Review

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Review

The Milk Fat Globule Membrane: from Neurocognitive Development to Infant Formula Supplementation

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Abstract: The milk fat globule membrane (MFGM) surrounds and protects the fat globules present in milk. The addition of MFGM to infant formula has gained recognition as a means to enhance its nutritional profile. MFGM is rich in bioactive components such as phospholipids, glycoproteins, and enzymes, which are believed to support various aspects of infant development. Studies suggest that MFGM supplementation in formula may contribute to improved neurocognitive development. The incorporation of MFGM in infant formulas is an area of ongoing research and development to provide optimal nutrition for infants who are not exclusively breastfed. The European Food Safety Authority (EFSA) and the Food and Agriculture Organization of the United Nations (FAO) provide guidelines for the essential composition of infant formula and the food-safety of milk and milk products. To elucidate the mechanisms by which MFGM contributes to neurocognitive development, the review highlights the metabolic pathways in the brain and brain gut-axis of the newborn and their correlation with MFGM.

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1. Introduction

Milk fat globule membrane (MFGM) is a tripartite phospholipid layer that surrounds lipid droplet and is secreted into milk by epithelial cells of the mammary gland. Many investigations have shown MFGM important nutritional value, from the first description on the protective role of human MFGM [1] to the comprehensive and pioneering review of the nutraceutical properties of bovine MFGM in 2005 [2], until the emerging evidence of a positive role of MFGM in the neurodevelopment, as recently reviewed in [3]. At the beginning in vitro assays highlighted the role of MFGM glycoproteins as specific bacterial and viral ligands, while a detergent-like lytic action on pathogens for free fatty acids and monoglycerides released from the hydrolysis of the lipid core were shown [2]. In the following years, many clinical trials have been performed to assess the MFGM properties, as reviewed [4,5]; among them, some clinical trials have reported the correlation between MFGM enriched formula and neurocognitive improvement. More recently, in a randomized control trial (RCT) [6] growth parameters and several biomarkers have been evaluated in infants who received an infant formula with added bovine MFGM, in comparison with standard cow's milk formula or human milk until 2 years of age, there were no changes, only iron and high-density lipoprotein cholesterol were higher in infants who received infant formula with the addition of bovine MFGM. Another more recent RCT has assessed neurodevelopmental results at 5.5 years of age in children previously assigned to cow milk-based infant formula or a similar formula enriched with bovine MFGM and lactoferrin [7]. This evaluation revealed enhanced cognitive outcomes across various domains,

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encompassing measures of intelligence and executive function. These benefical outcomes persist beyond the period of formula feeding, indicating that exposure to the formula's constituents during infancy might influence the brain's functionality. Another study [8], as a follow-up of the COGNIS study, supports the evidence that the long-term effects of early nutrition on glycemic variability in healthy children rely on nutrition during the first 1000 days of life, in this case the tested formula was enriched with multiple bioactive components, such as MFGM, arachidonic acid, docosahexaenoic acid, gangliosides, nucleotides, synbiotics and sialic acid. All these evidences support the extreme importance of introducing MFGM in commercial infant formula as a regular basis, for the development of future health outcomes in formula-fed infants.

2. MFGM Preparation for Their Addition into Infant Formula

Considering the role of MFGM for the infant health and development, it is important to focus on the preparation steps of MFGM and their addition into infant formula. From dairy industry perspective, it is not economical the preparation of MFGM that is usually performed at laboratory level, with centrifugation of whole milk and subsequent washing of the milk fat globule [9]. Raw bovine milk, collected from the farms, undergoes processing before commercialization; one of the principal treatments of raw milk is heating [10], consisting in pasteurization and commercial sterilization with ultra-high-temperature, in order to reduce or eliminate safety concerns for the consumer. The MFGM acts as a natural emulsifying agent, which protects fat globules against coalescence, agglomeration and enzymatic action [11], heating milk above 60 ◦C causes the denaturation of the MFGM proteins and their association with whey proteins [9,12]. Moreover heating induces the release of membrane phospholipids into the serum phase, the newly formed membrane was more disordered than the natural MFGM; in fact it has been shown [13] that MFGM protein composition was differently affected by pasteurization, with a higher thermo-sensitivity observed in human and caprine MFGM proteins than bovine MFGM proteins. Moreover industrially commercialized milk is generally homogenized, which results in a MFGM degradation and a homogeneous dispersion of the fat molecules in the milk. It is noteworthy that the composition of the commercial MFGM preparations is heterogeneous and may lead to different nutritional and nutraceutical properties between the available products for the addition into infant formula [14]. For commercial preparation of MFGM, the aqueous streams in the butter making process, buttermilk and butter serum represent two suitable sources of MFGM fragment-rich materials. During butter making process, the emulsion containing 25-46% fat is broken during mechanical destabilization of churning; partial disintegration of MFGM tripartite structure occurred and MFGM fragments are recovered in the serum fraction called the buttermilk. Other polar lipids are preserved in the aqueous phase of butter, called the butter serum, and can be recovered subsequent to butter melting for the production of anhydrous milk fat [15]. It has been suggested that partitioning of the MFGM fraction between butter and buttermilk is partly related to the globule size (normally ranging in size between 0.2 and 15 μm), butter serums are rich in sphingomyelin, as compared to buttermilk, while phosphatidyl ethanolamine is enriched in buttermilk. The addition of milk polar lipids from butter serums could be recommended for infant formula since their profile is close to human breast milk [15]. As shown in [16], buttermilk is enriched in whey proteins, while butter serum in typical MFGM proteins. In addition, buttermilks and butter serums composition can be influenced by external parameters, including the cow's diet, such as maize silage or grass. The impact of these variations in the fatty acid composition of milk polar lipids on their functional and biological roles is yet to be elucidated [15].

As infant formula is the recommend solution, when breastfeeding is not possible, it has been developed a similarity index to check how an infant formula composition is next to human milk [17]. Infants receive roughly 50% of energy from fat in milk or formula. The fat of human milk is a very complex mixture consisting of about 200 different lipids, and the lipid composition is suitable for optimal adsorption and nutritional value and varies throughout lactation. To standardize the preparation of commercial MFGMs it is important to develop high reproducible and high-sensitive methods for the analysis of both their protein and lipid composition. A combination of proteomic and

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lipidomic analyses has been employed to highlight compositional variations in six MFGM preparations (whey or cream derived), the composition of lipids was more variable than that of proteins among the tested samples [18]. The analysis of phospholipid classes revealed that the lipid composition of human MFGM and cow MFGM was more similar than the other dietary derived lipids [19], nevertheless dairy fat is not suitable as the sole fat ingredient in the infant formulas due to lower amounts of linoleic acid and α-linolenic acid than required. The similarity index evaluation for milk-fat-containing formulas, revelead higher values in saturated fatty acid, monounsaturated fatty acid, and n − 3 polyunsaturated fatty acid, compared to the formulas containing vegetable oils as the primary fat source [17]. Moreover, the infant formulas containing milk fat were higher in similarity to breast milk in short and medium chain fatty acids; the positioning of saturated fatty acids in the middle position of triacylglycerol is crucial for their efficient absorption in the infant intestine. Infant formulas commonly utilize lecithin derived from oil plants as a source of polar lipids, which serves as an emulsifier to stabilize vegetable oils as small lipid droplets and/or MFGM from dairy fat. Another feature of milk-fat formulas, is the incorporation of cholesterol associated with sphingomyelin in cellular membranes, and it is regarded to have health effects, such as short- and long-term reduction of cardiovascular risk factors in infants [17]. Instead, the role of phytosterols in vegetable oils formula is less clear, and their oxidation products can have consequences on human health [20].

3. Infant Formula Features As Defined by the European Regulation

The preparation of an infant formula is strictly determined by the European Regulation [21–23]. In the past, the benchmark for "adapted" cow's milk-based formula was the composition of human milk. However, since 2005, merely replicating human milk composition has become insufficient; the new ultimate standard for all infant formulas should be the functional outcomes observed in breast-fed infants [24]. The regulation EU 609/2013 [21] establishes the composition of an infant formula, as " food intended for use by infants during the first months of life and satisfying by itself the nutritional requirements of such infants until the introduction of appropriate complementary feeding". Article 9 of the Regulation outlines the overall compositional and informational requirements, specifying that the infant formula's composition should be appropriate for the infants (infant is a child under the age of 12 months). Additionally, the article mandates that substances added to infant formula should be bioavailable for the human body, possess nutritional or physiological effects, and be suitable for infants, as supported by generally accepted scientific data. Article 15 shows the Union list of substances that may be added, they should belong to the following categories: vitamins, minerals, amino acids, carnitine and taurine, nucleotides, choline and inositol; unfortunately the MFGM preparations are not included. As much as regard the addition of substances in infant formula, indications can be found in the Scientific Opinion of the EFSA Panel [23] delivered in 2014 on the essential composition of infant and follow-on formula. The minimum content of a nutrient is defined and considered adequate for the majority of infants in the first six months of life; the Panel emphasizes that maximum amounts should be interpreted not as target values but rather as upper limits of a range which should not be exceeded. Nutrients and substances should be added to infant formulas only in amounts that serve a nutritional or other benefit. The addition in amounts higher than those serving a benefit or the inclusion of unnecessary substances in formulas may put a burden on the infant's metabolism and/or on other physiological functions. The average amount of daily energy intake coming from an infant formula (consumed in the first six months of life) is taken to be equivalent to 500 kcal/day; in particular minimum and maximum content of energy and macronutrients in infant formulas are described [23], for energy compositional requirements a minimum energy content of 60 kcal/100 mL and a maximum energy content of 70 kcal/100 mL are needed. The allowed sources of protein in infant formulas include cow's milk protein, goat's milk protein, isolated soy protein and protein hydrolysates of unspecified origin and unspecified degree of hydrolysis. The Panel emphasizes that the safety and suitability of each specific formula containing protein hydrolysates has to be established by clinical studies. It is interesting that goat milk formulas have been poorly commercialized in the last ten years, even if they are approved by EFSA. Recently a 16-week multi-center double-

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blind randomized controlled trial, has been reported [25], in which it has been shown that goat milk formula provides adequate growth, has a good tolerability, and is safe to use in infants. The Commission Delegated Regulation EU 2024/2684 amended the Delegated Regulation EU 2016/127 as regards the protein-related requirements for infant and follow-on formula manufactured from protein hydrolysates [26]. As a matter of facts, upon a request from the EU Commission, the EFSA issued a scientific opinion in 2023 about the nutritional safety and suitability of that infant and follow-on formula. In that opinion [27] , the Authority concluded that the protein hydrolysate in question is a nutritionally safe and suitable protein source for use in infant and follow-on formula, as long as the formula in which it is used contains a minimum of 0,57 g/100 kJ (2,4 g/100 kcal) protein.

Considering that breast milk has an average total fat content of 24-59 g/L accounting for the 50% of energy intake, the total fat proposed by the Panel is 4.4-6.0 g per 100 kcal; among polar lipids the range of linoleic acid shall be 500 -1200 mg/100 kcal; alpha-linolenic acid 50 -100 mg/100 kcal; docosahexaenoic acid 20-50 mg/100 kcal; trans-fatty acids ≤ 3% of fatty acids. Conjugated-linoleic acid is currently not permitted to be added to formulas in addition to the amount naturally present in the fat ingredients, and it is considered to be a novel food ingredient in this context. Also, the use of sesame oil and cottonseed oil is not permitted in infant formulas. The Panel EFSA moreover underlined that there is no necessity to add arachidonic acid, eicosapentaenoic acid, non-digestible oligosaccharides, “probiotics” or “synbiotics”, chromium, fluoride, taurine and nucleotides to infant formulas. There is also no necessity to use phospholipids as a source of long-chain polyunsaturated fatty acids instead of triacylglycerols in infant formulas or to use triacylglycerols with palmitic acid predominantly esterified in the sn-2 position instead of triacylglycerols from other fat sources. Then in 2016, the Commission delegated regulation EU 2016/127 [22] has introduced some new points supplementing the previous Regulation EU 2013/609 [21]. As a new requirements the addition of docosahexaenoic acid to infant formula and follow-on formula is mandatory. Other long-chain (20 and 22 carbon atoms) polyunsaturated fatty acids may be added, the content of which shall not exceed 2 % of the total fat content for n-6 long-chain polyunsaturated fatty acids (1 % of the total fat content for arachidonic acid). The eicosapentaenoic acid (20:5 n-3) content shall not exceed that of docosahexaenoic (22:6 n-3) acid content. The amount of phospholipids in infant formula shall not be greater than 2 g/l. In this Regulation it is also pointed out that the name of infant formula shall be “infant milk” when it is manufactured entirely from cow’s milk or goat’s milk protein; while if other sources of protein are used, the name shall be “infant formula” [22]. Moreover since fatty acids composition of breast milk reflects the diet of the mother [26], there is no standard value in the European regulation; in this context the similarity index [17] can be used in the preparation of an optimal infant formula. Even if these parameters are not controlled by legislation, they assume a significant role in infant metabolism, particularly concerning lipid absorption and cellular metabolism.

4. Donated Human Milk and MFGM

Human breast milk is the preferred choice for feeding newborns. For infants unable to be breastfed, donated human milk (DHM) is a vital alternative. DHM is obtained from healthy lactating women through Donated Human Milk Banks (DHMB), adhering to local regulations, and is primarily used to nourish preterm infants in neonatal intensive care units. Upon donation, the milk undergoes Holder pasteurization, a process that alters milk composition, mostly the structure of MFGM [29]. Figure 1 is a schematic representation of the milk fat globule in raw own mother milk, in donated human milk and in MFGM-enriched infant formula. The structure of the biological assembly of milk fat globule is completely changed upon pasteurization, it could be hypothesized that also the biological function is completely different. The structure of milk fat in infant formula is more different, as shown also by electron microscopy in [4]

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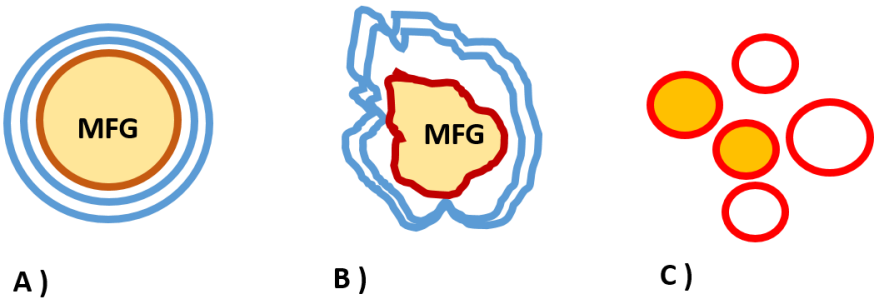

Figure 1. The unique structure of milk fat globule (MFG), surrounded by a tripartite membrane. A) MFG from raw breast milk from the own mother. B) MFG from pasteurized milk from donated human milk. C) Infant formula with the addition of MFGM preparation.

The regulation on pasteurization for milk for human consumption is well described and detailed, the main objectives of the EU legislation consist of consumer health protection at all stages of the food chain. The consumption of milk must meet the hygienic requirements and the official controls are focused on the pasteurization regime, reaching the prescribed temperature and time of pasteurization. FAO and WHO give the following definition for pasteurization: "Pasteurization is a microbiocidal heat treatment aimed at reducing the number of any pathogenic micro-organisms in milk and liquid milk products, if present, to a level at which they do not constitute a significant health hazard" The process criteria are given as the following: "According to validations carried out on whole milk, the minimum pasteurization conditions are those having bactericidal effects equivalent to heating every particle of the milk to 72 °C for 15 seconds (continuous flow pasteurization) or 63 °C for 30 minutes (batch pasteurization) [30].

The regulation of donated human milk in the European Union primarily falls under the framework for the safety and quality of human tissues and cells. Therefore, human milk banks must ensure proper screening of donors and apply safe processing techniques, such as pasteurization, to eliminate potential pathogens. There are different directives that ensure the safe collection, processing, and distribution of donated human milk, particularly for vulnerable populations such as premature and sick infants. Among the most relevant rules, the Directive 2004/23/EC [31] establishes standards of quality and safety for the donation, procurement, testing, processing, storage, and distribution of human tissues and cells, including human milk when used for medical purposes . Moreover, Directive 2006/86/EC lays down technical requirements for the traceability of tissues and cells and establishes protocols for reporting adverse events or reactions related to the use of donated milk [32] . Recently Regulation EU 2024/1938 prescribes standards of quality and safety for substances of human origin intended for human application, the so called "SoHo"[33] .

5. Milk Fat and Brain Metabolism

As previously reviewed [34] infant metabolism is ketogenic, During the first months of life, an infant's dietary protein needs decrease rapidly, leading to a decline in the protein concentration of human milk as lactation progresses and matures. However, infant formula does not adjust to these time-dependent changes, maintaining a higher total protein concentration than human milk. A recent clinical trial [35] compared the serum metabolomes of breast-fed and formula-fed infants. It found elevated ketogenesis in breast-fed infants, characterized by increased levels of 3-hydroxybutyrate, acetate, and formate. In contrast, formula-fed infants exhibited metabolic patterns dominated by protein catabolism, with higher levels of urea, essential amino acids, and their breakdown products. Supplementing infant formula with milk fat globule membrane (MFGM) shifted newborns' metabolic profiles toward fat utilization, similar to breast-fed infants, rather than protein utilization typical of formula-fed infants. This supplementation also altered the gut microbiota to more closely resemble that of breast-fed infants [35]. The high levels of ketone bodies in the blood of breast-fed infants support their rapid brain growth and development. During this stage, the brain's glucose metabolism is

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lower than in adults, allowing efficient utilization of ketone bodies as an alternative energy source. Ketone bodies are utilized during brain development, they can support brain energy requirements (30-70%) and also serve as precursors for synthesizing cholesterol, long-chain fatty acids, and amino acids critical for brain maturation [36,37]

Fat and energy intake in the early postnatal period is extremely important for neurocognitive development. Magnetic resonance imaging (MRI) has been used to evaluate neonatal brain development; while breast milk intake and long-chain polyunsaturated fatty acid supplementation has been proven beneficial in term infants, the impact in preterm infants is less well understood [38]. Brain injury is more likely to occur in extremely preterm infants and can give rise to cognitive impairment; since MFGM can affect gut microbiota and modulate the gut-brain axis, an interventional study evaluate the synergistic posiive effects of infant formula supplemented with bioactive compounds (synbiotics,LC-PUFAs, and MFGM)on microbiota maturation and infant neurodevelopment [39].

6. Conclusions

Fat plays a crucial role in infant formulas, constituting the second largest component and contributing approximately half of the formula's energy content. While some formulas solely rely on vegetable oils as their fat source, others incorporate milk fat. The objective of formula production is to closely mimic the ideal benchmark—the fat found in breast milk—often regarded as the gold standard.

From the perspective of an infant, living in Europe, who cannot be breast-fed, it would be important to choose an infant formula with the addition of MFGM, but the European regulation does not include this possibility.

There is a need for standardization of MFGM materials preparation, or better it is important to develop new methods for checking the protein and lipid composition in a very easy and rapid way; this would be helpful for a future introduction in the European regulation.

Noteworthy, the choice of an infant formula in Europe can be affected by digital marketing. Worldwide digital technologies are progressively being harnessed for the marketing of food products. The adoption of digital platforms by manufacturers and distributors of breast-milk substitutes for product promotion is accelerating [40]. Despite substantial evidence highlighting exclusive and prolonged breastfeeding as pivotal factors in securing lifelong health for children, women, and communities, the adherence to recommended breastfeeding practices remains inadequate. Digital marketing, using strategies across a wide range of online channels and social media platforms, boosts infant formula purchases, offering superior cost-effectiveness compared to traditional marketing methods. This is a worldwide global dimension that goes further European law and it is uncontrolled. Some concerns are raising for example in US for infant formula of European origin [41], but it could be the same for a European mother, who wants to buy an infant formula containing MFGM. In the United States, many parents of infants are using imported European formulas purchased over the Internet from third party vendors, that do not meet all FDA-labeling requirements and could be a risks for the consumers.

In conclusion MFGM and MFG are unique structures in the human milk and in the milk of other lactating mammals; they have no correspondent assemblies among blood and other body fluids, nor in cellular organelles. They are shaped perfectly to deliver energy and information to the breast-fed infants. At the moment, the arising message is that nothing can replace the presence of MFGM in human milk.

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