition for the promotion of optimal growth and development. Human milk has a highly complex composition of macro- and micronutrients, functional proteins and lipids, enzymes, and hormones.[1] A key component of human milk is the milk fat globule membrane (MFGM). Naturally occurring in mammalian milk, MFGM has important functional proteins and lipids that, when fed to infants, have been associated with positive outcomes in brain structure, cognitive development, immune system development, and gut health.[2] While scientific advancements on MFGM and MFGM components continue to expand, the purpose of this monograph is to provide an overview of the current evidence. This monograph presents the composition, structure, and physiology of MFGM. It will also summarize the body of evidence spanning 7 decades, including preclinical studies examining mechanisms of action as well as clinical trials studying the benefits of MFGM in the diets of infants and young children. Finally, applications in maternal and adult nutrition will be noted.

III.

INTRODUCTION

NUTRITION AND THE FIRST 1000 DAYS

Infancy and early childhood represent a critical time period for nutrition, as proper dietary intake during this phase is vital to supporting not only early growth and development but also optimal health outcomes later in life.[3] Nutritional and environmental influences are especially important during the first 1000 days of life, when physical growth and development in the brain, immune system, and GI tract are happening at astonishing speed.[4]

The supply of nutrients to the fetus and the infant during the first 1000 days, from conception to 2 years of age, depends on the diet of the mother during pregnancy and infant feeding. The challenge of optimizing nutrition, therefore, is to identify and promote a diet containing an appropriate blend of nutrients that best support healthy growth and development at each stage of life.[5]

BRAIN DEVELOPMENT

The acceleration of physical growth during this time can be observed as a child's weight triples in the first year of life and length increases by 75% at two years of age.[6] More difficult for clinicians to observe is the rapid brain growth.

Brain growth is exponential; it doubles in size in the first 2 years, reaching approximately 85% of adult weight.[7,8] Additionally, remarkable brain processes are occurring during this time, including synaptogenesis and myelination (Figure 1).[9-11]

Synaptogenesis is the formation of synapses between the neurons. Toddlers have over 1000 trillion synapses, the most they will have in their entire life, and are creating synapses at a rate faster than at any other time in their life (Figure 1A).[10-12]

During myelination, nerve axons are wrapped with multiple layers of cell membrane by oligodendrocyte glial cells, a process that accounts for a large portion of brain growth during late gestation and the first 2 years of life[13], but which can also continue up to 5-10 years of age (Figure 1B).[14]

Meeting the nutritional demands of the first 1000 days requires an appropriate balance of nutrients, such as proteins, carbohydrates, lipids, and micronutrients. For instance, infants and toddlers need a high proportion of dietary fat—up to 50% of total calories in the first 2 years—in order to support rapid myelination.[15] Therefore, it is imperative to investigate human milk composition and what components can be targeted to ensure the optimal breast milk substitute when necessary.

IMMUNE AND DIGESTIVE SYSTEM DEVELOPMENT

An infant's immune system relies on T-cell and B-cell maturity that gradually develops with environmental exposures.[20] By 6-12 months, an infant's immune system begins to mount antibody responses as maternal protective factors decline.[21] Immune health requires specific proteins (eg, mucins and lactadherin) to help foster the development of host protective bacteria within the gastrointestinal (GI) tract.[22] In addition, nutrients such as iron, zinc, copper, selenium, and vitamins C and E are needed for enzymatic and antioxidant support.[4,23]

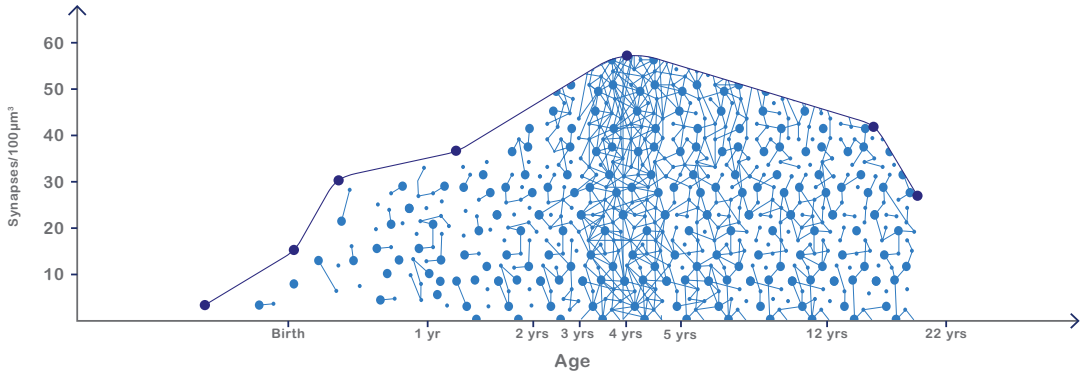
Clinical Pearl

The first 1000 days of life is a period of rapid growth and development, especially brain development.[6,7,16,17] Remarkable brain processes happen during this time, including synaptogenesis and myelination, which affect learning and memory later in life.[18,19]

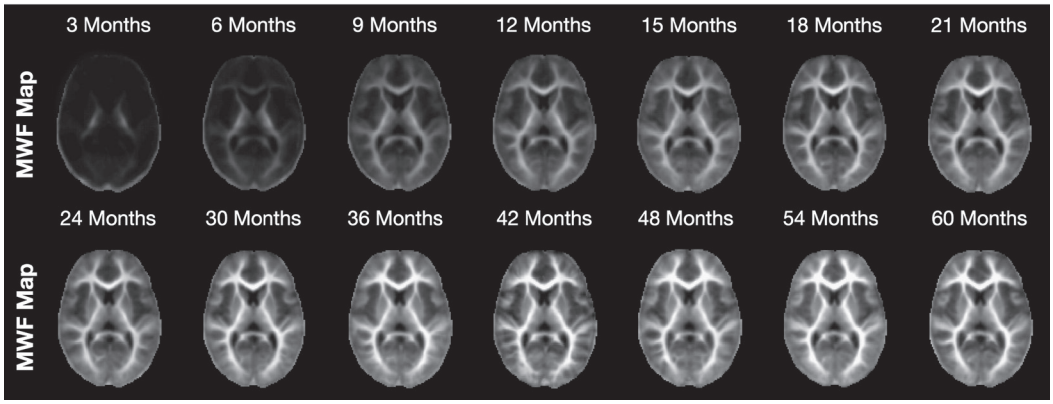
The mammalian intestinal tract is the largest immune organ in the body.[24] An infant's digestive tract undergoes profound growth, morphological changes, and functional maturation after the first feed.[25,26] Nutrition is a critical component in the establishment of normal GI maturation and function from digestion and absorption to barrier function and development of the mucosal immune system. The functional nutrients of milk support a microenvironment for gut maturation and immune function.[26]

FIGURE 1. MYELINATION AND SYNAPTOGENESIS OCCUR AT A HIGH RATE EARLY IN LIFE[10,11]

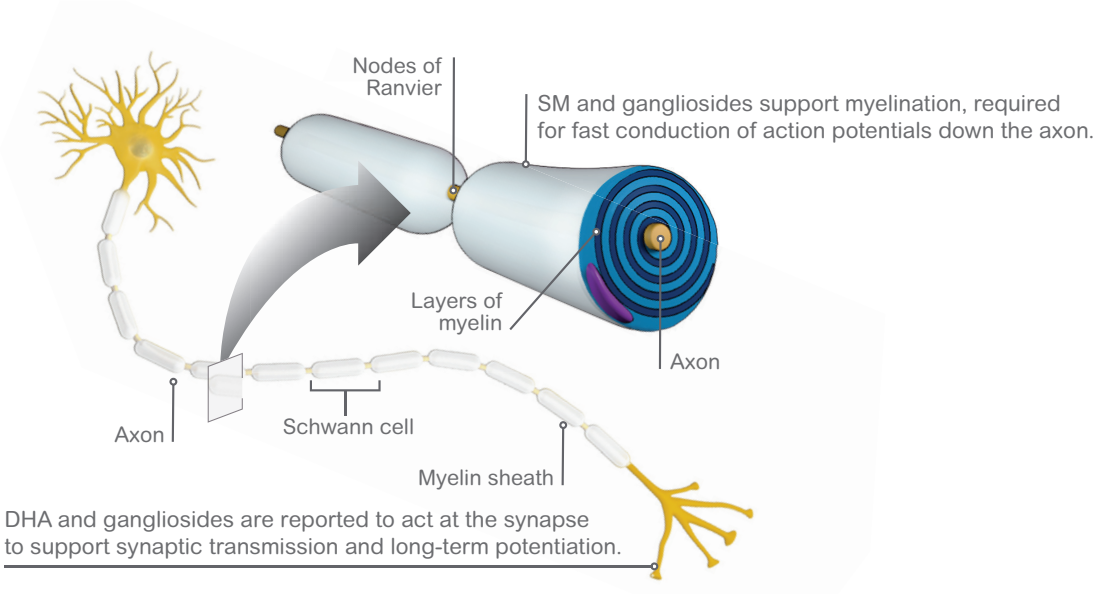
1A. Mean synaptic density in prefrontal cortex at various ages.[11] The rate of synaptogenesis peaks between 3-5 years of age.



1B. Axial slices of myelin water fraction (MWF) in average templates from ages 3 to 60 months.[10] MWF is a surrogate measurement of myelin content. Myelination occurs at its fastest rate during the first 3 years of life.



1C. MFGM components and DHA support synaptic transmission and myelination.



INTERPLAY OF GUT MICROBIOTA AND BRAIN, IMMUNE, AND GUT HEALTH IN EARLY LIFE

Colonization of the human body by microorganisms begins during the first moments of life, and microbial makeup gradually shifts, reaching adult-like composition around 3 years of age.

The microbiota present in the gut not only have an influence locally in the intestinal environment but also beyond the GI tract. Gut microbiota interact with many organs, including the brain, lungs, and skin.[27] Some of these interactions are well known, such as the gut-brain axis.[28] Gut microbiota and the central nervous system (CNS) are believed to communicate via bidirectional pathways between the GI tract and brain. These pathways are mainly driven by neural, endocrine, immune, and metabolic mediators.[29]

The composition of the infant intestinal microbiota can be influenced by multiple factors, including gestational age, mode of birth delivery, birth weight, maternal microbiome and diet, antibiotic exposure, and early-life nutrition.[30,31]

INSIGHTS FROM HUMAN MILK RESEARCH

Research into human milk's composition and functionality has inspired innovations to bring formula for infants closer to human milk. One example is the addition of prebiotic oligosaccharides, which have been demonstrated to support development of the gut immune system.[32] Another example is the addition of the long-chain polyunsaturated fatty acids (LCPUFA), including docosahexaenoic acid (DHA) and arachidonic acid (ARA), which are in human milk but were originally not added to infant formula.

Based on years of data supporting benefits to health, cognition, and visual development, DHA is now widely recognized to be necessary in the diets of infants and young children during the first 24 months of life.[33,34] As related to the design of infant formula, research has highlighted the importance of understanding and approximating human milk's nutrient composition and functional benefits.

Nutrients in human milk perform three major distinct roles. They may be sources of energy, provide structural building blocks, or act as functional compounds.

Functional compounds can be defined as constituents that affect biological processes and thus have an impact on body function or condition beyond basic nutrition[1,35] and represent a large and complex category that includes proteins, oligosaccharides, and lipids, among others, and have diverse functions and potential health benefits.[36]

Some nutrients can fulfill more than one role. For instance, lactose is a primary carbohydrate energy source, but it also functions as a prebiotic, as it may reach the colon partially undigested and thereby influence the development of gut bacterial flora.[37, 38]

Protein/lipid components in MFGM support brain myelination and synaptogenesis, but also contribute to gastrointestinal integrity.[1] These features support brain development, immune, and gut health.[39,40]

Key takeaways

- The first 1000 days of life is a period of rapid growth and development, especially brain development. Remarkable brain processes happen during this time, including synaptogenesis and myelination, which affect learning and memory later in life.
- Optimal nutrition during infancy and early childhood is important to supporting early growth and development.
- Human milk is the ideal source for infant nutrition. Understanding human milk's unique composition and function is essential for optimizing health and developmental outcomes when formula feeding is necessary.
- MFGM has been produced during lactation throughout the course of mammalian evolution. Increasing evidence has suggested that MFGM has long-lasting benefits in brain development and immune and gut health.

IV.

WHAT IS THE MILK FAT GLOBULE MEMBRANE (MFGM)?

ORIGIN OF MFGM

Milk fat globules, or droplets, have a triacylglycerol core encased in a membrane. They are secreted in a unique manner by lactocytes, which are specialized epithelial cells within the alveoli of the lactating mammary gland.

1. First, fat synthesized within the endoplasmic reticulum (ER) accumulates in droplets between the inner and outer phospholipid monolayers of the ER membrane.
2. As these droplets increase in size, the two monolayers separate further and eventually pinch off, surrounding the droplet in a phospholipid monolayer vesicle that allows it to disperse within the aqueous cytoplasm.
3. Lipid droplets then migrate to the apical surface of the cell, where the plasma membrane subsequently envelops the droplet and extrudes together with it, fully encasing the fat droplet in an additional bilayer of phospholipids.
4. The milk fat globule thus released into the glandular lumen, measuring 3-6 µm in average diameter, is surrounded by a phospholipid trilayer containing associated proteins, carbohydrates, and lipids derived primarily from the membrane of the secreting lactocyte. This trilayer is collectively known as MFGM (Figure 2).[41,42]

Although MFGM only makes up an estimated 2-6% of the total milk fat globule,[41] it is an especially rich phospholipid source, accounting for 60-70% of total milk phospholipids.[43,44] In contrast, the inner core of the milk fat globule is composed predominantly of triacylglycerols.

MFGM is unique to mammalian milk, including human and bovine, and is not present in nondairy food products. The secretion process described earlier is distinct from lipid secretion in non-mammary cells.[42] Interestingly, the genes associated with the production of the milk fat globule and MFGM appear to be the most conserved lactation genes throughout evolution, indicating a significant physiologic role for the MFGM structure.[39]

Although many of the components within MFGM are identified as functional and have been linked to cognitive and other health benefits (as discussed below), indeterminate amounts of these components have traditionally been lost during commercial dairy processing. More recent advances in technology have facilitated the separation of MFGM from the fat globule, allowing bovine MFGM components to be added in concentrated form.[45]

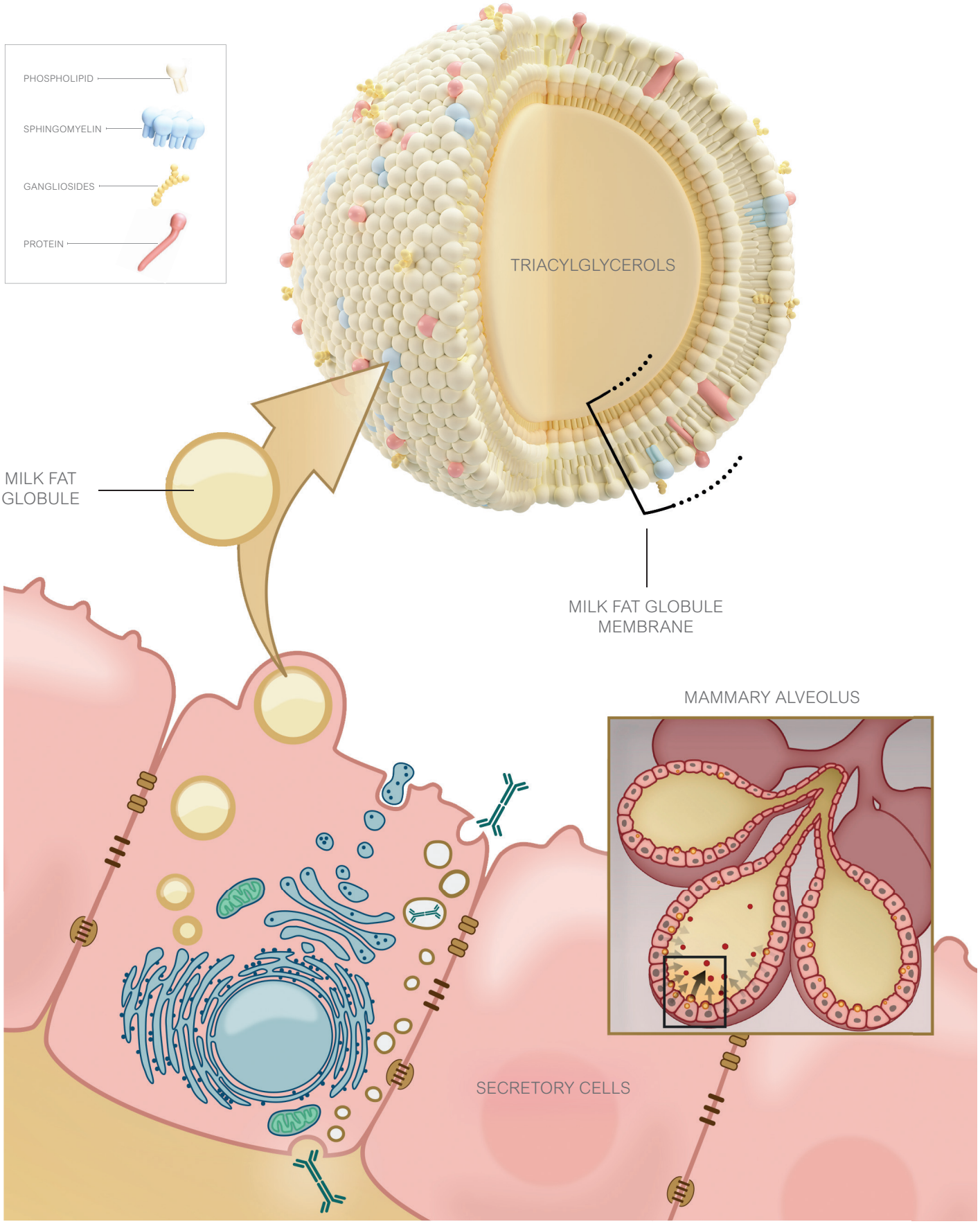
While differences exist, bovine MFGM generally contains a similar lipid and protein composition to that of human MFGM.[46,47] In addition, proteins, neutral lipids, and phospholipids within bovine MFGM appear to be efficiently digested in conditions that are physiologically similar to those of an infant's stomach and small intestine.[48] Thus, the enrichment of infant formula with MFGM components represents an opportunity to match the composition and functionality of breast milk more closely, and thereby provide health benefits for formula-feeding infants.

Clinical Pearl

While MFGM in human milk and bovine milk is compositionally similar, it has historically been discarded in the manufacturing of infant formula.

Recent advances in technology have facilitated the separation of MFGM from the fat globule, allowing bovine MFGM components to be added in concentrated form.

FIGURE 2. FORMATION OF MFGM[41,42]
During their production in the mammary gland, milk fat globules become encased in a tri-layer membrane rich in polar lipids and proteins, collectively known as MFGM.

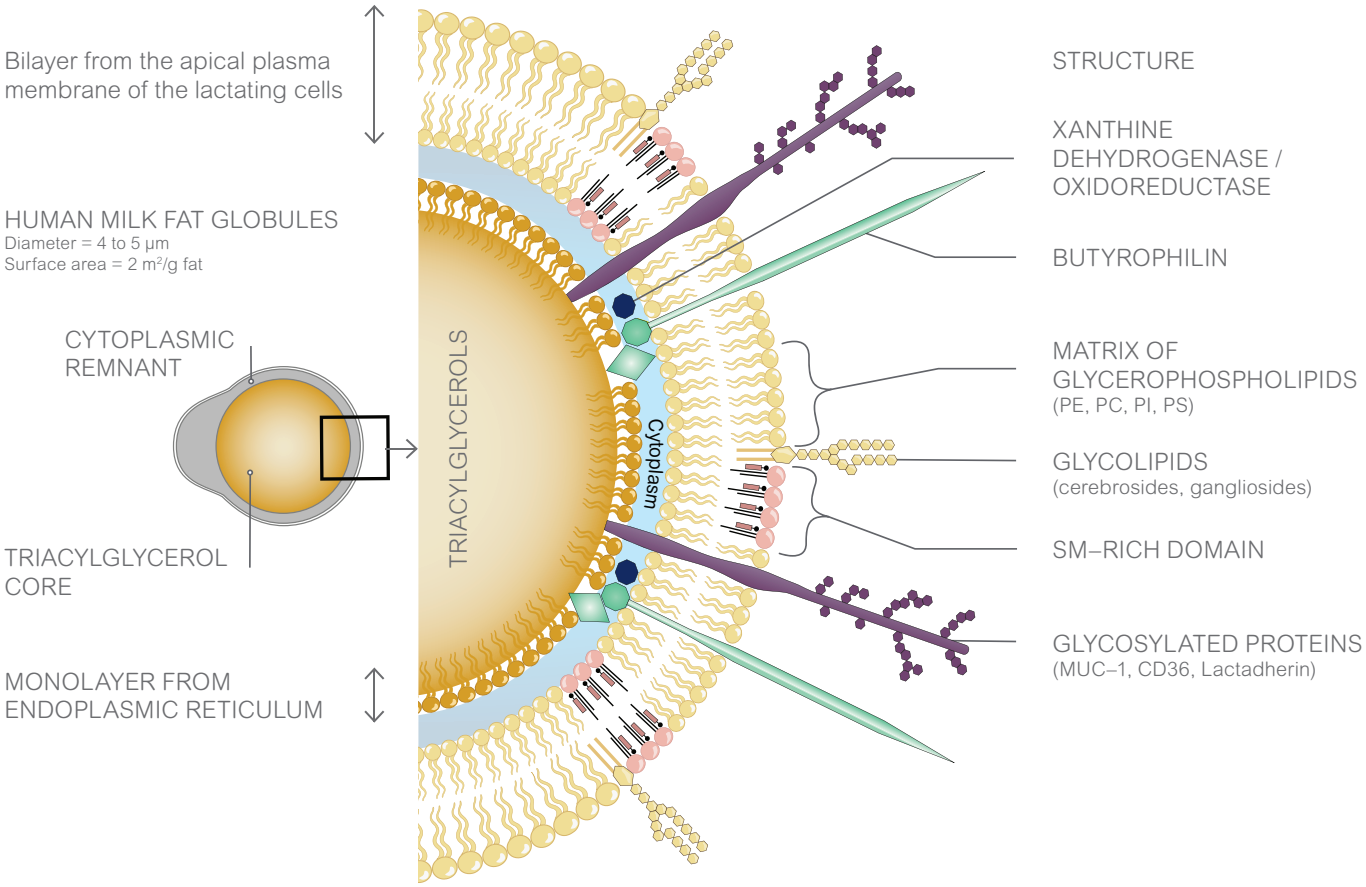


STRUCTURE AND COMPONENTS OF MFGM

MFGM is made of various lipids and proteins, including (but not limited to) phospholipids, glycolipids, proteins, glycoproteins, and cholesterol. Specific lipids and proteins are localized to different layers of the membrane,with carbohydrate chains of glycoproteins and glycolipids directed toward the outer surface of the milk fat globule.The lipid:protein weight ratio in MFGM is approximately 1:1.[49]

As a quantitatively minor presence within milk, MFGM likely contributes little to energy production, but its constituents may confer structural and functional benefits (Figure 3).[41,45] Many of these components are known to play important roles within the brain, gut, and elsewhere in the body. Key individual components of MFGM are highlighted in the sections below and summarized in Table 1.

FIGURE 3. MFGM: A TRILAYER STRUCTURE WITH A LATERAL ORGANIZATION OF POLAR LIPIDS[41,45]



CD36=cluster of differentiation 36. MUC-1=glycoprotein mucin-1. PC=phosphatidylcholine. PE=phosphatidylethanolamine. PI=phosphatidylinositol. PS=phosphatidylserine. SM=sphingomyelin.

TABLE 1: SUMMARY OF KEY MFGM COMPONENTS AND MECHANISTIC FUNCTIONS

MFGM COMPONENT		FUNCTION
POLAR LIPIDS		
Phospholipids	Phosphatidylcholine	• Major structural component of biological membranes[70] • Involved in synthesis of sphingomyelin and the regeneration of choline[70] • Key constituent of the intestinal mucus barrier; may possess anti-inflammatory functions[71-73]
	Sphingomyelin	• Key component of the myelin sheath; insulates axons and supports efficient transmission of nerve impulses[60,61] • Contributes to membrane structure in the gut epithelium[62,63] • Sphingomyelin and its metabolites (ceramide, sphingosine, ceramide-1-P, and sphingosine-1-P) act as second messengers in cell signaling, with regulatory effects on cell proliferation, cell survival, apoptosis, and inflammation[63,65]
Gangliosides		• Structural components within cellular membranes of most body tissues, especially the brain[88] • Involved in neurotransmission and synapse formation[88,89] • May be involved in improved gut microflora and antibacterial defense in the gut[93]
PROTEINS		
Mucin (MUC-1, MUC-4, MUC-15)		• May enhance triacylglycerol (TAG) digestion efficiency[41] • May possess antibacterial and antiviral properties[97,98,100]
Butyrophilin		• May enhance TAG digestion efficiency[41]
Lactadherin		• May enhance TAG digestion efficiency[41] • May possess antimicrobial and antiviral properties[97,98,100] • Induces anti-inflammatory responses[230] • Maintenance and repair of the intestinal epithelium[133]
CD36		• May enhance TAG digestion efficiency[41]
Xanthine oxidase		• Antibacterial properties through the production of reactive oxygen species[231]
CARBOHYDRATES		
Oligosaccharides		• Conjugate with glycoproteins or glycolipids of the MFGM[105]
Sialic acid		• Involved in synaptic transmission and functions that occur during brain development[87,109] • Needed for proper development of gangliosides[110]

LIPIDS

The lipid fraction of MFGM is rich in phospholipids, glycosphingolipids, and cholesterol. Phospholipids make up approximately 30% of the total lipid weight of MFGM, the most prominent being sphingomyelin (SM), phosphatidylcholine (PC), and phosphatidylethanolamine, which account for up to 85% of phospholipids.[41,49] Other important polar lipids present in the membrane include the glycerophospholipids phosphatidylserine (PS) and phosphatidylinositol (PI), as well as gangliosides, which are sphingolipids containing sialic acid and an oligosaccharide side chain. Each of these lipid classes is known to play functional roles within the body, including within the central nervous system (CNS) development, immune system, and gut.[52-54] For example, phospholipids and sphingolipids play central roles in cerebral neurogenesis and migration during fetal development, as well as promote neuronal growth, differentiation, and synaptogenesis during the first year of life.[50,51]

Choline-Containing Phospholipids: Sphingomyelin and phosphatidylcholine

Sphingomyelin (SM)

In addition to being a major phospholipid in both human and bovine MFGM, SM is the most abundant class of sphingolipid. Some SM also contains PC (see below). SM concentration in human milk remains relatively constant throughout lactation and ranges from 5.0-16.5 mg/100 mL.[55,56] SM levels in bovine milk are similar or somewhat lower than in human milk. However, in some commercially available bovine, milk-based formulas that do not include a bovine-derived MFGM ingredient, SM levels are typically lower than in human or bovine milk.[57-59]

SM has been shown to play several important roles within the body. In the CNS, SM is a key component of the myelin sheath, which insulates axons and supports efficient transmission of nerve impulses.[60,61]

SM and other sphingolipids are also present in the gut epithelium, where they contribute to membrane structure, modulate growth factor receptors, and provide binding sites for microorganisms, microbial toxins, and viruses.[62,63] Within the plasma, SM is a key lipid on the monolayer of plasma lipoproteins that contributes to cell membrane structure and participates in lipid transport within blood circulation.[64] Furthermore, SM and its metabolites (ceramide, sphingosine, ceramide-1-phosphate, and sphingosine-1-phosphate) act as second messengers in cell signaling, with regulatory effects on cell proliferation, cell survival, apoptosis, and inflammation.[63,65]

Phosphatidylcholine (PC)

Choline is found in various metabolic forms in humans and animals. Milk and dairy products are an important source of choline for developing infants as requirements are increased for organ development and membrane biosynthesis. In mature breastmilk, choline is mainly present in the form of water-soluble metabolites, including phosphocholine, glycerophosphocholine, and free choline, followed by lipid-soluble metabolites such as PC and SM.[66,67]

PC makes up approximately 18-33% of the total phospholipid content in human milk[41,59,68,69] and is one of the two most abundant glycerophospholipids present within MFGM. Bovine milk has similar concentrations of PC to human milk.[57-59]

PC is also a major structural component of biological membranes, including all plant and animal cells, and is usually the major component of the animal or plant tissue lipid extract known as lecithin. PC is a valuable source of choline, and PC is involved in the regeneration of choline and its metabolites, such as the neurotransmitter acetylcholine. PC is also involved in the synthesis of SM.[70]

In addition to its membrane effects, PC is a key constituent of the intestinal mucus barrier and has been proposed to exert anti-inflammatory functions in models of colitis and arthritis.[71-73]

Gangliosides

Gangliosides are complex glycosphingolipids containing an oligosaccharide side chain plus sialic acid (N-acetylneuraminic acid, or NANA). Gangliosides are quantitatively less abundant in MFGM relative to phospholipids (9-40 mg/L vs 40-80 mg/L for SM alone).[74-80] Rather than a single compound, gangliosides represent a class of different molecules that varies in the nature and length of their carbohydrate side chains and in the number of attached sialic acids.[81] The quantity and composition of gangliosides in breast milk varies over the course of lactation. However, the GD3 ganglioside is present in both human colostrum and mature human milk, as well as in bovine milk.[77,78] The concentration of gangliosides in bovine milk appears to be similar or somewhat lower than that in human milk.[75,79]

Gangliosides are important structural components within the cellular membranes of most body tissues, including nuclear and plasma membranes and endoplasmic reticulum. Gangliosides are expressed more predominantly in nervous tissue. They are especially abundant in the brain, where they are concentrated within the grey matter and constitute approximately 6-10% of the total human brain lipid mass.[82-87] Additionally, gangliosides are enriched at the synaptic membrane of neurons and are functionally involved in neurotransmission and synapse formation.[88,89] Moreover, gangliosides play a role in nervous system development through stem cells; membranes and organelles within neurons and glia; ion transport mechanisms; and receptor modulation, including neurotrophic factor receptors.[90] Brain ganglioside accretion occurs at an accelerated rate in the early years of life, coinciding with the most active period of myelination, axonal outgrowth, and synaptogenesis.[91,92] Alongside the growth of brain size, total brain ganglioside concentration also increases 3-fold from early fetal development to 5 years of age.[91]

Outside the CNS, ganglioside concentrations are much lower. However, they are found in other areas of the body, such as the intestinal mucosa, and may contribute to improved gut microflora and antibacterial defense.[93] Furthermore, gangliosides appear to play a role in cell-cell recognition and adhesion, as well as signal transduction within cell surface microdomains, alongside other sphingolipid components and cholesterol.[94]

PROTEINS

In addition to the polar lipids, the outer layer of MFGM contains a number of glycosylated and nonglycosylated proteins. Proteomic analysis has revealed at least 191 different known proteins in human MFGM and comparable numbers in bovine milk protein concentrates (For reference: 133 in buttermilk protein concentrate, 244 in whey protein concentrate).[41] Quantitatively, this represents only 1-2% of milk's total protein content.[95] However, MFGM proteins are of significant interest because many are known to have functional and health-benefiting properties. Almost half of the identified MFGM proteins have membrane/protein trafficking or cell signaling functions.[96]

Additionally, the glycosylated proteins, including mucins (MUC-1, MUC-4, MUC-15), butyrophilin, lactadherin, and CD36, have been suggested to enhance triacylglycerol (TG) digestion efficiency.[41] Furthermore, lactadherin and MUC-1, in addition to the nonglycosylated protein xanthine oxidase, have been shown in preclinical studies to possess antimicrobial properties.[96-101] Lactadherin has also been shown to bind to intestinal epithelial cells and support wound healing in vitro and may thus aid in infant gut development.[102]

CARBOHYDRATES

Oligosaccharides

Oligosaccharide concentrations are considerably higher in human milk than in either bovine milk or infant formula.[103] The majority of human milk oligosaccharide is in the freely soluble form.[104,105] However, within the MFGM structure, oligosaccharides are present primarily in the form of conjugates with either glycoproteins or glycolipids. As noted earlier, these components of MFGM have been shown to exert various functional activities as previously outlined.

Sialic Acid

Sialic acid represents a class of 9-carbon sugar derivatives of neuraminic acid, the most common of which is N-acetylneuraminic acid (NANA). Sialic acid is widely distributed in animal tissues. It sometimes appears in free form but primarily as a component of gangliosides (bound to the oligosaccharide side chain) and glycoproteins. The highest known concentration of sialic acid occurs in the human brain.[106]

In human milk, sialic acid is primarily bound to oligosaccharides rather than glycoproteins (ratio 3:1). In contrast, typical infant formula without bovine MFGM added as an ingredient has been found to have total sialic acid levels <25% of that in breast milk, and the majority is protein-bound (ratio 1:3).[107] The MFGM glycoproteins MUC-1 and MUC-15 are high in sialic acid content.[103]

Breastfed infants have significantly higher amounts of sialic acid in saliva[108] and 22% higher concentrations of protein-bound sialic acid in the brain frontal cortex, compared with infants fed formula without a bovine-MFGM ingredient.[106] Additionally, evidence suggests a contributory role for sialic acid in synaptic transmission and several other functions during brain development.[53,87,109,110]

Key takeaways

- MFGM is a complex structure composed of a variety of lipids and proteins generally localized to different membrane layers, as well as carbohydrates that reside toward the outer surface of the globule.
- Lipids in MFGM, such as choline-containing phospholipids (including sphingomyelin and phosphatidylcholine) and gangliosides, play important roles in the support of brain development
- Sphingomyelin is the most abundant class of sphingolipid in MFGM. It is an important component of the myelin sheath, which provides insulation of axons for efficient transmission of nerve impulses and supports a large portion of brain growth during the first 2 years of life.
- Gangliosides are complex glycosphingolipids containing an oligosaccharide side chain plus sialic acid. Gangliosides are important structural components within cellular membranes, especially within nervous tissue in the brain and are abundant at the synaptic membrane of neurons and aid in neurotransmission and synaptogenesis.
- MFGM contains glycosylated and nonglycosylated proteins that have demonstrated roles in membrane/protein trafficking, cell-signaling functions, and other aspects relating to infant immunity and gut development.
- Oligosaccharides and sialic acid are carbohydrates found in MFGM; a small percentage of the oligosaccharides in human milk serve as conjugates for glycoproteins and glycolipids. Sialic acid is a key component of gangliosides and is thought to be involved in synaptic transmission and functions that occur during brain development.

V.

SCIENTIFIC EVIDENCE FOR HEALTH BENEFITS OF MFGM

ADVANCES IN MFGM RESEARCH AND TECHNOLOGY

Although much is still being learned about the significance of MFGM and its components to infant nutrition, this research rests on more than 70 years of foundational research into milk lipid composition and function.[111]

In the 1950s, scientists developed methods to extract and purify the proteins of MFGM.[112] Emerging analytic methods in the 1970s allowed researchers to characterize the specific content of fatty acids within the MFGM of both human and bovine milk, leading to the recognition of sphingomyelin (SM) and other phospholipids as major components of the membrane.[113,114]

During the 1980s, additional studies compared the changes in milk phospholipid composition across the full lactation cycle[69] and further characterized the relative compositions of SM, other phospholipids, and gangliosides in human milk, bovine milk, and infant formula. This research contributed to the recognition that these components may play important roles in growth, as well as brain development, immune, and gut health during the critical period of early infancy.[14,30,57,69,75,115] At the same time, advances in dairy processing technology have facilitated the process of purifying and concentrating MFGM from bovine milk, allowing for ingredients such as polar milk lipids and other MFGM components to be added to infant formulas.[45,111]

HETEROGENEITY BETWEEN DIFFERENT COMMERCIAL MFGM SOURCES

Commercial sources of MFGM generally have the same key components of naturally occurring MFGM: polar lipids, proteins, and sialic acid. However, the relative concentration of these components may vary among commercial products.[116] This heterogeneity between different commercial MFGM preparations can result from the source material (eg, bovine cream or whey) and the manufacturing techniques used to isolate and concentrate MFGM components.[117]

The parallel development in the scientific interest regarding the functionality of MFGM components and the technological advances have driven preclinical and clinical research forward (see highlighted historical depiction of MFGM research on pages 24-25).

“

… the heterogeneous nature of various bovine commercial MFGM fractions … must be considered when evaluating and describing potential functional benefits of these products shown in clinical trials.”[116]

7 DECADES OF RESEARCH
MFGM & its Components

Advances in **analytical methods** led to recognition of MFGM & its components

1950 **Polonovski et al.**[112]
Extraction of xanthine dehydrogenase from milk

1968 **Morrison**[114]
PL levels in milks

1972 **Bracco et al.**[113]
Lipid composition of MFGM

1984 **Bitman et al.**[69]
PL levels over lactation

1986 **Zeisel et al.**[57]
PL and SM in HM, BM, IF

1986 **Laegrid et al.**[247]
GG composition of HM and BM

1988 **Hundrieser and Clark**[115]
Method to separate and measure PL classes

KEY GUIDE Analytical Pre-clinical Clinical

SM: sphingomyelin; **PL**: phospholipid; **HM**: human milk: **BM**: bovine milk; **GG**: ganglioside; **IF**: infant formula

Preclinical studies explore mode of action in the brain, gut, and immune system

1993 **Mei and Zheng**[125]
Exogenous GG on learning and memory in rats

1997 **Fong et al.**[127]
GG improves learning and memory in aged rats

1998 **Rueda**[208]
GG improves gut flora in preterm infants

1998 **Carlson**[248]
Phospholipids reduced complications associated with prematurity

2003 **Oshida et al.**[61]
Dietary SM and myelination in rats

2003 **Wang, et al.**[106]
Brain GG in BF and FF infants

2007 **Susuki et al.**[121]
GG, myelination and nerve function

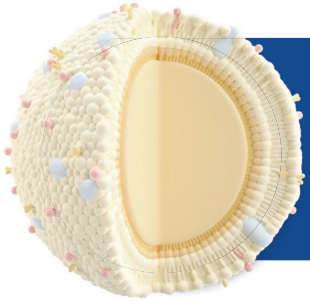
2008 **Clare et al.**[100]
Antimicrobial functions of MFGM

2008 **Jung et al.**[124]
GG improves spatial learning and memory in rats

2009 **McJarrow et al.**[89]
A review on dietary GG and brain development

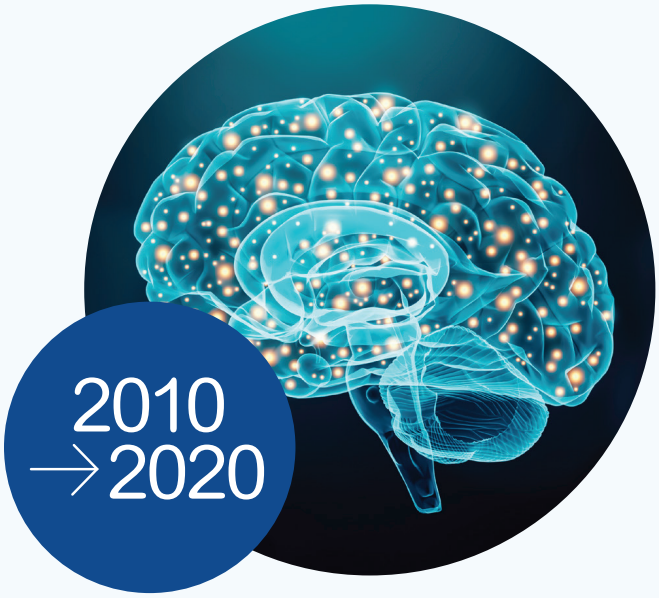
2009 **Schnabl et al.**[249]
GG can be taken up by intestinal cells

2009 **Vickers et al.**[140]
Complex milk lipid supplementation improves cognition in rats



Over 2000 studies published on MFGM and its components and the evidence continues to grow…

Clinical evidence supports MFGM's role in cognition and immune health in early life



2011 **Zavaleta et al.**[211]
MFGM enriched foods improve diarrhea in infants

2012 **Gurnida et al.**[158]
Complex milk lipids associated with cognitive development in 6 month infants

2012 **Veereman-Wauters et al.**[162]
MFGM improves parent reported behavior regulation and febrile episodes in children

2013 **Tanaka et al.**[160]
SM-enriched formula improves multiple development measures in preterm infants

2014 **Billeaud et al.**[47]
Safety and tolerance of MFGM enriched formulas

2014 **Timby et al.**[155]
MFGM and neurodevelopments in infants

2014 **Timby et al.**[250]
MFGM and cardiovascular markers in infants

2015 **Timby et al.**[46]
MFGM and infections in infants

2016 **Ten Bruggencate et al.**[251]
MFGM and immune outcomes in adults

2017 **Timby et al.**[210]
MFGM and infant oral microbiota

2019 **Li et al.**[217]
MFGM in infant formula supports normal growth and tolerance

2019 **Li et al.**[164]
MFGM and Lactoferrin support cognitive development in infants

2019 **Norris et al.**[229]
Safety of maternal supplementation with GG-enriched complex lipids during pregnancy

2019 **Nieto-Ruiz et al.**[169]
Long term follow-up after feeding infant formula with multiple functional ingredients, incl MFGM

2019 **He et al.**[216]
Metabolome analyses of MFGM intervention trial in infants

Clinical evidence on **long-term** cognitive benefits to 5.5 - 6 year olds

2021 **Chichlowski et al.**[213]
Gut microbiota and metabolome of infants fed formula with MFGM and lactoferrin

2021 **Xia et al.**[157]
GG-enriched complex lipid ingredient in formula and developmental milestones

2022 **Lazarte et al. [abstract]**[168]
13 years follow-up after feeding infants with complementary foods supplemented with MFGM

2022 **Nieto-Ruiz et al.**[173]
Follow-up of children at 6 years old, who were fed with a combination of functional components including MFGM and DHA during infancy

2023 **Deoni et al. [abstract]**[154]
MFGM intake associated with enhanced brain myelination and cognitive function

2023 **Colombo et al.**[165]
5.5 years follow-up after feeding infant formula with MFGM+LF

BENEFITS TO BRAIN DEVELOPMENT AND COGNITIVE FUNCTION

Metabolic activity is highest in infancy and early childhood, reflecting the energy demands associated with rapid growth and development during this time period, although the brain still remains highly metabolically active with remodeling that continues well into adult life.[118] Important insights can be derived from basic research into the neural effects of functional nutrients not only during early development but also at different stages throughout life. Numerous preclinical and clinical studies have elucidated some of the roles for MFGM and MFGM-derived components in the development of brain structure, learning, memory, and other cognitive functions.

PRECLINICAL DATA

Components of MFGM

Sphingomyelin

Loss of myelination results in defects of nerve impulse conduction, as seen in human multiple sclerosis and in animal models of neural disease.[60] In developing rats with impaired sphingomyelin synthesis, dietary SM was shown to restore myelination of the central nervous system, as measured by brain weight, myelin weight and thickness, and axon diameter.[61]

Gangliosides

Double knockout mice that lack brain gangliosides have shown severe disruptions in axon myelination, demonstrating that gangliosides are critical to axon stability and function.[119,120] Other mice deficient in specific gangliosides demonstrated impaired stability of paranodal junctions in myelinated nerve fibers and a slowing of nerve conduction.[121] Furthermore, in vitro and in vivo studies in different animal models suggest parenteral administration of gangliosides (by subcutaneous, intraperitoneal, and intraventricular injections) supports learning and memory.[122-126] In other studies, ganglioside administration to rats with aging or compromised brain function appeared to alleviate genetic and lesion-induced memory deficits and improve spatial learning and memory,[127,128] although lack of benefit has also been seen in this model as well.[129]

Phosphatidylcholine and Choline

Studies in pregnant rodents have shown PC treatments support learning and memory in offspring.[130] As noted, hydrolysis of membrane PC can also generate free choline, although the rates of such conversion are not easily defined.[70] In a mouse model, experimental inhibition of choline uptake and metabolism in embryos was associated with neural tube defects.[131] In rats, choline treatments improved memory and learning,[132] and the most positive effects of oral choline on brain function occur during the periods of peak neurogenesis and synaptogenesis, which when extrapolated to humans would correspond to a period beginning in utero and continuing to 4 years of age.[133]

Phosphatidylserine

There is some evidence from adult animal models that suggests PS may attenuate some of the neural effects of aging. In rats, long-term PS treatment (oral or intraperitoneal) was found to diminish some of the neural changes usually seen in aging rodents, such as loss of dendritic spines and decrease in neurotransmitter release.[134,135] Similarly, chronic oral PS treatment also improved spatial memory and passive avoidance retention in aged rats with age-associated cognitive dysfunction.[136]

Sialic Acid

A study using an in vivo model demonstrated that adding sialic acid to the diet of piglets improved learning and memory.[137] This finding also coincided with a dose-related increase in the amount of sialylated glycoproteins in the frontal cortex. In another model, malnourished rat pups injected intraperitoneally for 7 days with sialic acid were found to have increased brain ganglioside and glycoprotein sialic acid concentrations and a decrease in behavioral abnormalities.[123] When later tested in a Y maze as adults, the sialic acid-treated rats learned the maze more quickly than control rats.

Combination of MFGM-derived Components

Several preclinical studies have been conducted using MFGM and combinations of MFGM-derived components. In a study by Brink et al. (2019), growth-restricted rat pups were randomized to receive 1 of 5 treatments from postnatal day 2 to postnatal day 21. Treatments included bovine whey-derived MFGM ingredient (in the form of MFGM-10, Arla Foods Ingredients, Denmark), bovine phospholipid and ganglioside concentrate (PL-20), sialic acid at 200 mg/kg body weight, sialic acid at 2 mg/kg body weight, or nonfat milk as a control. The rat pups underwent several behavioral tests in adulthood, including T-maze Spontaneous Alternation, Novel Object Recognition, and Morris Water Maze. The group that received MFGM exhibited higher T-maze scores compared with the group receiving 2 mg/kg body weight sialic acid (P=0.01), with authors theorizing that MFGM compared to its individual components may have a larger impact on neurodevelopment in rat pups.[138]

In contrast, Liu et al. (2014)[139] investigated neonatal piglets fed formulas containing 0% (control), 0.8%, or 2.5% of PL-20, a supplement that provided milk phospholipids and gangliosides from postnatal day 2 to postnatal day 28. Piglets that were fed 0.8% and 2.5% PL-20 made choices more rapidly and with fewer errors in a spatial T-maze cognitive test compared to controls, implying improved spatial learning. In piglets fed 0.8% or 2.5% PL-20, mean brain weights were about 10% higher than control. Additionally, multiple brain areas had more gray and white matter than control piglets. This suggests that the additional phospholipids and gangliosides were incorporated into the developing brain, promoting myelination and growth.

Similarly, Vickers et al. (2009) demonstrated that administration of complex milk lipids to rats from postnatal day 10 through adulthood (day 80) led to significant improvements in learning and memory tasks compared with control animals.[140]

Since myelination and white matter integrity are often altered in obesity, Arnoldussen et al. (2021) investigated the effect of a 3% whey protein lipid concentrate high in MFGM components (MFGM-10) in obesity-induced mice fed a high-fat diet over 24 weeks. The study found the 3% MFGM-10 group experienced a decrease in high-fat, diet-induced neuroinflammation and an attenuation in the reduction of hippocampal-dependent spatial memory and hippocampal-functional connectivity.[141] Conversely, a another study demonstrated that maternal diets of complex milk lipids during pregnancy did not alter the long-term behavioral function of the offspring.[142]

Clinical Pearl

Multiple MFGM components may be more impactful on neurodevelopmental outcomes than individual components of MFGM.

Several studies have investigated potential mechanisms of action related to changes in the brain lipidome that may help explain the role of MFGM in neurocognitive development.

Fraser et al. (2022) investigated the role of a dietary MFGM on lipid profiles in different neonatal brain regions in 10-day-old male piglets. Piglets were fed infant formula with no, low (4%), or high (8%) levels of SureStart™ MFGM Lipid 100 (NZMP, Fonterra) daily for 21 days. Upon examination, piglets fed high-MFGM formula had altered lipid abundance in the hippocampus, indicating MFGM may alter the lipidome of some brain regions.[143]

In addition, Davies et al. (2022) found rodents fed diets with bovine MFGM (MFGM-10) had altered plasma phospholipid composition. Predominantly, there were increased SM levels in systemic circulation with some similar, but nonsignificant, trends in central regions of the brain.[144]

Similarly, Moukarzel and colleagues (2018) found that adding bovine MFGM (MFGM-10) to formula reduced differences in brain phospholipid and fatty acid composition between mother-reared and formula-fed rat pups.[145] Additionally, a study found that male pigs fed a milk-replacement *ad libitum* with MFGM whey protein concentrate (MFGM-10) experienced increases in serum lipoprotein levels with similar patterns to those found in breastfed infants.[146]

In addition to brain lipid alterations, researchers have evaluated the impact of MFGM on expression of genes involved in brain function. Results showed MFGM-10 increased mRNA expression of genes involved in brain function and long-term cognition in both normal- and restricted-growth rat pups. This included upregulation of brain-derived neurotrophic factor, glutamate receptor-1, glucagon-like peptide 1 receptor, and St8 alpha-N-acetyl-neuraminide alpha-2,8-sialytransferase 4. Authors suggested the upregulation of these genes supported the differences found in behavioral tests performed (T-maze and passive avoidance).[147]

Several studies have investigated the effect of MFGM in combination with other nutrients on brain development and cognitive function. For example, Mudd et al. (2016) evaluated the impact of the combination of prebiotics, bovine MFGM (MFGM-10), and lactoferrin on neurodevelopment in piglets. They observed differences in microstructure maturation of the internal capsule and cortical tissue concentrations. This finding suggests that piglets who received the experimental formula were more advanced developmentally compared with control piglets.[148] A similar study in rats that utilized this combination of nutrients (in differing amounts of each ingredient) found a significant increase in total dendritic spine density in hippocampal dentate gyrus neurons compared with the control group.[149]

CLINICAL DATA

The suitability and efficacy of formula with added MFGM, complex milk lipids, or MFGM components in infants and children has been investigated in several randomized, controlled trials (refer to section VI: Suitability and Tolerance). In line with preclinical findings, most of these studies have demonstrated evidence for clinically relevant benefits in terms of cognition, behavior, immunity, and gut health.

Many studies have aimed to address measures of cognitive development when MFGM components, including gangliosides and SM, have been added to the diets of pediatric populations. These trials have been conducted in both infants and children of different age groups and are summarized in Table 2.

Components of MFGM

Sialic Acid

Sialic acid has been suggested as a potential factor in breast milk supporting optimal brain development and function, although there are limited human data.[87] Breastfed infants have significantly higher levels of salivary sialic acid than formula-fed infants.[150] Both breastfed and formula-fed infants have significant amounts of gangliosides in brain tissue; however, protein-bound sialic acid was found to be 22% higher in the frontal cortex gray matter of breastfed infants compared with formula-fed infants,[106] echoing similar findings in an animal study.[137]

Phosphatidylserine

Phosphatidylserine (PS) has been clinically evaluated for neurocognitive benefits. In a small, randomized controlled trial of children ages 4-14 years with attention deficit-hyperactivity disorder (ADHD), diets with PS (200 mg per day) for 2 months led to improvement of symptoms of ADHD, as well as short-term auditory memory.[151] In an adult study of cortical activity after mental stress, diets with PS for 6 weeks led to a more relaxed state compared with subjects on a control diet.[152]

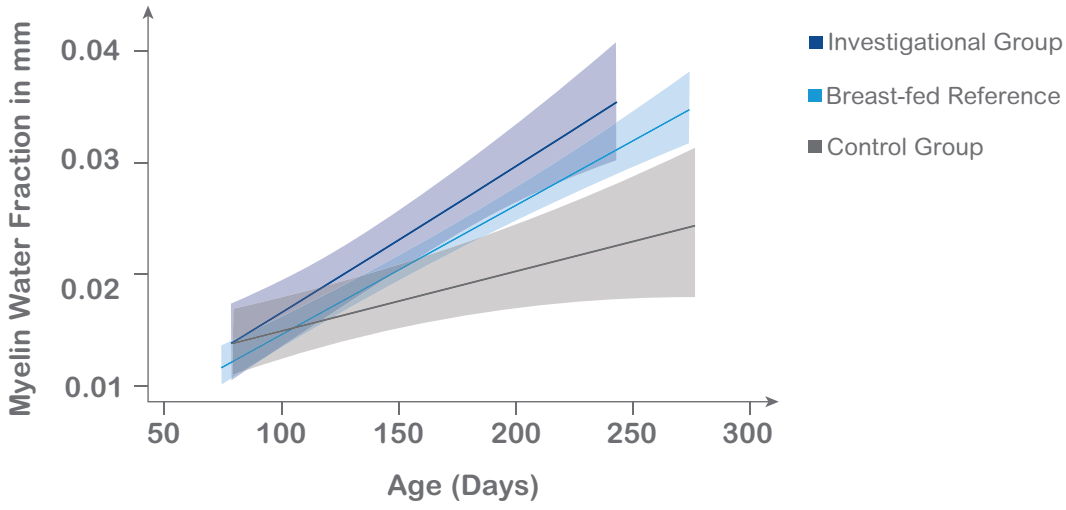
Combination of MFGM-derived Components

Studies Reporting Impact on Brain Myelination

Deoni et al., 2018[252]
This longitudinal study examined brain and neurocognitive development in children who were exclusively breastfed (n=62) or exclusively formula fed (n=88) for 3 months or longer. The formula-fed group was further divided into 3 groups based on formula composition; composition varied in proportional amounts of long-chain fatty acids (docosahexaenoic and arachidonic acid), iron, choline, phosphatidylcholine, sphingomyelin, and folic acid. To obtain longitudinal measures of development, participants had MRI scans performed at 6-month increments from time of recruitment until 2 years of age, and yearly thereafter. The level of myelination was evaluated in 8 brain regions: the frontal, temporal, occipital, parietal, and cerebella white matter as well as the body, genu, and splenium of the corpus callosum. Formula composition was associated with significant developmental differences. This study reported that sphingomyelin and phosphatidylcholine in infant formula positively correlated with myelination throughout the brain.

Schneider et al., 2022[153]
This multicenter, double-blind, randomized controlled trial evaluated the impact of an infant formula with multinutrients on developmental myelination and cognitive and behavioral development in the first 6 months of life. The experimental formula included a blend of SM (from a uniquely processed WPC with alpha-lactalbumin and phospholipids), DHA, ARA, iron, vitamin B12, and folic acid. The experimental formula (n=42) was compared with standard formula (n=39) or nonrandomized breast milk (n=108).

FIGURE 4. MFGM COMPONENTS ARE ASSOCIATED WITH IMPROVED BRAIN MYELINATION[153]
Infants in the investigational group fed formula supplemented with a myelin blend* had significantly higher myelin volume and myelin growth rate compared to the control group.



*The myelin blend includes DHA, ARA, sphingomyelin, phospholipids, iron, folic acid, and vitamin B12.

TABLE 2: CLINICAL STUDIES REPORTING THE ROLE OF MFGM COMPONENTS IN SUPPORTING BRAIN MYELINATION, COGNITIVE FUNCTION, AND SOCIAL EMOTIONAL FUNCTION

Clinical Study	Population	Intervention	Original, Secondary Analysis, or Follow-up	Main Findings
Deoni et al. (2018)[252]	Healthy, neurotypical children from 3 months to 9 years of age	Formula with different composition of long-chain fatty acids, iron, choline, sphingomyelin, phosphatidylcholine and folic acid	Longitudinal, secondary analysis	Sphingomyelin and phosphatidylcholine in infant formula positively correlated with myelination through the brain.
Schneider et al (2022)[153]	Full-term infants	Myelin blend containing long chain fatty acids, iron, folic acid, vitamin B12, and phospholipids including sphingomyelin	Original	Significant differences in myelin structure, volume, and rate of myelination were observed in favor of the test myelin blend at 3 and 6 months of life vs standard formula.
Deoni, D'Sa (2023)[154]	Healthy, full-term infants	Formula with MFGM	Original	Brain imaging results reveal significantly enhanced myelination rate and overall content in infants receiving formula with bMFGM compared to standard formula, particularly throughout motor-related regions.
Timby et al. (2014)[155]	Full-term infants	Formula with MFGM	Original	Feeding infants a formula with MFGM until 6 months of age improved their cognitive performance at 12 months relative to control infants and resulted in similar performance in breastfed infants.
Xia et al. (2021)[157]	Healthy term infants	Formula with MFGM	Original	At 12 months, composite social, emotional, and general adaptive behavior scores were significantly higher in the MFGM formula than standard formula. Short-term memory was significantly higher at 12 months, and serum gangliosides were significantly higher at 4 months in formula with MFGM vs standard formula.
Gurnida et al. (2012)[158]	Healthy term infants	Complex milk lipid	Original	Feeding infants a formula with MFGM-derived complex milk lipids until the age of 6 months resulted in motor skills and cognitive performance similar to breastfed infants and better than infants fed standard formula. Increased serum ganglioside levels in the MFGM group did not differ from the breastfed group.
Tanaka et al. (2013)[160]	Very low birth weight preterm infants	Dietary sphingomyelin	Original	Very low birth weight preterm infants showed improvements across multiple developmental measures when fed with formulas with phospholipids enriched for sphingomyelin over a period of 8 weeks compared with formulas with lower sphingomyelin content. Red blood cell sphingomyelin levels in the phospholipid formula group increased.
Veereman-Wauters et al. (2012)[162]	Healthy preschool children	MFGM concentrate	Original	Preschool children who consumed chocolate milk formula with MFGM for 4 months were rated to have better behavioral and emotional regulation by their parents compared with those fed standard formula.
Li et al. (2019)[164]	Healthy term infants	Formula with MFGM + lactoferrin	Original	Infants who received formula with MFGM and lactoferrin had an accelerated neurodevelopmental profile at day 365 and improved language subcategories at day 545. Formulas were associated with age-appropriate growth and significantly fewer diarrhea and respiratory-associated adverse events through 545 days of age.
Colombo et al. (2023)[165]	Healthy young children (age 5.5-6 years at follow-up)	Formula with MFGM + lactoferrin	Follow-up of Li et al. 2019[164]	Formula with MFGM and lactoferrin fed through 12 months of age supported improved neurodevelopmental outcomes between 5.5-6 years.
Nieto-Ruiz et al. (2019)[169]	Healthy term infants	Combination of MFGM, synbiotics, probiotics, LCPUFA, gangliosides, nucleotides, and sialic acid	Original (COGNIS trial)	No differences in growth and neurodevelopment found in infants taking formula with MFGM components, LCPUFAs, and synbiotics vs standard formula.
Nieto-Ruiz et al. (2020)[170]	Healthy term infants (age 4 years at follow-up)	Combination of MFGM, synbiotics, probiotics, LCPUFA, gangliosides, nucleotides, and sialic acid	Original (COGNIS trial)	Children fed formula with MFGM through 18 months experienced long-term benefits on language development at 4 years of age.
Nieto-Ruiz et al. (2020)[171]	Healthy term infants	Combination of MFGM, synbiotics, probiotics, LCPUFA, gangliosides, nucleotides, and sialic acid	Original (COGNIS trial)	No major behavioral differences between children who received the test formula and those who were breastfed at the 2.5-year evaluation.
Cerdo et al. (2022)[172]	Healthy term infants	Combination of MFGM, synbiotics, probiotics, LCPUFA, gangliosides, nucleotides, and sialic acid	Secondary analysis of Nieto-Ruiz et al. 2019[169] (COGNIS trial)	An exploratory study suggesting links between gut maturation and infant brain development.
Nieto-Ruiz et al. (2022)[173]	Healthy young children (age 6 years at follow-up)	Combination of MFGM, synbiotics, probiotics, LCPUFA, gangliosides, nucleotides, and sialic acid	Original (COGNIS trial)	Formula with MFGM components, LCPUFAs, and synbiotics seems to be associated with long-term effects on neurocognitive development and brain structure in children 6 years of age.

At 3 and 6 months, magnetic resonance imaging (MRI) results showed significant differences in myelin structure, volume, and rate of myelination in the experimental group compared with the standard formula group. Further, effects were shown at approximately 6 months of age for whole brain myelin and for cerebellar, parietal, occipital, and temporal regions, which are known to be involved in sensory, motor, and language skills, including functions such as inhibitory control over reflexive behaviors, target-directed head-eye coordination, reaching to grasp, and hand-to-hand transfer (Figure 4). No significant differences were found for early behavior and cognitive scores. It is worth noting that the absence of any measurable group differences in cognitive and behavioral outcomes this early in development is in line with other clinical evidence where there is consistently a latency seen between brain structural and behavioral benefits.

Clinical Pearl

In recent years, multiple clinical studies using MRI have shown MFGM and its components may impact myelination in infants and children.

Deoni, D'Sa, 2023[154]
Healthy, full-term infants were drawn from a longitudinal neurodevelopmental study with continuous enrollment since 2010. Matched cohorts of 28 and 23 infants were selected who had multiple MRI scans between 0 and 2 years of age and had exclusively received either standard or formula containing bovine MFGM (bMFGM+). Both formulas differed nutritionally only in bMFGM content. 3T MRI scanning was performed to quantify myelin development throughout the brain, and cognitive abilities were assessed using the Mullen Scales of Early Learning (MSEL). Following MRI processing and alignment, mean longitudinal trajectories of myelination were calculated for each infant group and compared throughout the brain using a series of general, nonlinear mixed-effects models. Development of verbal, non-verbal, and overall cognitive ability was also compared between groups using linear mixed-effects models. Brain imaging results revealed significantly enhanced myelination rate and overall content in infants receiving bMFGM+ formula compared to standard formula, particularly throughout motor-related regions (corpus callosum, cerebellum, and parietal cortex). Nonverbal cognitive MSEL scores were also significantly higher in the bMFGM+ group.

In summary, current clinical evidence suggests MFGM or its components may play a beneficial role in supporting healthy cognitive development, cognitive function, and behavioral regulation in infants and young children.

Studies Reporting Cognitive Function Outcomes Up to 18 Months of Age

Timby et al., 2014[155]
Timby and colleagues conducted a clinical study to determine the potential impact of added MFGM in formula on cognitive development in infants. In this randomized, double-blind trial, term infants (<2 months old) were assigned to consume either a standard formula (n=80) or an MFGM-added test formula (n=80) until 6 months of age. The test formula had a whey-derived MFGM ingredient (MFGM-10), providing 4% of total protein content as MFGM protein. A breastfed reference group (n=80) was also included. Cognitive assessment was done using the Bayley Scales of Infant and Toddler Development-III (BSID-III) at 12 months of age.

The MFGM-fed infants exhibited mean cognitive scores that were significantly higher compared with the control group (105.8 vs 101.8, $P<0.008$) but not significantly different from the breastfed reference group (Figure 5). In contrast, there were no significant differences in motor domain scores among the 3 groups, and both experimental and control formula groups scored lower than the reference group in the verbal domain.

The authors concluded that infant formula with MFGM-10 demonstrated a positive effect on cognitive function. The authors also noted that the cognitive effect was persistent and measurable 6 months after the end of formula feeding. Further, the 4-point difference in Bayley cognitive score is clinically relevant, being similar in size to the difference shown between breastfed and formula-fed infants in multiple studies.[156]

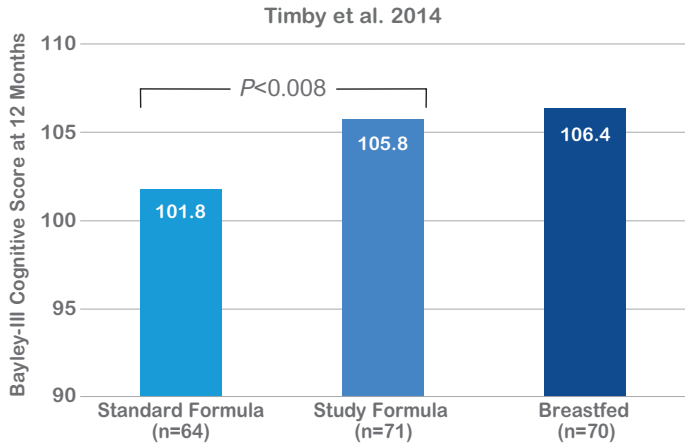
Xia et al., 2021[157]
A recent multicenter, double-blind, randomized controlled trial conducted in China evaluated the effect of formula that had a bovine-derived MFGM (SureStart™ MFGM Lipid 100) on neurodevelopment in healthy term infants (n=108) compared with infants receiving standard formula (n=104), as well as breastfed infants (n=206) from <14 days until 12 months of age. At 12 months, BSID-III was used to evaluate neurodevelopment.

Results showed the composite social emotional and general adaptive behavior scores at 12 months were significantly higher in the MFGM-formula group compared with the standard formula group ($P=0.048$ and 0.004, respectively). Moreover, short-term memory in infants receiving the formula with MFGM was 6.86 points higher than the standard formula group at 12 months ($P=0.008$), although significance was not demonstrated at the 6-month evaluation. Serum total gangliosides at 4 months of age were also significantly higher in the MFGM-formula group compared with the standard formula group and were not different from breastfed infants.

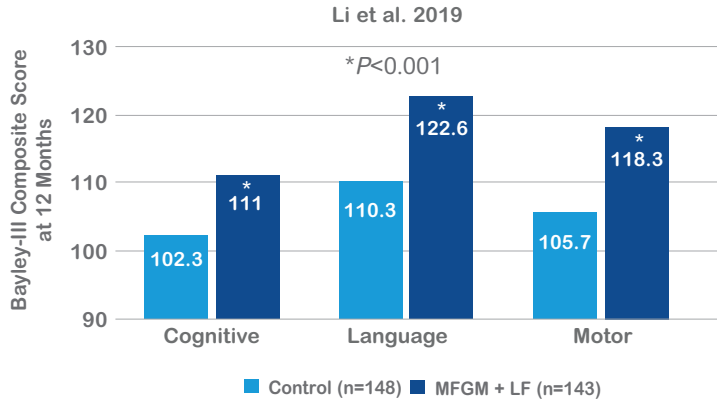
FIGURE 5: MFGM SUPPORTS COGNITIVE FUNCTION IN INFANTS[155,164]

Clinical tests have compared children fed formula with and without added MFGM during infancy. At 12 months, an MFGM group scored 4 points higher on Bayley-III cognitive tests than a standard formula group, similar to breastfed infants (5A). At 18 months, an MFGM group demonstrated accelerated development in 3 important domains: cognitive, language, and motor (5B). MFGM was also associated with language development through increased sentence complexity (5C).

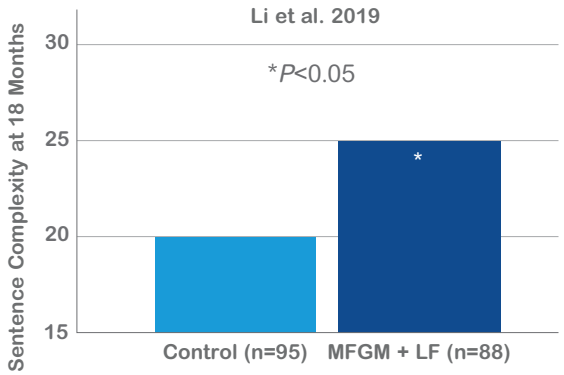
5A



5B



5C



These results add to the body of evidence on the suitability of MFGM in infancy and showed that the addition of this cream-derived MFGM ingredient supports increased serum ganglioside levels and improved social emotional, general adaptive, and short-term memory measures of neurodevelopment as evaluated by the BSID-III assessment.

Gurnida et al., 2012[158]
Another study addressed the cognitive effects of formula that had a ganglioside-enhanced, MFGM-derived complex milk lipid in term infants. In this randomized, double-blind, controlled trial, healthy infants (2-8 weeks of age) were assigned to receive either standard infant formula that had 6 mg gangliosides/100 g (control; n=30) or a test infant formula with added complex milk lipids to increase ganglioside concentration to 9 mg/100 g (test; n=29) until 6 months of age. The level of gangliosides (measured as GD3 only) in the test infant formula was approximately 11-12 µg/mL. This level is within the range of total ganglioside levels in human milk (3.4-16.2 µg/mL).[75,76,78] A breastfed reference group (n=32) was also included.

Results showed serum ganglioside levels in the test group (measured as GM3 [monosialodihexosylganglioside], GD3, and total gangliosides) were significantly higher than controls at 6 months but did not differ significantly from levels in the breastfed group.

Cognitive outcomes were measured at 6 months of age using the Griffiths Mental Development Scale, a validated screening tool that provides assessment across 5 domains, including locomotor, personal-social, hearing and speech, hand and eye coordination, and performance.[159] The test group had significantly increased scores for hand and eye coordination, performance, and total score (general intelligence quotient [IQ]) at 6 months compared with the control group. However, there were no significant differences in cognitive performance compared with the breastfed reference group.[158]

The authors concluded that a ganglioside ingredient that permits more closely matching the intake of breastfed infants may provide some advantages in cognitive development, particularly those aspects related to motor skills. However, the positive effects cannot be solely attributed to gangliosides since the ingredient used in this trial contained other polar lipids as well.

Tanaka et al., 2013[160]
The neurobehavioral effects of feeding formula with SM-enriched phospholipids were assessed in a trial of preterm infants. In this double-blind controlled trial, very low birthweight (VLBW) preterm infants (birthweight <1500 g) were randomized either to a control group (n=12) or a test group (n=12). In the control group, preterm infants were fed formula that had phospholipids derived from egg yolk lecithin with SM at 13% of total phospholipid. In the test group, preterm infants were fed formula that had milk-derived phospholipids containing 20% SM.The total amount of phospholipid added was the same for both groups, and the SM contents were both lower than that reported for mature preterm milk (42.4±8%).[160,161]

Infants in the test group had significantly higher percentages of SM in total phospholipids after 4, 6, and 8 weeks of feeding compared with those in the control group.

Mental and psychomotor development was assessed using the Bayley Scales of Infant Development II (BSID-II), including the Behavior Rating Scale (BRS) at 6, 12, and 18 months of corrected age. Visual recognition memory was assessed with the Fagan test score (novelty preference rate). Although the 2 groups did not differ in Bayley Mental Development Index (MDI) and Psychomotor Development Index (PDI) scores, the test group had significantly higher BRS scores than the control group at 12 and 18 months, including orientation, emotional, motor quality, and overall scores. Additional significant differences in outcomes included higher scores on the Fagan test at 12 months and the sustained attention test at 18 months in the high-SM group compared to the control group.

This pilot study suggested a relationship between dietary SM content and neurobehavioral development in VLBW infants.

Studies Reporting Cognitive Function and Behavior Outcomes up to 14 Years of Age

Veereman-Wauters et al., 2012[162]
Potential effects of MFGM on behavioral outcomes have also been examined in young children. In another randomized, double-blind, controlled trial, healthy preschool children (2.5-6 years of age) consumed a fortified milk beverage for a period of 4 months. The beverage for the control group (n=97) had 60 mg/day of endogenous phospholipids, while the test group beverage (n=85) had a total of 500 mg/day of dairy-derived phospholipids due to the addition of MFGM concentrate (provided as INPULSE, Bullinger SA, Belgium). At the end of the trial, a validated behavioral questionnaire (the Achenbach System of Empirically Based Assessment, ASEBA) was completed by parents and teachers. The ASEBA questionnaire is considered a gold standard for assessing emotion and behavior in preschool children;[163] it includes/encompasses internal problem (emotional, anxious/depressed, somatic, and withdrawn), external problem (attention and aggressiveness), and total problem scores that are standardized for gender and age.

The investigators observed significant differences in internal, external, and total behavioral problem scores in favor of the test formula group, as reported by parents (but not by teachers).

Li et al., 2019[164] *and Colombo et al., 2023*[165]
(Clinical Trial ID: NCT02274883)
A recent clinical trial focused on short- and long-term neurodevelopmental outcomes in infants who received formula that had MFGM and lactoferrin (LF) added as ingredients. In the initial trial, healthy term infants whose families chose exclusive formula feeding were randomized at 10-14 days of age to receive either routine bovine milk-based formula (n=228) or test formula (n=223) through 1 year of age.[164] The test formula consisted of routine bovine milk-based formula with bovine whey-derived MFGM (MFGM-10; 5 g/L) and bovine LF (0.6 g/L) added as ingredients. Neurodevelopmental outcomes were evaluated at 1 year and again at 1.5 years withthe BSID-III, Ages & Stages Questionnaire (ASQ), MacArthur-Bates Communicative Development Inventories (CDI), and Carey Toddler Temperament Scales (TTS).

Results showed cognitive, language, and motor scores were significantly higher in the MFGM + LF group than the control group at 1 year of age (Figure 5B,C). Few group differences in cognitive scores existed at 1.5 years of age. However, children in the MFGM + LF group had higher scores for some language domains at 1.5 years on the MacArthur-Bates CDI.

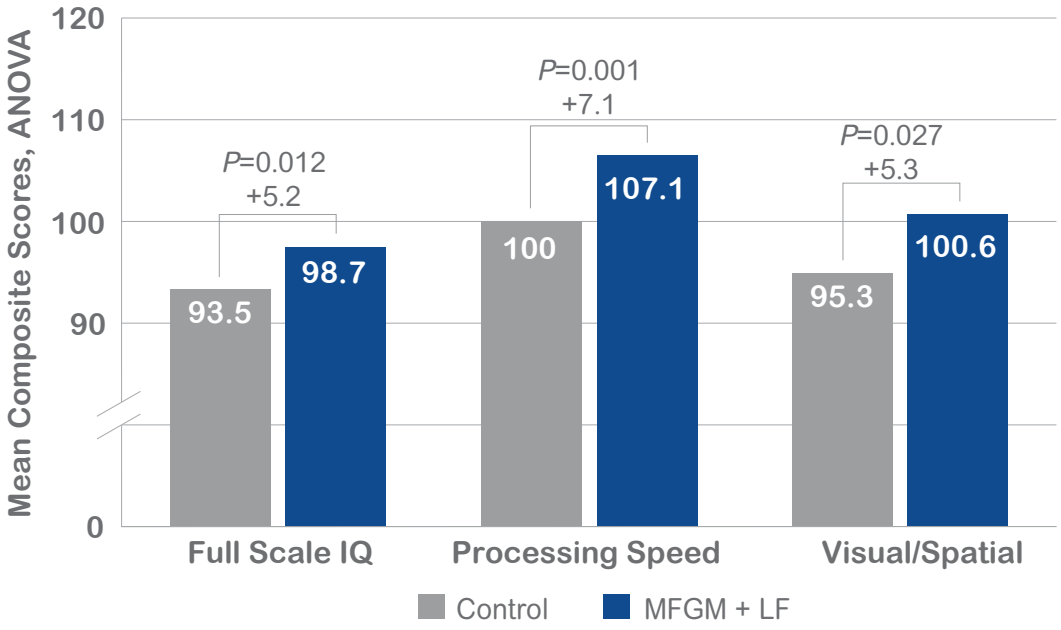
In a subsequent long-term, follow-up assessment, children from the trial were invited to follow up between 5.5 and 6 years of age for further assessment of neurodevelopmental outcomes.[165] Approximately 40% of eligible participants enrolled and completed follow-up assessments (control, n=59; MFGM + LF, n=57), although there were few socio-environmental characteristics between the follow-up groups. Outcomes included the Wechsler Preschool & Primary Scale of Intelligence (WPPSI-IV), Stroop Task, Dimensional Change Card Sort (DCCS) Task, and Child Behavior Checklist (CBCL).

Children in the MFGM + LF group had significantly higher composite scores for visual-spatial, processing speed and full-scale IQ from the WPPSI-IV with mean differences of approximately 5 to 7 points for all groups (Figure 6). For context, WPPSI-IV mean full-scale IQ scores have been shown to be lower by approximately 7 points in children with ADHD as compared with matched controls from a normative sample of US children.[166]

The MFGM + LF group also exhibited significantly higher achievement in measures of executive function, including higher scores on the Stroop Task and the border phase of the DCCS Task, and a significantly higher phase passed in the DCCS Task.

FIGURE 6. IMPROVED NEURODEVELOPMENTAL OUTCOMES AT 5.5-6 YEARS WITH MFGM AND LACTOFERRIN[165]

Follow-up, long-term study in healthy term infants who were fed formula with or without added MFGM and lactoferrin through 1 year of age and then evaluated at 5.5 years of age for further assessment of neurodevelopmental outcomes using the Wechsler Preschool & Primary Scale of Intelligence (WPPSI-IV).



Taken together, these studies demonstrated that an MFGM ingredient together with LF in infant formula supported improved neurodevelopmental outcomes in children that were not only apparent at 12 months of age (the end of the feeding intervention period) but also at 18 months of age and sustained through the preschool age of 6 years.

There is a significant growing body of evidence that has demonstrated beneficial effects of MFGM and its components on neurocognitive outcomes, whereas clinical evidence for lactoferrin on these outcomes is absent; thus, the effects seen in this study may be largely attributable to MFGM.[164]

Timby and colleagues (2021) also conducted a long-term, neurodevelopment follow-up, but that assessment did not show that children who consumed formula with added MFGM in infancy had significantly different neurodevelopmental outcomes at 6.5 years of age compared with a reference group fed standard infant formula.[167] Important to note are the differences in cognitive development evaluations used within the aforementioned studies, as well as the discrepancy in the duration of the interventions (specifically to 6 months of age vs 365 days of age in Colombo et al).[165,167] These differences in measures used and duration of interventions could help explain the contrariety seen in significant and nonsignificant findings between these studies, as only one of the same measures was used in both studies (CBCL). Moreover, the investigators also suggested that the optimal ages and methods for follow-up on neurodevelopment are subject to discussion.[167]

Lazarte et al, 2022[168]
Long-term effects were seen in a follow-up assessment involving adolescents who had received either whey protein concentrate with added MFGM or skim milk as infants, ages 6-11 months. The MFGM group demonstrated significant advantages in strategic working memory tasks at 14 years of age, even when covariates were appropriately controlled.

Nieto-Ruiz et al., 2019,[169] *Nieto-Ruiz, et al., 2020,*[170] *Nieto-Ruiz et al., 2020,*[171] *Cerdo T et al., 2022,*[172] *and Nieto-Ruiz et al., 2022*[173] *(Clinical Trial ID: NCT02094547)*
The COGNIS study (A Neurocognitive and Immunological Study of a New Formula for Healthy Infants) was a prospective, randomized, double-blind nutritional trial. This trial evaluated the effects of formulas for infants with or without multiple functional components on development through infancy and up to 6 years of life. Healthy term infants (n=170) ages 0-2 months were randomized to groups either receiving standard formula (n=85) or test formula (n=85) that had MFGM components (10% of total protein content), synbiotics (a mix of fructooligosaccharides and inulin at a 1:1 ratio), probiotics (Bifidobacterium infantis IM1 and Lactobacillus rhamnosus LCS-742), long-chain polyunsaturated fatty acids (LCPUFAs), gangliosides, nucleotides, and sialic acid during the first 18 months of life. A group of 50 infants who were exclusively breastfed for at least 2 months were included as a control group. At 2, 3, and 4 months of age, neurological development was evaluated using the general movements assessment, while visual function was assessed at 3 and 12 months of age through testing of cortical visual evoked potentials (cVEPs). Anthropometric evaluation was completed at all time points through 18 months of age.[169]

Results showed no significant differences in growth or neurological development between infants fed standard formula, experimental formula, or breast milk. Breastfed infants had significantly better visual function at 3 and 12 months of age; however, a secondary analysis showed no difference between breastfed and experimental formula-fed infants in response to minimum visual stimulus.[169]

A similar study measuring auditory event-related potential (ERP) showed that children at 24 months who received infant formula with added MFGM during the first year of life demonstrated differences in perception between familiar and unfamiliar auditory stimuli, as well as lower ERP amplitudes. This might indicate a higher degree of maturation of neural circuits and shorter latency for native unfamiliar stimuli, suggesting a potential for improved myelination.[174]

From the same study, a subsequent, long-term evaluation of children's behavior at 2.5 years found no major behavioral differences between children who received the test formula with added MFGM and those who were breastfed at the 2.5-year evaluation. Notably, some differences were observed between the group receiving the standard formula and the breastfed group at 2.5 years.[171]

Clinical Pearl
Recently, long-term follow-up studies reported that diets with MFGM during infancy leads to advantages in intelligence at 5.5-6 years of age.

The COGNIS trial also evaluated the effect of the experimental formula on language development in children at 4 years of age and found scores were similar to those of breastfed children, even when confounding variables were controlled.[170]

Investigators subsequently examined a potential relationship between gut microbiota maturation and neurodevelopmental outcomes within the same group of infants at 12 months and 4 years of age.[172] Through exploratory analysis of stool samples, microbial enterotypes were characterized as mixed (aerobic and anaerobic), Bacteroides dominant, Firmicutes enriched, or Lachnospiraceae dominant. Shifts in enterotype were associated with age progression, changing from mixed at baseline, to Lachnospiraceae-dominant, to Bacteroides-dominant or Firmicutes-enriched. At 12 months of age, infants fed the experimental formula who also experienced a rapid microbial enterotype trajectory had higher language and expressive scores than breastfed infants. Infants receiving experimental formula with slow trajectories had no difference in neurodevelopmental outcomes compared with breastfed infants. These exploratory findings set the stage for future research into the mechanistic roles of dietary components on gut microbiota maturation and the developing infant brain.

Lastly, 108 children from the original COGNIS study participated in a follow-up at 6 years of age to analyze long-term effects of the experimental formula on neurocognitive function and brain structure.[173] Structural MRI was used for the assessment of brain structure, while neurocognitive performance was evaluated with the Kaufman Brief Intelligence Test (K-BIT), the Oral Language Test of Navarra Revised (PLON-R), and the Computerized Battery for Neuropsychological Evaluation of Children (BENCI).

There were no statistically significant differences between the standard and experimental formula groups on the neurocognitive tests. Children who had received the experimental formula had higher IQ and higher vocabulary scores compared with children who had been breastfed, while the standard formula group did not differ from the experimental or breastfed group on these tests. The experimental formula group had greater brain volume measures, with the exception being the left orbital, and greater cortical thickness measures as compared with the standard formula group. Neither group differed from the breastfed group, with exceptions being the right parietal volume (the standard formula group had a lower volume than the breastfed and experimental formula groups) and the left inferior circular insular sulcus cortical thickness (the experimental group had greater thickness than breastfed and standard groups). Greater right parietal volume was associated with better verbal comprehension and working memory, and these measures further correlated with status and intake of certain polyunsaturated fatty acids during the first 18 months of life.[173]

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The results of the current study demonstrate an extension of the effects of formula with added MFGM and LF in infancy and early childhood ... and adds to a growing and consistent body of evidence that suggests the use of dietary MFGM components in infancy may confer durable benefits on cognitive development.”[165]

BENEFITS TO IMMUNITY AND GUT HEALTH

Like the central nervous system, other organ systems also exhibit rapid development and functional adaptation during infancy and early childhood. In particular, the gastrointestinal (GI) tract is essential to overall growth and the development of immunity and tolerance, as it provides both the absorption site for all nutrients and serves as a major interface for immune interaction with the environment.[36] As discussed later, numerous preclinical and clinical studies of MFGM and its components suggest beneficial roles in the maintenance of gut health and immunity via mechanisms that include immune modulation and direct antipathogenic activity.

PRECLINICAL DATA

Individual Components of MFGM

Sphingomyelin (SM)

Sphingolipids, including SM, are present in the apical membrane of the gut epithelium and involved in cell regulation.[62] They are also important for maintaining membrane structure and modulating growth factor receptors. Additionally, sphingolipids serve as binding sites or competitive binding inhibitors for microorganisms, microbial toxins,and viruses.[63]

In one study, oral SM was associated with more rapid intestinal maturation in rats.[175] In another rodent model, SM was associated with beneficial effects on colon tumorigenesis.[176] Evidence for the effects of SM on inflammation is conflicting, as SM has been found to both increase[177] and decrease[178] inflammation in different mouse models of colitis.

Gangliosides

Gangliosides are also present in intestinal mucosal cell membranes, although at lower concentrations than within the central nervous system.[87] In both in vitro and in rabbit intestinal loops, gangliosides have been shown to prevent the binding of enterotoxins to intestinal cells, which may contribute to protection against bacterial disease.[75] Other preclinical studies have shown that gangliosides contribute to improved gut microflora, gut immunity, and defense against infections.[93] Dietary treatments with GD3, a ganglioside species enriched in human colostrum and bovine MFGM, decreased the incidence and pathologic severity of necrotizing enterocolitis (NEC) in newborn rats, in part by modulating the mucosal immune response in favor of anti-inflammatory cytokines such as interleukin-10 (IL-10).[179]

Sialic Acid

In addition to being a structural component of gangliosides, in vitro studies suggest sialic acid may also influence the intestinal immune response by acting as a decoy receptor or competing with pathogens for receptor sites on intestinal epithelial cells.[180]

Phosphatidylcholine (PC)

Also a cell membrane component, PC represents more than 90% of the phospholipids in the intestinal mucus layer and, therefore, may contribute to intestinal defense against invasive pathogens. In vitro, PC protected against Clostridium difficile toxin-induced intestinal barrier injury[181] and was shown to attenuate neutrophil activation.[182]

Protein

There are several functional protein components of the MFGM, including the glycoproteins lactadherin, mucin (MUC-1), and butyrophilin, which have been shown in preclinical studies to affect immune response.[183] These components influence the immune system through several mechanisms, including interference with microbe adhesion to intestinal epithelia, bactericidal action, support of beneficial microbiota, and modulation of other parts of the immune system.[52]

Lactadherin is a cell adhesion molecule that interacts with integrins and is associated with the membrane through binding to phosphatidylserine. Depletion of lactadherin led to intestinal mucosal injury in mice, while in vitro administration of lactadherin promoted experimental wound healing of intestinal epithelial cells.[102] In another study, mice that were fed prophylactically with bovine whey glycoprotein fraction, containing lactadherins (LP14 and PAS6/7), did not develop diarrhea after exposure to rotavirus.[184]

MUC-1 is a rod-like glycoprotein (located on the surface of MFGM and many epithelial cells) that can act as a decoy for binding of infective agents. Bovine MUC-1 was found to inhibit rotavirus infectivity in human cell lines, including Caco-2 cells.[97,98] It also prevented the appearance of antiviral antibodies in the serum of mice after oral challenge with rotavirus.[97] MUC-1 mucin has also been shown to inhibit binding of S-fimbriated Escherichia coli to buccal epithelial cells. Both MUC-1 mucin and lactadherin are resistant to digestion in the infant stomach, a property that is likely relevant to their functional effects in supporting gut immunity.[185]

Although there is no known evidence for direct antimicrobial activity of butyrophilins, some data suggest proteins of this family may be costimulatory molecules that contribute to immune homeostasis. For example, butyrophilin can bind to and stimulate xanthine oxidase.[186] MFGM-derived xanthine oxidase was demonstrated to exert direct antibacterial effects against several pathogens in vitro due to the generation of hydrogen peroxide under specific conditions.[100]

Combination of MFGM-derived Components

Considering the functions demonstrated by its constituents, MFGM may be capable of modulating immune function in the gut through distinct but potentially complementary mechanisms. Glycosylated proteins (MUC-1, MUC-15, butyrophilin, and lactadherin) and glycosylated sphingolipids from MFGM may

promote the development of healthy infant gut microbiota by favoring beneficial Bifidobacterium species.[187] Several studies in rodents have shown that MFGM also promotes an intestinal flora more like that of mother's milk-fed animals.[188,189] Additionally, Berding and colleagues (2016) found an experimental formula with a combination of MFGM (MFGM-10), lactoferrin, and prebiotics in piglets was shown to modulate the composition of the gut microbiota, although the study did not differentiate between specific outcomes and individual functional components of the formula tested.[190] MFGM (MFGM-10) has also been shown in preclinical models to play a role in maintaining probiotic viability against physiological conditions in the GI tract.[191]

In addition to microbiome development, MFGM components have also been shown to impact the maturation of the intestinal mucosal barrier. This impact occurs through the promotion of intestinal proliferation and differentiation and increasing tight protein junctions,[188,189,192,193] as well as the inhibition of inflammation.[193-195]

One proposed mechanism for the role of MFGM in intestinal barrier function is its role in promoting the generation of short-chain, fatty acid-producing bacteria, which can directly facilitate intercellular tight junctions and build up the primary epithelial intestinal barrier.[196]

Huang et al. (2019) investigated the effect of MFGM on low birth weight (LBW) mice, which are commonly found to have impaired mucosal integrity and immunity. Researchers found the LBW mouse pups receiving MFGM experienced an increase in expression of tight junction proteins and a decrease in expression of proinflammatory cytokines and inhibition of inflammatory signals, such as the Toll-like receptor (TLR) 2 and TLR4.[193]

Similarly, Zhang et al. (2018) found MFGM (MFGM-10) inhibited the expression of TLR4 and subsequently reduced the incidence of NEC, increased the survival rate, and attenuated the severity of bowel damage in a NEC rat model.[195] MFGM (MFGM-10) also induced higher expression of tight junction proteins and MUC-1 and lower expression of NLRP3 inflammasome activation in a short bowel model in rats that underwent small-bowel resection.[194]

Another key to the immunomodulatory function of MFGM may be that its structure inhibits the binding of pathogens (eg, bacteria, viruses, and even toxins) to host cells largely due to the presence of glycoproteins and glycolipids.[197] Several preclinical studies have demonstrated inhibitory effects of MFGM against several pathogens. Both whole bovine MFGM and its extracted lipid components were found to exhibit dose-dependent inhibition of rotavirus infectivity in vitro.[198] In a similar in vitro model, Monaco et al. (2021) found whey protein concentrate (WPC) high in MFGM components (MFGM-10) was 1.5-4.8-fold more effective in reducing infectivity of two strains of rotavirus compared with a WPC control in both animal (MA104) and human (Caco-2) cell lines. These effects on virus infectivity may explain potential mechanisms of action that contribute to beneficial effects of MFGM within infant formula that may reduce rotavirus-associated diarrhea incidence in infants.[199]

Antibacterial effects of MFGM have included decreased gastric colonization and inflammation after Helicobacter pylori infection in mice;[107] inhibition of Shiga toxin gene expression by Escherichia coli O157:H7;[200] and decreased colonization and translocation of Listeria monocytogenes.[201] Formula with MFGM (MFGM-10) has also been shown to protect against C difficile toxin-induced inflammation in rat pups.[188] Moreover, a combination of MFGM (MFGM-10), lactoferrin, and prebiotics in formula fed to piglets was also associated with reduced proportions of opportunistic pathogens, including lower relative abundance of Mogibacterium, Collinsella, Klebsiella, Escherichia/Shigella, Eubacterium, and Roseburia in the ascending colon.[190]

Recent studies have also begun to explore the impact MFGM may have on the microbiota-gut-brain axis. Stressful events during the early postnatal period have been shown to have particularly detrimental effects on host development and physiology by leading to long-lasting changes in several systems, including the GI, endocrine, peripheral, and central nervous system. Studies have shown causal relationships between early-life stress, mood disorders, and gut dysfunction, which can be attributed at least partially to a dysfunctional communication between the gut and brain via the microbiota-gut-brain axis. Maternal separation (MS) is a well-established rat model of early-life stress and gut-brain axis dysfunction. In rats, MS has been shown to induce depressive and anxiety-like behaviors, increase gut epithelial barrier permeability and visceral sensitivity, and lead to stress hyper-responsivity.[202] Collins et al. (2022) investigated the effects of MFGM in rats from birth on various gut-brain signaling pathways in MS. They found that visceral hypersensitivity was improved to a greater extent by MFGM received from birth, which they suggested has potential clinical relevance, as visceral sensitivity is a hallmark of irritable bowel syndrome in humans.[202] A similar study by O'Mahony and colleagues (2020) also found MFGM (MFGM-10) attenuated MS-induced visceral hypersensitivity, with effects being even greater when MFGM was combined with a prebiotic blend.[203] Thompson et al. (2017) evaluated the effect of a combination of MFGM (MFGM-10), prebiotics, and lactoferrin on the physiological impacts of stress on sleep quality. They found that the combination of these ingredients enhanced sleep quality, which was related to changes in gut bacteria.[204] These results indicate MFGM may ameliorate some of the long-term effects of early-life stress, although more studies are needed in human models.

In summary, a significant body of preclinical experimental data have demonstrated that the MFGM, or components thereof, play roles in neural development and function, as well as support gastrointestinal immune defense and gut health.

“

… intervention with MFGM and prebiotic blend significantly impacted the composition of the microbiota as well as ameliorating some of the long-term effects of early-life stress.”[203] Although more studies are needed in human models.

CLINICAL DATA

A difference in the incidence of infectious outcomes, particularly gastroenteritis and acute otitis media, has been consistently reported between formula-fed and breastfed infants.[205,206] As with preclinical studies, clinical trials have also demonstrated that MFGM and many of its components are associated with beneficial effects on promoting immune and gut health. Possible mechanisms of action include modulation of the immune system, altering the composition or function of the GI tract microbiota, or a combination thereof, reflecting the complex interplay between the gut microbiota and the immune system.[207]

The levels of various MFGM components found in infant formula, such as SM, sialic acid, and gangliosides, can vary considerably from the average amounts found in breast milk.[57,78,150] Therefore, it is possible that formula with added MFGM and its derived components may result in a composition of infant formula closer in some respects to that of human milk. This could, in turn, potentially support outcomes to cognition, immunity, and gut health closer to those reported in breastfed infants.

Individual Components of MFGM

Gangliosides

The effect of formula with added gangliosides on gut microbiota was investigated in a randomized trial of preterm infants. Infants who were fed formula with added gangliosides at a concentration of 1.43 mg/100 kcal had significantly reduced mean counts of pathogenic E coli at 3 and 7 days after birth, and a lower percentage of fecal samples positive for E coli at 7 and 30 days compared with those fed control formula. Infants in the test group also had increased mean Bifidobacteria counts at the end of the 30-day trial compared with controls.[208] These findings suggest a potential role for gangliosides in supporting the development of beneficial gut flora. However, it should be noted that the ganglioside preparations used in this trial were porcine-derived and may not necessarily reflect the ganglioside profile present in human MFGM.[208]

Lactadherin

A cohort study evaluated the correlation between breast milk levels of the MFGM protein lactadherin and symptomatic rotavirus infection.[209] Increased concentrations of lactadherin in the breast milk of 200 mothers in Mexico City were associated with significantly reduced symptoms of rotavirus infection in their breastfed infants. This finding remained significant after adjusting for breast milk levels of secretory immunoglobulin A (sIgA), which is known to have antiretroviral activity (Table 3).[209] However, the dietary effect of lactadherin has not been independently evaluated in any clinical trial.

Combination of MFGM-derived Components

The previously described study by Timby and colleagues, which demonstrated improved measures of cognition in term infants receiving formula with added MFGM,[155] was also analyzed for disease symptoms and medication use in the first year of life.[46] In particular, the cumulative incidence of acute otitis media was analyzed between the 2 randomized feeding groups (standard formula or test formula with added MFGM-10 to 6 months of age) and compared with a breastfed reference group.

The test group experienced a significant reduction in episodes of acute otitis media up to 6 months of age compared with infants fed standard formula (1% vs 9%, P=0.034), with no difference in otitis media incidence compared with the breastfed group (0%, P=1.0). In addition, a significantly lower incidence and longitudinal prevalence of antipyretic drug use was seen in the test group (25%) compared with the standard formula group (43%) (Table 3, Figure 7). No differences were seen for other antibiotic-treated bacterial infections. Differences in serum anti-pneumococcal immunoglobulin G (IgG) concentrations were also found, leading the authors to speculate that MFGM may reduce the risk of otitis media through modulatory effects on the humoral immune system. Additionally, a more recent secondary data analysis by Timby and colleagues (2017) examined infant oral microbiota and reported Moraxella catarrhalis (a genus of bacteria associated with otitis media) was less prevalent in infants fed formula with MFGM compared with infants fed standard formula.[210]

Another clinical trial evaluated the effects of complementary food with MFGM on health outcomes in infants older than 6 months.[211] This randomized, double-blind controlled trial conducted in Peru enrolled 499 primarily breastfed term infants at 6-11 months of age. Infants were assigned to receive a daily milk-based, complementary food that included either WPC with MFGM (MFGM-10, with an average daily intake of 5.9 g) or an equal amount of additional protein from skim milk (control) for 6 months.

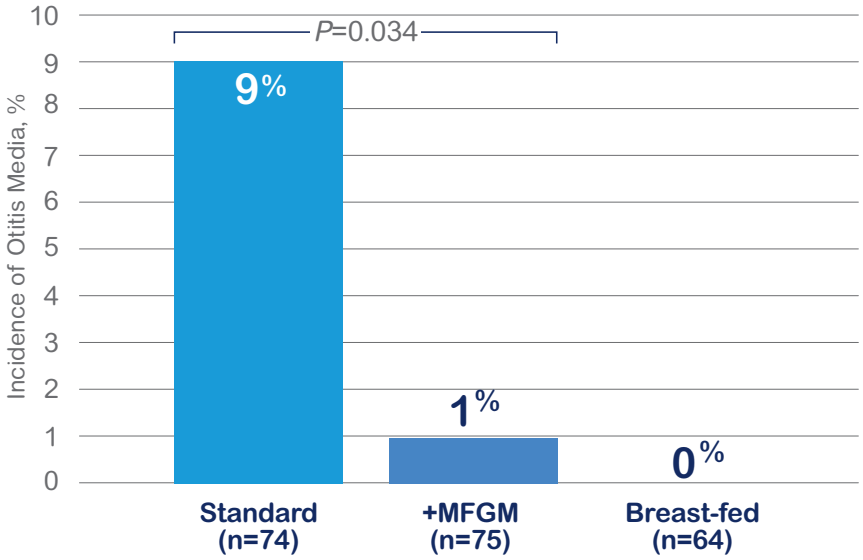
Results showed that the group with the MFGM diet had a significantly lower prevalence of diarrhea during the study compared with the control group (means 3.84% vs 4.37%, P<0.05), as well as a significant reduction (46%) in episodes of bloody diarrhea compared with controls (P=0.025). The most common pathogen isolated during the diarrhea episodes was E coli (45% of pathogens) (Table 3).[211] The authors concluded that the addition of an MFGM protein fraction to complementary food could have beneficial effects on severe diarrhea in infants, especially in vulnerable populations.

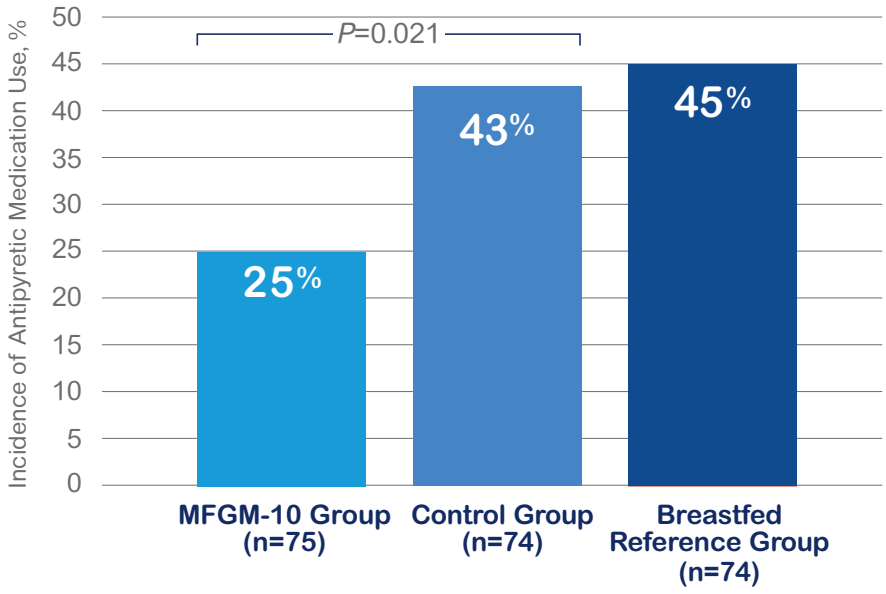
A third clinical trial in healthy term infants fed formula with or without added bovine MFGM (MFGM-10; 5 g/L) and bovine LF (0.6 g/L) through 12 months assessed immune outcomes. This study demonstrated that the MFGM + LF formula group was associated with significantly reduced incidence of all respiratory events, upper respiratory infections, all gastrointestinal events, and diarrhea up to 18 months of age, compared to control.[164]

In addition, the study by Veereman-Wauters et al. in preschool-age children (2.5-6 years of age) also reported the effect of consuming formula with MFGM on health outcomes. In this randomized controlled trial, the group of children receiving the MFGM milk beverage reported a significant reduction in the number of days with fever, and particularly the number of short febrile episodes (<3 days), compared with the control group (Table 3).[162]

FIGURE 7. MFGM IMMUNE HEALTH OUTCOMES[46]

Timby et. al (2015) compared healthy term infants fed formula with MFGM, infants fed standard formula, and a breastfed infant reference group. Formula with MFGM was associated with reduced risk for otitis media (7A) and reduced use of antipyretic medications (7B).





It is well known that the infant gut microbiota plays an important role in long-term health, and nutrition provided in early life is a strong determinant of the microbiome.

Zhao and colleagues (2022) investigated the effect of MFGM on stool microbiome and found several key components of MFGM, including lactadherin, sialic acid, and phospholipid, were positively correlated with Bifidobacterium within the stool samples, a genus of bacteria reported to play an important role in maintaining a healthy gut environment in infants. Interestingly, they also found an in vitro analysis of MFGM stimulated the growth rate of Bifidobacterium.[212]

In a secondary data analysis of the trial described in Li et al. (2019),[164] Chichlowski et al. (2021) also found subtle differences in the stool microbiome and metabolome in infants 4 months of age, in which a combination formula that included MFGM and lactoferrin increased the abundance of the Bacteroides species. This species has been found in higher amounts in breastfed infants and is reported to be a potential marker of relative eubiosis.[213]

Contrastingly, in exploratory analyses of the trial described in Timby et al. (2014),[155] Lee and colleagues (2021) did not find MFGM to induce significant compositional changes in fecal microbiota, although they did note similarities to breastfed infants regarding serum metabolome.[214] Similarly, He et al. (2019) observed only moderate differences between fecal microbiota of MFGM formula-fed vs standard formula-fed infants but did report an influence of MFGM on the fecal metabolome.[215]

In a subsequent exploratory evaluation of serum metabolites, He and colleagues (2019) found differences between breastfed and formula-fed infants but no significant differences between infants fed formula with or without MFGM, except for higher-end products of fat metabolism in the MFGM group.[216] In addition to metabolic comparisons, serum cytokine patterns have also been evaluated in infants receiving formula with MFGM. In a secondary data analysis of the trial described by Li et al. (2019),[217] Li et al. (2021) found that the cytokine profile of MFGM-fed infants approached that of breastfed infants.[218]

Overall, emerging evidence points to potential, although perhaps subtle, effects of MFGM on the gut microbiota and metabolome.

Key takeaways

- As our understanding of the role of MFGM components has improved, so too have our advances in purifying and concentrating MFGM from bovine milk to enable its use in formulas for infants.
- Numerous preclinical studies have demonstrated the roles of MFGM and its components (eg, sphingomyelin, phosphatidylcholine and other phospholipids, gangliosides, sialic acid, membrane proteins)—both alone and in combination—in the development of brain structure, memory, and other cognitive functions and in the maintenance of gut health and immunity.
- Consistent with preclinical data, clinical research strongly suggests MFGM and its components exhibit clinically relevant benefits in cognition development, while also establishing a consistent suitability/tolerance profile. Further, longer-term follow-up studies have begun to support the persistence of cognitive effects later in childhood from MFGM consumption during infancy, while emerging clinical data also support its role in brain myelination. The clinical evidence also strongly supports a role for MFGM and its components in immunity, which may be mediated through several pathways within the gut.
- MFGM and its components are being studied in other populations, including maternal consumption, but evidence to date is limited.

TABLE 3: CLINICAL STUDIES REPORTING BENEFITS OF MFGM COMPONENTS ON IMMUNE, GUT HEALTH, AND OTHER HEALTH OUTCOMES

Clinical Study	Population	Intervention	Original or secondary analysis	Main Findings
Timby et al. (2015)[46]	Full-term infants	Formula with MFGM	Secondary analysis of Timby et al. 2014[155]	Feeding infants a formula with MFGM until 6 months of age reduced the incidence of acute otitis media, from inclusion until 6 months of age, relative to control infants but resulted in similar incidence in breastfed infants. It also decreased the incidence and longitudinal prevalence of antipyretic use compared with control infants during the intervention period.
Timby et al. (2017)[210]	Healthy term infants	Formula with MFGM	Secondary analysis of Timby et al. 2014[155]	Formula with MFGM yielded moderate effects on oral microbiome; Moraxella catarrhalis was less prevalent in infants fed MFGM formula than in those fed standard formula and may be associated with a decrease in otitis media.
Zavaleta et al. (2011)[211]	Healthy infants and young children	MFGM protein fraction	Original	Daily intake of complementary food containing MFGM as the protein source, for 6 months, reduced the prevalence of diarrhea and bloody diarrhea compared with food containing skim milk protein.
Veereman-Wauters et al. (2012)[162]	Healthy preschool children	Chocolate milk formula with MFGM	Original	Young children fed a chocolate milk formula with MFGM, for a period of 4 months, had reduced febrile episodes compared with those fed the standard formula.
Zhao et al. (2022)[212]	Healthy term infants	Formula with MFGM	Original	Key components of MFGM in the diet were positively associated with stool Bifidobacterium.
Chichlowski et al. (2021)[213]	Healthy term infants	Formula with MFGM + LF	Secondary analysis of Li et al. 2019[164]	Infant formula with bovine MFGM and lactoferrin was associated with slight differences in stool microbiome and metabolome in infants at 4 months of age, including increased prevalence of Bacteroides species.
Lee et al. (2021)[214]	Healthy term infants	Formula with MFGM	Secondary analysis of Li et al. 2019[217]	Formula with MFGM decreased some gaps in metabolism between formula-fed and breast feed infants.
He et al. (2019)[215]	Healthy term infants	Formula with MFGM	Secondary analysis of Timby et al. 2014[155]	Fecal metabolome of infants fed a formula with MFGM showed significant reduction of several metabolites, including lactate, succinate, and amino acids and their derivatives compared to infants fed a standard formula.
He et al. (2019)[216]	Healthy term infants	Formula with MFGM	Secondary analysis of Timby et al. 2014[155]	Infants consuming formula with MFGM had higher levels of fat metabolism end products compared with infants receiving standard formula.
Li et al. (2021)[218]	Healthy term infants	Formula with MFGM	Secondary analysis of Li et al. 2019[217]	The cytokine profile of the MFGM group was similar to breastfed infants.
Rueda et al. (1998)[208]	Healthy preterm infants	Formula with gangliosides	Original	A formula with gangliosides resulted in reduced fecal E coli counts and increased fecal counts of Bifidobacteria throughout 30 days of postnatal age.

VI.

SUITABILITY AND TOLERANCE

As a component naturally present in human and bovine milk, MFGM has a long history of safe use in infants and children. Studies designed to assess growth and tolerance have continued to show infant formulas with MFGM are suitable and well-tolerated to 12 and 24 months,[47,155,217,221-223] with the longest-term follow-ups to date now extending to 6.5 years of age.[165,167,224]

In multiple trials, infants fed experimental MFGM-containing formulas to 4, 6, or 12 months of age reported no safety or tolerance concerns, including fussiness, gassiness, and stool consistency.[46,47,164,218,220-222]

However, there have been contrasting reports in the literature regarding the incidence or prevalence of rash with formulas with added MFGM. In one clinical study of infants receiving formula with added bMFGM, Billeaued et al reported that eczema incidence was low but increased in the group receiving added MFGM in formula.[47] However, Hedrick et al reported that there were no significant group differences in eczema incidence or the overall incidence of adverse reactions within the skin system between infants receiving formula with bovine MFGM or standard formula.[221] Additionally, two separate studies using the same source of bovine MFGM (MFGM-10) in formula demonstrated no association between bMFGM and increased risk of eczema through 6 or 18 months of age.[155,164]

Clinical studies have also demonstrated the suitability and tolerance of formulas that have MFGM-derived complex milk lipids[158] or SM in infants.[160] Similarly, suitability and tolerance of complementary foods with MFGM have been demonstrated in infants through 12-17 months of age,[211] with follow-up to 14 years of age,[168] as well as in foods for children[162] and as a supplement for adults.[225]

A large, double-blind, randomized trial compared 4 micronutrient, 8 metabolic, and 3 inflammatory markers in children who received either human milk, standard formula, or formula with MFGM during the first year of life. Blood samples taken at baseline (≤120 days), 6 months, 1 year, and 2 years of age showed the MFGM group had higher iron and high-density lipoprotein cholesterol (HDL-C) levels than the standard formula group at 2 years of age. Markers of glucose metabolic health were similar between formula and breastfed groups after 180 days. In addition, micronutrient and cardiometabolic markers were generally similar through 2 years of age in infants who received either type of formula during infancy.[219]

This growing body of evidence suggests that added bMFGM in infant formula is well-tolerated and associated with adequate growth throughout the first year of life and supported normal iron status.

VII.

EMERGING SCIENCE ON MFGM IN MATERNAL POPULATIONS

Additional clinical studies have addressed the effect of dietary MFGM in populations other than infants. Given the rapid accretion of these complex lipids into neuronal tissues in utero, research has been conducted to investigate whether maternal ganglioside consumption may affect maternal ganglioside levels and subsequent pregnancy outcomes. Limited evidence suggests at leastsome ganglioside species are able to transfer across the placenta,[226] though most of these data exist in preclinical models.[142,227]

Albert and colleagues (2020) investigated the effects of daily consumption of no milk, standard milk, or milk with gangliosides in pregnant women, on maternal ganglioside levels and pregnancy outcomes. Results showed maternal consumption of milk with MFGM (as a source of gangliosides) was not associated with differences in maternal serum ganglioside levels, fetal cord blood ganglioside levels, or the majority of pregnancy and neonatal outcomes compared with the control group.[228]

Norris et al. (2021) evaluated the effect of maternal consumption during pregnancy of milk with complex milklipids (ie, gangliosides and phospholipids) from MFGM on fetal growth, compared with mothers receiving no milk or milk without MFGM. Consumption of milk with MFGM did not affect size at mid-pregnancy or fetal growth trajectories, but no adverse effects were seen, leading the authors to suggest diets with MFGM during pregnancy is safe and could potentially serve as a way to increase ganglioside and phospholipid supply early in life.[229]

VIII.

SUMMARY AND CONCLUSIONS

Optimal nutrition during the early years of life is essential to healthy growth and development. Past and ongoing research into human milk has expanded our understanding of its composition, function, and variability, as well as the nutritional needs of infants. A considerable number of studies have identified MFGM as an important lipid-protein structure present in all mammalian milk, and a critical source of sphingomyelin, phospholipids, glycolipids, glycoproteins, cholesterol, and carbohydrates that have important functional roles within the brain, immune system, gut, and elsewhere in the body.

An increasing body of evidence supports both suitability and efficacy of MFGM and its constituents. Preclinical studies have demonstrated effects of MFGM-derived functional components on brain structure and function, intestinal development, and immune defenses. Similarly, pediatric clinical trials have reported beneficial effects on cognitive and immune outcomes, with some studies showing meaningful, long-term effects on cognitive development.

In populations ranging from premature infants to preschool age children, diets with MFGM added have been associated with clinically relevant improvements in cognition and behavioral milestones as well as infection outcomes, including fever, diarrhea and otitis media. In addition, emerging clinical data supports the role of MFGM in brain myelination. Further, there is emerging evidence evaluating the effects of MFGM supplementation in maternal and pregnant populations.

IX.

APPENDIX: INFANT AND CHILD COGNITIVE DEVELOPMENTAL ASSESSMENTS

- Ages & Stages Questionnaire (ASQ).[232] Provides reliable and accurate developmental and social-emotional screening for children between birth and age 6 years.
- Auditory event-related potential (ERP). Measures of cortical brain activity related to auditory stimuli.
- Carey Toddler Temperament Scales (TTS).[233] Measures nine clinically important categories to describe childhood temperament: activity level, rhythmicity, adaptability, approach to novelty, emotional intensity, quality of mood, sensory sensitivity, distractibility, and persistence.
- Child Behavior Checklist (CBCL).[234] Brief parent-report measure used to detect behavioral and emotional problems in children and adolescents.
- Computerized Battery for Neuropsychological Evaluation of Children (BENCI).[235] A computerized tool that evaluates neuropsychological functions and neurodevelopmental domains in children, such as immediate and delayed memory, attention, visual motor coordination, verbal fluency and comprehension, processing speed, and executive functions.
- Dimensional Change Card Sort (DCCS) Task.[236] This test measures rule learning and flexibility in cognitive reasoning in three sequential phases. Preswitch phase (6 trials): the child sorts multidimensional stimuli (colored shapes) based on color. Postswitch phase (6 trials): sort same stimuli based on shape. Border phase (most complex phase; 12 trials): sort criterion is based on border presence (ie, border present=color, border absent=shape).
- General Movements Assessment.[237] In the early stages of life, this test is predictive of motor and cognitive development in term infants. It measures a series of gross movements of variable amplitude and speed involving all body parts; evaluation of such movements includes the quality of general movements exhibited by the infant using a video recording of the baby.
- Griffiths Mental Development Scale.[159] Assesses multiple aspects of cognitive development. There are five scales: locomotor, personal-social, hearing and speech, eye and hand coordination, and performance.A numeric score is produced from these, and a General IQ (total) is produced from the five scores.
- Kaufman Brief Intelligence Test (K-BIT).[238] Evaluates verbal and nonverbal intelligence through two subsets: vocabulary and matrices. K-BIT provides a general IQ based on the sum of scores obtained by the subsets.
- MacArthur-Bates Communicative Development Inventories (CDI).[239] Parent-reported instruments that capture important information about children's developing abilities in early language, including vocabulary comprehension, production, gestures, and grammar.
- Oral Language Test of Navarra Revised (PLON-R).[240] Standardized test that allows early detection or screening of oral language development in children ages between 3 and 6 years. It also provides a total score for language development.
- Stroop Task.[165,224] This test comes in two forms: Red/Yellow and Day/Night. It measures inhibitory control and rule learning. The child must inhibit intuitive, congruous responses to simple stimuli and provide an incongruous response (eg, apple=yellow, sun=night) on 16 trials. The number correct is the outcome variable.
- Sustained-attention Test.[241] The free play, sustained-attention test of Colombo measures attention during 4 minutes of free play.
- The Achenbach System of Empirically Based Assessment (ASEBA).[242] The ASEBA assesses competencies, strengths, adaptive functioning, and behavioral, emotional, and social problems.
- The Bayley Scales of Infant Development, currently in the 3rd edition (BSID-III).[243] Evaluates mental and psychomotor development of infants and is a standardized test tool that is widely used in clinical research.
- The Fagan Test of Infant Intelligence.[244] This test is used to determine novelty preference rate, where a higher novelty preference rate indicates better intellectual development. There is some evidence that intellectual development can be predicted from the speed of habituation and the novelty preference rate.
- Visual Evoked Potentials (VEPs). VEPs are used to measure the neurotransmission speed via the optic nerves. Latency (ms) of 16 Hz pattern simulation is measured to evaluate the level of myelination.
- Wechsler Intelligence Scale for Children, now in the 5th edition.[245] This test generates a Full Scale IQ, which represents a child's general intellectual ability. It also provides five primary index scores (ie, Verbal Comprehension Index, Visual Spatial Index, Fluid Reasoning Index, Working Memory Index, and Processing Speed Index) that represent a child's abilities in more discrete cognitive domains.
- Wechsler Preschool & Primary Scale of Intelligence (WPPSI-IV).[246] This is a standardized IQ test for ages 4 years to 6 years 11 months.

X.

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August 2023