Review

Not peer-reviewed version

Bioactive Functions of Lipids in Milk Fat Globule Membrane: A Comprehensive Review

Junyu Pan , Meiqing Chen , Ning Li , Rongwei Han , Yongxin Yang , Shengguo Zhao , Nan Zheng , Yangdong Zhang *

Posted Date: 16 August 2023

doi: 10.20944/preprints202308.1184.v1

Keywords: milk fat globule membrane; phospholipids; ganglioside; cholesterol; development

Review

Bioactive Functions of Lipids in Milk Fat Globule Membrane: A Comprehensive Review

Junyu Pan [1,2], Meiqing Chen [1], Ning Li [2], Rongwei Han [2], Yongxin Yang [2], Nan Zheng [1], Shengguo Zhao [1] and Yangdong Zhang [1*]

[1] Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China; buckyneng@foxmail.com (J.P.); mqchen1997@163.com (M.C.); zhengnan@caas.cn (N.Z.); zhaoshengguo@caas.cn (S.Z.)

[2] College of Food Science and Engineering, Qingdao Agricultural University, Qingdao 266109, China; lining20211114@163.com (N.L.); qauhan@qau.edu.cn (R.H.); qauyang@qau.edu.cn (Y.Y.)

* Corresponding: zhangyangdong@caas.cn

Abstract: Milk fat globule membrane (MFGM) is a complex tri-layer membrane that wraps droplets of lipids in milk. In recent years, it has attracted widespread attention due to its excellent bioactive functions and nutritional value. The milk fat globule membrane contains a diverse array of bioactive lipids, including cholesterol, phospholipids, and sphingolipids, which play pivotal roles in mediating the bioactivity of the milk fat globule membrane. We sequentially summarize the main lipid types in milk fat globule membrane in this comprehensive review and outline the characterization methods used to employed them. In this comprehensive review, we sequentially describe the types of major lipids found in the milk fat globule membrane and outline the characterization methods employed to study them. Additionally, we compare the structural disparities among glycerophospholipids, sphingolipids, and gangliosides, while introducing the formation of lipid rafts facilitated by cholesterol. The focus of this review revolves around an extensive evaluation of current research on lipid isolates from the milk fat globule membrane, as well as products containing milk fat globule membrane lipids, with respect to their impact on human health. Notably, we emphasize clinical trials encompassing a large number of participants. The summarized bioactive functions of milk fat globule membrane lipids encompass regulation of human growth and development, influence on intestinal health, inhibition of cholesterol absorption, enhancement of exercise capacity, and anti-cancer effects. By offering a comprehensive overview, the aim of this review is to provide valuable insights into the diverse biologically active functions exhibited by lipids in milk fat globule membrane.

Keywords: milk fat globule membrane; phospholipids; ganglioside; cholesterol; development

1. Introduction

Milk fat, a highly regarded and widely consumed nutrient, not only serves as a vital energy source for the human body but also constitutes a significant reservoir of essential fatty acids and vitamins. Approximately 98% of the fat in milk exists as milk fat globules, which are mainly composed of triacylglycerol inside and surrounded by milk fat globule membrane (MFGM) [1,2]. MFGM with a thickness of 10-20nm has a mass of 2-6% of the milk fat globules and acts as a natural emulsifier, avoiding fat from coalescing in the presence of enzymes [3,4]. The emulsifying properties of MFGM help enhance the texture and flavour in various food products. For instance, the incorporation of MFGM in the bread production process serves to retain moisture in bread crumbs, thereby impeding bread aging and hardening. The nutrients in MFGM, especially sphingolipids, phospholipids and proteins, make it an excellent source for the development of nutraceuticals, especially infant development supplements [5]. The diverse bioactive functions of lipids in milk fat globule membranes have led to the emergence of various MFGM-related industrial products. Examples include Lacprodan MFGM 10 and Lacprodan PL 20, which serve as supplements for phospholipids [6]. These products capitalize on the bioactive properties of MFGM lipids, catering to specific nutritional needs.

Both fresh milk and by-products derived from dairy processing serve as effective sources of MFGM. The process of isolating MFGM from milk typically involves several steps: separation of milk fat globule, cream washing, MFGM releasing, and MFGM collection [4]. During the permeation process, the addition of reverse osmosis water helps maintain a constant feed rate after filtration to eliminate whey and casein. Finally, acidification was employed to isolate the MFGM[7]. Industrial processes typically utilize by-product milk (e.g., buttermilk, cheese whey, etc.) to separate MFGM. For instance, the removal of casein in buttermilk can be achieved through rennet-induced coagulation, followed by filtration to eliminate whey proteins. The MFGM is subsequently collected through diafiltration steps [8]. It must be noted that these by-products often undergo high-temperature heat treatment, leading to protein denaturation on the MFGM or reactions with whey protein or sugars, which can alter the emulsifying properties of the milk fat globule membrane [9].

As shown in Table 1, dietary supplementation with milk fat globule membrane lipids have demonstrated numerous beneficial bioactive functions. For instance, the addition of MFGM to milk powder has been found to mitigate the impact of phytosterols on infant nutrition by competing with cholesterol for absorption[10] . Furthermore, the intake of milk-derived phospholipids has been demonstrated to suppress the endocrine stress response in individuals exposed to high-intensity stress, with phospholipid-supplemented subjects exhibiting a faster recovery rate[11] . In a mouse study, ganglioside-rich MFGM isolates showed potential in reducing carrageenan-induced paw edema, indicating their anti-inflammatory properties[12]. In this comprehensive review, keywords such as milk fat globule membrane, lipids, sphingolipids, phospholipids, health, and development were searched on Google Scholar and Web of Science platforms. The review encompasses an examination of the types of lipids present in MFGM, characterization methods employed, and the diverse functions they exhibit. The purpose of this review is to offer an insightful understanding of the nutritional value of lipids in MFGM, offering valuable insights for both daily dietary considerations and production practices.

Table 1. Bioactive functions of lipids in milk fat globule membrane.

Lipid	Participants	Dose	Time	Results	Reference
Sphingomyelin	Low-birth-weight preterm babies	20% of total phospholipids in milk	18 months	Supplementation of sphingomyelin in milk has a positive effect on the neurobehavioral development of low-birth-weight preterm infants.	[32]
Sphingomyelin	Wistar Rat	810 mg/100g Sphingomyelin/diet	28 days	Sphingomyelin contributes to myelination in developing rats.	[33]
Sphingomyelin and Phosphatidylcholine	Children aged 0-5	62mg/L 85mg/L	90 days	Sphingomyelin and phosphatidylcholine have significant effects on neural and cognitive development.	[35]
Phospholipids	Healthy preschool children aged 2.5 to 6 years	250mg/100mL	6 months	High phospholipid concentration in milk is beneficial to children's behavior regulation, and the frequency of fever is significantly reduced.	[36]

Phospholipids	Infants aged ≤14 days	647mg/L	4 months	Diarrhea, vomiting, ear infections, conjunctivitis, and eczema were significantly reduced in infants fed the milk fat globule membrane phospholipid formula.	[44]
Sphingomyelin	Male Sprague-Dawley Rat	19.5±1.4% dose	7 weeks	Compared with egg-origin sphingomyelin, milk-origin sphingomyelin had a stronger effect in inhibiting the absorption of fat and cholesterol in the rat intestinal tract.	[49]
Phospholipids	Menopausal women	0.3, 0.5g/day	4 weeks	Phospholipids may reduce specific interactions involved in cholesterol absorption in the gut.	[54]
Phospholipids	Overweight men and women	40g/day	8 weeks	Milk-derived phospholipids significantly reduce fasting and postprandial plasma cholesterol concentrations. Milk fat enclosed by MFGM does not impair lipoprotein profiles.	[53]
Phospholipids	Men and women with serum low-density lipoprotein cholesterol (LDL-C) <5.0 mmol/L	187.5mg/day	8 weeks	The intake of phospholipids reduces cholesterol levels in the body, mainly by inhibiting the absorption of cholesterol in the gut.	[51]
Sphingomyelin and Phosphatidylserine	Healthy men with an average age of 41.5 years	13.5g/day -	3 weeks	High doses of phospholipids can dampen the activity and reactivity of the hypothalamic-pituitary-adrenal axis (HPAA) and make subject a blunted psychological stress response.	[11]
Phospholipids, Sphingolipids and Exercise	15-week-old male SAMP1 and ICR rat	356 ± 9 mg/day diet (contain 16.6% phospholipids)	28 weeks	Milk fat globule membrane combined with exercise can improves muscle function deficits.	[40]
Phospholipids and exercise	Seniors aged 71-75	1g tablet contain 16% phospholipid milk fat globule	8 weeks	Participants taking globular membrane tablets performed better in tapping and stepping.	[69]

		membrane per day			
Phospholipids and exercise	Older women aged 82-84	1g milk fat globule membrane tablet per day	12 weeks	Exercise and phospholipid supplementation may improve frailty in older adults.	[41]
Ganglioside	--	2mL breast milk	--	Gangliosides in breast milk protect infants from diarrhea caused by enterotoxins.	[65]
Ganglioside	Preterm infants	1.43mg/100 kcal	30 days	Formulas supplemented with gangliosides can promote the growth of bifidobacteria, thereby inhibiting the growth of E. coli and other potentially pathogenic microorganisms in the gut of premature infants.	[67]
Phospholipids and Gangliosides	Piglets	0.8 or 2.5% Lacprodan PL-20	26 days	Supplementation with gangliosides and phospholipids improved spatial learning in piglets and affected brain development.	[38]
Ganglioside	Infants aged 2 to 8 weeks	11~12 μg/mL	16 weeks	Formula with increased ganglioside content in the diet is beneficial for cognitive development in healthy infants aged 0-6 months.	[61]
Ganglioside	Wistar Rat	0.2%, 1.0%CML	80 days	Dietary gangliosides benefit cognitive development in infants.	[60]
Sphingomyelin and glycosphingolipids	Female CF1 Rat	0.025或0.1 g/100 g	4 weeks	Milk-derived sphingolipids and glycosphingolipids can suppress colon cancer in female mice at an early stage, reducing the appearance of aberrant crypt foci.	[55]
Sphingomyelin	Male Fischer-344 Rat	0.11% w/w	13 weeks	Diets containing sphingomyelin are protective against colon cancer in Fischer-344 rats.	[70]

2. Lipids in milk fat globule membrane

The primary lipids present in the tri-layer membrane of milk fat globules include phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SM), cholesterol, and gangliosides [13–16]. The surface layer of the bilayer primarily consists of glycolipids, cerebrosides, and gangliosides, while the inner layer of the bilayer mainly contain PE, PI, PS. Phospholipids are the predominant components of the monolayers[17]. Table 2 provides a summary of the compositional differences between major phospholipids in bovine and human milk [18]. It highlights the variations in phospholipid composition between these two milk sources, which may contribute to their distinct nutritional profiles.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 August 2023 doi:10.20944/preprints202308.1184.v1

Structurally, sphingomyelin consists of long chains of bases, such as sphingosine, which form the backbone of the molecule [19]. On the other hand, glycerophospholipids are composed of, phosphoric acid, glycerol, fatty acids, hydroxyl compounds and fatty acids [20]. The presence of glycerophospholipids with relatively high unsaturation levels contributes to improved fluidity of MFGM [21]. Gangliosides, on the other hand, are formed through glycosidic linkages between ceramides and residues of sialic acid [22].

Cholesterol is found primarily in the outer bilayer of MFGM and forms rigidly ordered domains known as lipid rafts when it binds with sphingomyelin [23] (Figure 1). In contrast, the disordered phase of the membrane mainly consists of phospholipids. Lipid rafts have the ability to bind to proteins and can induce signaling processes [24]. The structural role of lipids in milk fat globule membranes can be characterized by high-throughput synchrotron radiation X-ray diffraction (SR-XRD) and differential scanning calorimetry (DSC) [25]. Previous research has shown that the cholesterol enhances the order of the milk sphingomyelin bilayer membrane, with the ordered phase being achieved at 33 mol% cholesterol content. The ratio of cholesterol/sphingomyelin can influence the interfacial properties of MFGM, thereby impacting the functional properties of milk fat globules and their digestion mechanisms[26].

Table 2. Phospholipids in Human and Bovine Milk.

Polar lipids (%)	Bovine	Goat	Human
PI	8.97 ± 0.06	9.37 ± 0.06	7.85 ± 0.07
PC	33.12 ± 0.21	31.64 ± 0.21	24.39 ± 0.12
PS	9.07 ± 0.07	14.03 ± 0.05	13.12 ± 0.03
PE	23.42 ± 0.13	19.92 ± 0.10	25.33 ± 0.14
SM	25.40 ± 0.19	25.04 ± 0.17	29.28 ± 0.14

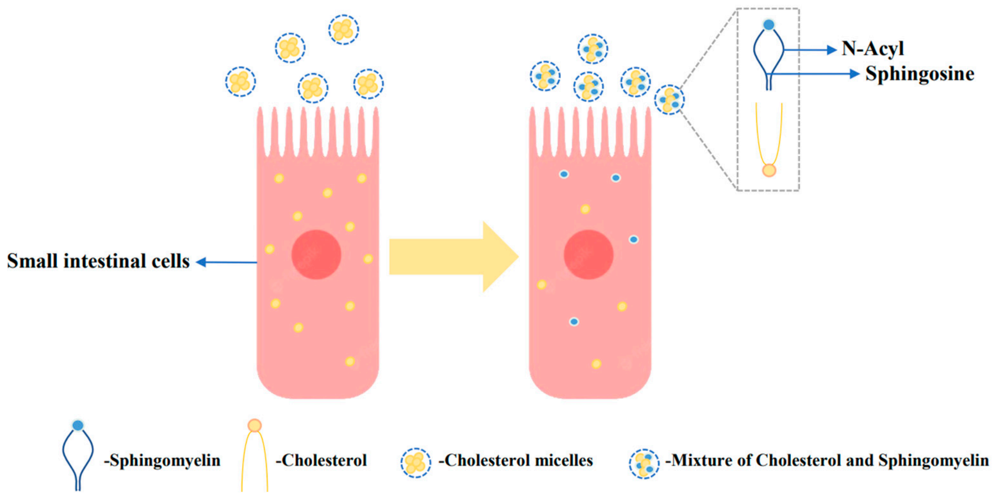

Figure 1. Structure of lipid rafts of milk fat globule membrane on cholesterol. Cholesterol in the milk fat globule membrane binds to sphingolipids in the outer bilayer to form rigid ordered domains, i.e., lipid rafts, and the disordered phase consists of phospholipids. The lipid rafts bind to proteins and can act as an induced signal.

3. Characterization of Lipids in Milk Fat Globule Membrane

Liquid chromatography with evaporative light scattering detector (ELSD) is a frequently employed method for quantifying and characterizing lipids. In the study by Zou et al., this technique was employed to determine the concentrations and relative proportions of PC, PI, PS, PE, and SM in the MFGM of colostrum, mature milk, and transitional milk from cow. The findings indicated that the concentration of polar lipids in total lipids reached its peak during the transitional stage of lactation. The relative content of sphingomyelin did not exhibit significant changes, but the level of phosphatidylcholine in mature milk was higher compared to the other two stages[27]. This study highlighted that the level of polar lipids in mature milk was significantly higher than that in colostrum, which is in accord with their earlier research [28]. In a comparative analysis conducted by Menard et al., the lipid profile of MFGM of buffalo and cow milk was examined using HPLC-ELSD. They indicated that the percentage of phosphatidylcholine of buffalo milk was higher than that in cow milk, while the percentage of sphingomyelin was lower when compared to cow milk.

Lipidomic, in combination with mass spectrometry, offers a powerful approach for identifying various lipid species based on different phospholipid classes. In the study by George et al., the relationship between growth characteristics and lipids in MFGM was explored, and the concentration and intake of lipids from different MFGM were compared. Through LC-MS analysis, this research identified a total of 166 MFGM lipid species originated from 10 fractions. The study further demonstrated that infants exhibit variations in the content and intake of different lipids exist in milk fat globule membranes, and the intake of MFGM lipids is positively correlated with infant development [29]. Similarly, Brink et al. utilized UPLC-MS to identify 338 milk fat globule membrane lipid species derived from 10 fractions, thereby completing the lipid characterization of different commercial MFGM materials. In a study by Ali et al., UPLC-ESI-Q-TOF-MS was employed to identify 100 milk fat globule membrane lipids derived from 7 fractions[30,31].

4. Phospholipids

4.1. The promoting effect phospholipid supplementation on development

The inclusion of sphingomyelin-enriched milk in the diet of premature infants has been investigated for its impact on motor, mental, and behavioral development. In a study involving 24 very low birth weight babies, the infants were separated into two groups. The trial group accepted sphingomyelin-enriched milk, where sphingomyelin accounted for 20% of all phospholipids in the milk, while the control group received milk with only 13% sphingomyelin content [32]. At eighteen months old, the babies in the group of sphingomyelin-enriched demonstrated significantly higher scores in various developmental assessments, including the Behavior Rating Scale of the BSID-II test, the Fagan test, Visual Evoked Potentials (VEPs), and the Sustained attention test, compared to the control group. These findings suggest that infants receiving sphingomyelin-enriched milk exhibited improved neurobehavioral development. This positive effect could be attributed to the role of sphingomyelin as an important component of the myelin sheath, as indicated by previous research [33,34].

Earlier research examining the neurocognitive development and longitudinal trajectories of brain and in kids who were fed different formulas for at least 3 months found significant developmental differences among the groups. Specifically, factors like long-chain sphingomyelin, iron, fatty acids, folic acid, and choline were closely associated with early myelination trajectories [35]. Another study compared the impact of formula milk and conventional milk on the central nervous system of 182 preschool children. The formula milk used in the trial group contained 8-9 times higher phospholipid concentration compared to non-formula milk. The trial group received 200 mL of formula milk without phospholipids, while the control group received formula milk containing 500 mg of phospholipids. The children were evaluated using the Achenbach System of Empirically Based Assessment. Results of the research indicated that formula milk with a higher phospholipid concentration had a more pronounced effect on children's behavioral regulation [36]. These investigations emphasise the significance of specific nutrients, particularly phospholipids, in formula milk for promoting healthy brain and behavioral development in children. The inclusion of appropriate levels of these nutrients in formula milk could positively impact the neurodevelopment.

Indeed, supplementation of phosphatidylserine and sphingomyelin has shown to enhance memory function [11]. In previous research involving 75 male aged 30 to 51, different test groups were given milk containing placebo, 0.5%, and 1% phospholipids (phospholipid content adjusted using Lacprodan PL 20) over a period of 42 days. The stress-protective effects of phospholipids were evaluated using the Trier Social Stress Test. It was concluded that high doses of phospholipids could inhibit the activity and responsiveness of the hypothalamic-pituitary-adrenal axis (HPAA) and result in a blunted psychological stress response. The age of subjects and the duration of phospholipid supplementation may influence these effects[37]. In a study involving piglets, Lacprodan PL-20 (0%, 0.8%, 2.5% v/v) was added to the diets of three groups of piglets. The development of the piglets was observed from day 2 to 28 of postpartum. The piglets' performance in the T-maze was measured on day 14, and brain MRI data were obtained on day 28 postpartum. The piglets supplemented with 0.8% and 2.5% gangliosides showed better performance in the maze, had higher brain weights, and exhibited more white and gray matter. This suggests that gangliosides enhance spatial learning in newborn piglets and influence brain development [38]. Similar findings were observed in mouse experiments, where supplementation of phospholipids from MFGM improved memory in mice [39]. These investigations emphasize the potential cognitive benefits of phospholipid supplementation, including improved capacity to memorize and spatial learning, in both human and animal testing. The specific effects may vary depending on factors such as dosage, duration of supplementation, and the age or developmental stage of the subjects.

Supplementation of phospholipids and sphingolipids, in combination with exercise, has been investigated for its potential to improve muscle movement and neuromuscular development, including the formation of neuromuscular junctions. In Yoshinaka's study, 71 subjects were divided into two groups. One group ingested 1g of milk fat globule membrane placebo (167 mg/placebo), while the other group received a placebo in the form of whole milk powder. Tablets containing MFGM were produced for the test group, and placebo tablets were composed of whole milk powder. Both groups engaged in low-intensity exercise, and the trial lasted for eight weeks. The test group demonstrated better performance in foot tapping and opening and closing steps, which could be attributed to the presence of sphingomyelin, one of the components that promote the development of nerve and muscle fibers [40]. Kim's research expanded on this by including a placebo plus exercise group and a milk fat globule membrane plus exercise group. This design allowed for a more targeted study to determine whether supplementation with milk fat globules or daily low-intensity exercise alone could improve frailty in the subjects[41]. These works suggest that dietary supplementation with phospholipid and sphingolipid supplements, in conjunction with exercise, may have positive effects on neuromuscular development and muscle movement.

4.2. Phospholipids have a regulating effect on gut health

Dietary supplementation of phospholipids have a immunomodulatory role in immune regulation, particularly in the regulation of gut microbial composition and inflammation. Compared to drugs, dietary modifications are often more cost-effective and sustainable in preventing infant diseases [42,43]. An investigation involving 119 infants aged ≤14 days divided them into three groups: those receiving standard infant formula, MFGM lipid-enriched formula (MFGM-L), and MFGM protein-enriched formula (MFGM-P). The aim of the research was to assess the prevalence of adverse reactions in infants. The data showed that infants fed MFGM-L formula had the lowest frequency of diarrhea among the three groups [44]. This finding was likely attributable to the modification of gut microbiome by phospholipids or to phospholipid-induced changes in the immune system [45]. The beneficial effects of phospholipids on the gut are further reflected in their interaction with microorganisms such as *Lactobacillus*. The interaction between phospholipids in MFGM and *Lactobacillus* has been shown to significantly influence *Lactobacillus* adhesion and enhance gut microbiota health [46]. Moreover, phospholipids in MFGM have been shown the ability of inhibiting the growth of *H.pylori* and reducing the levels of *E. coli* and *Salmonella* enteritidis [47].

4.3. Phospholipids regulate cholesterol metabolism

Increased serum cholesterol is a major contributor to cardiovascular disease (CVD), and supplementation with milk-derived lipids has been shown to modulate cholesterol levels. Lipids present in milk fat globule membranes, particularly sphingomyelin, have been found to inhibit cholesterol absorption in the gut [48]. Dietary sphingomyelin has a substantial impact on plasma and tissue cholesterol levels. As shown in Figure 2, milk-derived sphingomyelin exhibits a stronger effect in inhibiting rat cholesterol absorption, and this could attributed to the compatibility of sphingosine and N-acyl groups in the presence of cholesterol, promoting their mutual attraction[49,50]. In a study involving 34 subjects with low-density lipoprotein cholesterol (LDL-C) levels below 5.0 mmol/L, the effects of dietary supplementation with chocolate flavor buttermilk or placebo were investigated. The placebo formulation matched the macro/micronutrient content of buttermilk, except for the nutrients from MFGM. The research found that dietary supplementation with buttermilk containing a high concentration of milk globular phospholipids effectively inhibited cholesterol absorption [51]. The strong affinity of milk-derived sphingomyelin for cholesterol can effectively lower the cholesterol thermodynamic activity and reduce the monomer content between cholesterol micelles, thereby inhibiting cholesterol absorption[52]. These works indicated that supplementation with milk-derived lipids, particularly sphingomyelin, can have a beneficial impact on cholesterol absorption and may help in managing serum cholesterol levels.

In a test performed by Rosqvist et al. (2015), eight weeks of single-blind randomized controlled experiment was conducted with 57 overweight participants. The test group was supplemented with 40g of whipping cream, which served as a source of MFGM because of its enriched phospholipid level and relatively complete MFGM structure. The dietary phospholipid concentration in the experimental group was approximately 19-fold higher than that in the control group. The results indicated that blood lipids and LDL-C of participants without of supplementation of MFGM were significantly higher. Ingestion of MFGM did not increase cholesterol concentrations, potentially due to the influence of milk phospholipids on lipid metabolism and hepatic gene expression, affecting intra- and inter-organ lipid distribution[53]. Phospholipids have the ability to interfere with specific interactions in the gut, leading to the inhibition of cholesterol absorption without interfering with the gut microbiota[54]. This suggests that the mechanism of phospholipid regulation of cholesterol absorption involves specific interactions in the gastrointestinal tract, which effectively reduce the uptake of cholesterol without disrupting the balance of gut microbial communities.

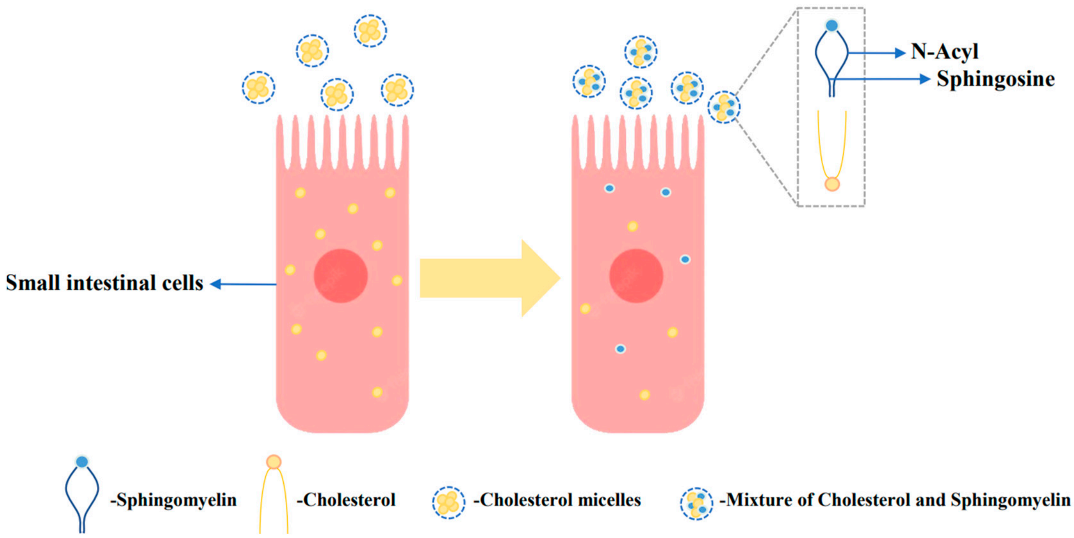

Figure 2. Inhibitory effect of sphingomyelin of milk fat globule membrane on cholesterol. In the presence of cholesterol, the mutual matching of sphingomyelin and N-acyl groups promotes the mutual attraction of cholesterol and sphingomyelin thereby inhibiting cholesterol absorption.

4.4. Anticancer effects of dietary phospholipids

Colon cancer is a prevalent gastrointestinal tumor with a significant global incidence. Previous research has suggested that milk-derived sphingomyelin can inhibit early colon cancer in mice[55]. A former research investigated the inhibitory effects of different dietary fats on colon cancer, a consistent diet (AIN-76A) containing low levels of sphingolipids was used as the baseline[56]. To assess the anticancer potential of sphingolipids, the AIN-76 feed was supplemented with anhydrous milk fat (AMF) and a combination of AMF and milk fat globule membrane (1:1). Microscopic analysis revealed a significantly reduced number of colonic lesions in mice fed milk fat globule membranes compared to those fed AIN-76 or anhydrous fat. This work proved that the anticancer function is attributed to sphingomyelin in MFGM (Snow et al., 2010), which has been supported by subsequent research [57]. Buttermilk, obtained by extracting lipid components using food-grade solvents, has shown promising inhibitory effects on cancer cell activity. The presence of phospholipids in the extracts appears to play a crucial role [58]. In one study, lipids from buttermilk powder were extracted by both food-grade ethanol and a non-food-grade solvent, such as a dichloromethane-methanol solution. The extracts were then fractionated using flash chromatography, and the resulting solution's inhibitory effects on nine human cancer cell lines were evaluated using an Absorbance Microplate Reader[59]. The aforementioned findings suggest that sphingolipids, particularly those derived from MFGM and buttermilk, could possess anticancer properties and exhibit inhibitory effects on colon cancer development and progression.

5. Gangliosides

5.1. Gangliosides promote brain development

Supplementation of gangliosides, a type of complex glycosphingolipids, has been found to promote neurodevelopment and cognitive function, potentially due to the presence of sialic acids in gangliosides, which play a role in synaptic growth and memory formation (Wang et al., 2003; Rahmann, 1995). When infants are fed formula with increased ganglioside content, it has been noted to have favourable influence on cognitive development [60]. In a study involving 60 infants aged 2-8 weeks, one group was fed standard formula milk powder while the other group was fed formula milk powder enriched with compound milk fat to adjust the ganglioside content to 9 mg/100g[61]. The cognitive development of the infants was assessed, and the group receiving the ganglioside-enriched formula showed improved outcomes. Moreover, prenatal supplementation of gangliosides by pregnant women has also been found to promote brain development in offspring [62]. This effect may be attributed to ganglioside supplementation enhancing the plasticity of the hippocampus, which is associated with learning and memory [63]. The above researches underlined benefits of ganglioside supplementation in promoting neurodevelopment, cognitive function, and brain plasticity in infants and offspring.

5.2 Inhibitory effect of gangliosides on intestinal pathogenic microorganisms

Indeed, dietary sphingolipids have been shown to modulate intestinal inflammation by influencing the gut microbiota [64]. Gangliosides, a type of sphingolipid, have demonstrated inhibitory effects on cholera toxin in rabbit intestinal loop [65]. The ability of sphingolipids, including gangliosides, to inhibit pathogenic microorganisms is attributed to their promotion of *Bifidobacterium* growth[66]. In a study involving 41 healthy preterm infants, one group was fed formula milk powder, while the other group was supplemented with gangliosides (1.43 mg/100 kcal) purified from porcine brain based on the same formula[67]. The infants' fecal samples were collected at 3 days, 7 days, and 30 days after birth. The results showed that the group receiving ganglioside supplementation had high *bifidobacteria* and low *E. coli*. Additionally, gangliosides were found to inhibit enterotoxicity and adherence of enteropathogenic *E. coli* strains in an in vitro cellular model [68]. To conclude, gangliosides could change the gut microbiome, promoting the development of helpful bacteria like *Bifidobacterium* and inhibiting the colonization and harmful effects of pathogenic microorganisms such as certain strains of *E. coli*. These effects on the gut microbiome might facilitate the benefits of ganglioside supplementation in promoting intestinal health and preventing gastrointestinal infections.

6. Conclusions

The article presents an overview of the types and characterization methods of the main lipids from milk fat globule membrane. It also summarizes current works on the biological activities and functions of these lipids, including study designs, experimental results, and functional mechanisms. The supplementation of lipids derived from milk fat globule membrane in the diet has been shown to promote development and improve immunity, making it a potential bridge between formula and breast milk. The article emphasizes the significance of studying milk fat globule membrane lipids in the context of food nutrition and clinical applications. While there is existing research on the topic, the article suggests that there is still much to be explored, indicating potential future research directions. It is noted that the composition of the milk fat globule membrane would be influenced by external factors like processing methods and satge of lactation. Therefore, accurately separating and purifying milk fat globule membranes and lipids in it is crucial for their commercial application. Overall, the article emphasizes the function of lipids in milk fat globule membrane various aspects of human health and nutrition. It calls for further research to better understand their functions, explore their potential applications, and develop effective methods for their utilization in the food industry.

Author Contributions: Junyu Pan: Conceptualization, Writing, Original draft. Meiqing Chen: Data curation, Review & Editing. Ning Li: Investigation. Yongxin Yang: Investigation, Review & Editing. Rongwei Han: Investigation, Data curation. Shengguo Zhao: Review & Editing. Nan Zheng: Conceptualization, Review & Editing, Validation, Funding Acquisition, Supervision. Yangdong Zhang: Investigation, Funding Acquisition, Supervision.

Funding: This research was funded by the National Key R&D Program of China, grant number 2022YFD1301004, the Agricultural Science and Technology Innovation Program, grant number ASTIP-IAS12, and China Agriculture Research System of MOF and MARA, grant number CARS-36.

Conflicts of Interest: The authors declare no conflict of interest.

Reference

1. Jukkola, A. and O.J. Rojas, Milk fat globules and associated membranes: Colloidal properties and processing effects. *Advances in colloid and interface science*, **2017**. *245*: p. 92-101. http//dx.doi.org/10.1016/j.cis.2017.04.010
2. Wei, W., Q. Jin, and X. Wang, Human milk fat substitutes: Past achievements and current trends. *Progress in lipid research*, **2019**. *74*: p. 69-86. http//dx.doi.org/10.1016/j.plipres.2019.02.001
3. Zheng, H., R. Jiménez-Flores, and D.W. Everett, Bovine milk fat globule membrane proteins are affected by centrifugal washing processes. *Journal of agricultural and food chemistry*, **2013**. *61*(35): p. 8403-8411. http//dx.doi.org/10.1021/jf402591f
4. Dewettinck, K., et al., Nutritional and technological aspects of milk fat globule membrane material. *International dairy journal*, **2008**. *18*(5): p. 436-457.
5. Fontecha, J., et al., Sources, production, and clinical treatments of milk fat globule membrane for infant nutrition and well-being. *Nutrients*, **2020**. *12*(6): p. 1607. http//dx.doi.org/10.3390/nu12061607
6. Yano, M., et al., Continuous supplementation of milk fat globule membrane with habitual exercise from a young age improves motor coordination and skeletal muscle function in aged mice. *Journal of Nutritional Science and Vitaminology*, **2019**. *65*(5): p. 405-413. http//dx.doi.org/10.3177/jnsv.65.405
7. Wang, C., et al., Advancement on Milk Fat Globule Membrane: Separation, Identification, and Functional Properties. *Frontiers in Nutrition*, **2021**. *8*. http//dx.doi.org/10.3389/fnut.2021.807284
8. Holzmüller, W. and U. Kulozik, Isolation of milk fat globule membrane (MFGM) material by coagulation and diafiltration of buttermilk. *International Dairy Journal*, **2016**. *63*: p. 88-91. http//dx.doi.org/10.1016/j.idairyj.2016.08.002
9. Lopez, C., C. Cauty, and F. Guyomarc'h, Unraveling the complexity of milk fat globules to tailor bioinspired emulsions providing health benefits: The key role played by the biological membrane. *European Journal of*

Lipid Science and Technology, **2019**. *121*(1): p. 1800201. http//dx.doi.org/10.1002/ejlt.201800201
10. Ostlund Jr, R.E., Phytosterols in human nutrition. *Annual review of nutrition*, **2002**. *22*: p. 533. http//dx.doi.org/10.1146/annurev.nutr.22.020702.075220
11. Hellhammer, J., et al., Effects of milk phospholipid on memory and psychological stress response. *British Food Journal*, **2010**. http//dx.doi.org/10.1108/00070701011080258
12. Palmano, K.P., et al., In vitro and in vivo anti-inflammatory activity of bovine milkfat globule (MFGM)-derived complex lipid fractions. *Nutrients*, **2020**. *12*(7): p. 2089. http//dx.doi.org/10.3390/nu12072089
13. Murthy, A.V.R., et al., Cholesterol strongly affects the organization of lipid monolayers studied as models of the milk fat globule membrane: Condensing effect and change in the lipid domain morphology. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, **2015**. *1848*(10): p. 2308-2316. http//dx.doi.org/10.1016/j.bbamem.2015.06.014
14. Liu, Z., et al., Comprehensive characterization of bovine milk lipids: phospholipids, sphingolipids, glycolipids, and ceramides. *Journal of agricultural and food chemistry*, **2020**. *68*(24): p. 6726-6738. http//dx.doi.org/10.1021/acs.jafc.0c01604
15. Calvo, M.V., et al., Comprehensive characterization of neutral and polar lipids of buttermilk from different sources and its milk fat globule membrane isolates. *Journal of Food Composition and Analysis*, **2020**. *86*: p. 103386. http//dx.doi.org/10.1016/j.jfca.2019.103386
16. Ferreiro, T., L. Gayoso, and J. Rodríguez-Otero, Milk phospholipids: Organic milk and milk rich in conjugated linoleic acid compared with conventional milk. *Journal of dairy science*, **2015**. *98*(1): p. 9-14. http//dx.doi.org/10.3168/jds.2014-8244
17. Raza, G.S., K.-H. Herzig, and J. Leppäluoto, Invited review: Milk fat globule membrane—A possible panacea for neurodevelopment, infections, cardiometabolic diseases, and frailty. *Journal of Dairy Science*, **2021**. *104*(7): p. 7345-7363. http//dx.doi.org/10.3168/jds.2020-19649
18. Yao, Y., et al., Lipid composition and structural characteristics of bovine, caprine and human milk fat globules. *International Dairy Journal*, **2016**. *56*: p. 64-73. http//dx.doi.org/10.1016/j.idairyj.2015.12.013
19. Beare-Rogers, J., A. Dieffenbacher, and J. Holm, Lexicon of lipid nutrition (IUPAC Technical Report). *Pure and applied chemistry*, **2001**. *73*(4): p. 685-744. http//dx.doi.org/10.1351/pac200173040685
20. Contarini, G. and M. Povolo, Phospholipids in milk fat: composition, biological and technological significance, and analytical strategies. *International Journal of Molecular Sciences*, **2013**. *14*(2): p. 2808-2831. http//dx.doi.org/10.3390/ijms14022808
21. Wang, L., et al., Comparison of the fatty acid composition of total lipids and phospholipids in breast milk from Japanese women. *Pediatrics International*, **2000**. *42*(1): p. 14-20. http//dx.doi.org/10.1046/j.1442-200x.2000.01169.x
22. Sipione, S., et al., Gangliosides in the brain: physiology, pathophysiology and therapeutic applications. *Frontiers in neuroscience*, **2020**. *14*: p. 1004. http//dx.doi.org/10.3389/fnins.2020.572965
23. Lopez, C., M.-N. Madec, and R. Jimenez-Flores, Lipid rafts in the bovine milk fat globule membrane revealed by the lateral segregation of phospholipids and heterogeneous distribution of glycoproteins. *Food Chemistry*, **2010**. *120*(1): p. 22-33.
24. Brown, D. and E. London, Functions of lipid rafts in biological membranes. *Annual review of cell and developmental biology*, **1998**. *14*(1): p. 111-136. http//dx.doi.org/10.1146/annurev.cellbio.14.1.111
25. Lopez, C., K. Cheng, and J. Perez, Thermotropic phase behavior of milk sphingomyelin and role of cholesterol in the formation of the liquid ordered phase examined using SR-XRD and DSC. *Chemistry and physics of lipids*, **2018**. *215*: p. 46-55. http//dx.doi.org/10.1016/j.chemphyslip.2018.07.008
26. Et-Thakafy, O., F. Guyomarc'h, and C. Lopez, Lipid domains in the milk fat globule membrane: Dynamics investigated in situ in milk in relation to temperature and time. *Food Chemistry*, **2017**. *220*: p. 352-361. http//dx.doi.org/10.1016/j.foodchem.2016.10.017
27. Zou, X.-Q., et al., Human milk fat globules from different stages of lactation: a lipid composition analysis and microstructure characterization. *Journal of agricultural and food chemistry*, **2012**. *60*(29): p. 7158-7167. http//dx.doi.org/10.1021/jf3013597
28. Zou, X., et al., Composition and microstructure of colostrum and mature bovine milk fat globule membrane. *Food Chemistry*, **2015**. *185*: p. 362-370. http//dx.doi.org/10.1016/j.foodchem.2015.03.145
29. George, A.D., et al., Healthy Breastfeeding Infants Consume Different Quantities of Milk Fat Globule Membrane Lipids. *Nutrients*, **2021**. *13*(9): p. 2951. http//dx.doi.org/10.3390/nu13092951
30. Ali, A.H., et al., Impact of technological processes on buffalo and bovine milk fat crystallization behavior

 doi:10.20944/preprints202308.1184.v1

and milk fat globule membrane phospholipids profile. *Lwt*, **2018**. *90*: p. 424-432. http//dx.doi.org/10.1016/j.lwt.2017.12.058
31. Brink, L.R., et al., Omics analysis reveals variations among commercial sources of bovine milk fat globule membrane. *Journal of dairy science*, **2020**. *103*(4): p. 3002-3016. http//dx.doi.org/10.3168/jds.2019-17179
32. Tanaka, K., et al., The pilot study: Sphingomyelin-fortified milk has a positive association with the neurobehavioural development of very low birth weight infants during infancy, randomized control trial. *Brain & Development*, **2013**. *35*(1): p. 45-52. http//dx.doi.org/10.1016/j.braindev.2012.03.004
33. Oshida, K., et al., Effects of dietary sphingomyelin on central nervous system myelination in developing rats. *Pediatric research*, **2003**. *53*(4): p. 589-593. http//dx.doi.org/10.1203/01.PDR.0000054654.73826.AC
34. Babin, F., et al., Nervonic acid in red blood cell sphingomyelin in premature infants: an index of myelin maturation? *Lipids*, **1993**. *28*(7): p. 627-630. http//dx.doi.org/10.1007/BF02536057
35. Deoni, S., et al., Early nutrition influences developmental myelination and cognition in infants and young children. *Neuroimage*, **2018**. *178*: p. 649-659. http//dx.doi.org/10.1016/j.neuroimage.2017.12.056
36. Veereman-Wauters, G., et al., Milk fat globule membrane (INPULSE) enriched formula milk decreases febrile episodes and may improve behavioral regulation in young children. *Nutrition*, **2012**. *28*(7-8): p. 749-752. http//dx.doi.org/10.1016/j.nut.2011.10.011
37. Schubert, M., et al., Milk-based phospholipids increase morning cortisol availability and improve memory in chronically stressed men. *Nutrition Research*, **2011**. *31*(6): p. 413-420. http//dx.doi.org/10.1016/j.nutres.2011.05.012
38. Liu, H., et al., Early supplementation of phospholipids and gangliosides affects brain and cognitive development in neonatal piglets. *The Journal of nutrition*, **2014**. *144*(12): p. 1903-1909. http//dx.doi.org/10.3945/jn.114.199828
39. Guan, J., et al., Long-term supplementation with beta serum concentrate (BSC), a complex of milk lipids, during post-natal brain development improves memory in rats. *Nutrients*, **2015**. *7*(6): p. 4526-4541. http//dx.doi.org/10.3390/nu7064526
40. Haramizu, S., et al., Habitual exercise plus dietary supplementation with milk fat globule membrane improves muscle function deficits via neuromuscular development in senescence-accelerated mice. *Springerplus*, **2014**. *3*(1): p. 1-17. http//dx.doi.org/10.1186/2193-1801-3-339
41. Kim, H., et al., Effects of exercise and milk fat globule membrane (MFGM) supplementation on body composition, physical function, and hematological parameters in community-dwelling frail Japanese women: a randomized double blind, placebo-controlled, follow-up trial. *PLoS One*, **2015**. *10*(2): p. e0116256. http//dx.doi.org/10.1371/journal.pone.0116256
42. Herrmann, F., et al., Infant formula enriched with milk fat globule membrane, long-chain polyunsaturated fatty acids, synbiotics, gangliosides, nucleotides and sialic acid reduces infections during the first 18 months of life: The COGNIS study. *Journal of Functional Foods*, **2021**. *83*: p. 104529. http//dx.doi.org/10.1016/j.jff.2021.104529
43. Ramiro-Cortijo, D., et al., Breast milk lipids and fatty acids in regulating neonatal intestinal development and protecting against intestinal injury. *Nutrients*, **2020**. *12*(2): p. 534. http//dx.doi.org/10.3390/nu12020534
44. Billeaud, C., et al., Safety and tolerance evaluation of milk fat globule membrane-enriched infant formulas: a randomized controlled multicenter non-inferiority trial in healthy term infants. *Clinical Medicine Insights: Pediatrics*, **2014**. *8*: p. CMPed. S16962. http//dx.doi.org/10.4137/CMPed.S16962
45. Zavaleta, N., et al., Efficacy of an MFGM-enriched complementary food in diarrhea, anemia, and micronutrient status in infants. *Journal of pediatric gastroenterology and nutrition*, **2011**. *53*(5): p. 561-568. http//dx.doi.org/10.1097/MPG.0b013e318225cdaf
46. Ortega-Anaya, J., A. Marciniak, and R. Jiménez-Flores, Milk fat globule membrane phospholipids modify adhesion of Lactobacillus to mucus-producing Caco-2/Goblet cells by altering the cell envelope. *Food Research International*, **2021**(3): p. 110471. http//dx.doi.org/10.1016/j.foodres.2021.110471
47. Cichosz, G., H. Czeczot, and M. Bielecka, The anticarcinogenic potential of milk fat. *Annals of Agricultural and Environmental Medicine*, **2020**. *27*(4): p. 512-518. http//dx.doi.org/10.26444/aaem/116095
48. Bruno, R.S., et al., Cardiometabolic health benefits of dairy-milk polar lipids. *Nutrition reviews*, **2021**. *79*(Supplement_2): p. 16-35. http//dx.doi.org/10.1093/nutrit/nuab085
49. Noh, S.K. and S.I. Koo, Milk sphingomyelin is more effective than egg sphingomyelin in inhibiting intestinal absorption of cholesterol and fat in rats. *The Journal of nutrition*, **2004**. *134*(10): p. 2611-2616. http//dx.doi.org/10.1093/jn/134.10.2611

50. Maulik, P. and G. Shipley, N-palmitoyl sphingomyelin bilayers: structure and interactions with cholesterol and dipalmitoylphosphatidylcholine. *Biochemistry*, **1996**. *35*(24): p. 8025-8034. http//dx.doi.org/10.1021/bi9528356
51. Conway, V., et al., Impact of buttermilk consumption on plasma lipids and surrogate markers of cholesterol homeostasis in men and women. *Nutrition, Metabolism and Cardiovascular Diseases*, **2013**. *23*(12): p. 1255-1262. http//dx.doi.org/10.1016/j.numecd.2013.03.003
52. Eckhardt, E.R., et al., Dietary sphingomyelin suppresses intestinal cholesterol absorption by decreasing thermodynamic activity of cholesterol monomers. *Gastroenterology*, **2002**. *122*(4): p. 948-956. http//dx.doi.org/10.1053/gast.2002.32539
53. Rosqvist, F., et al., Potential role of milk fat globule membrane in modulating plasma lipoproteins, gene expression, and cholesterol metabolism in humans: a randomized study. *The American journal of clinical nutrition*, **2015**. *102*(1): p. 20-30. http//dx.doi.org/10.3945/ajcn.115.107045
54. Vors, C., et al., Milk polar lipids reduce lipid cardiovascular risk factors in overweight postmenopausal women: towards a gut sphingomyelin-cholesterol interplay. *Gut*, **2020**. *69*(3): p. 487-501. http//dx.doi.org/10.1136/gutjnl-2018-318155
55. Schmelz, E.M., Colonic Cell Proliferation and Aberrant Crypt Foci Formation Are Inhibited by Dairy Glycosphingolipids in 1,2-Dimethylhydrazine-Treated CF1 Mice. *Journal of Nutrition*, **2000**. *130*. http//dx.doi.org/10.1093/jn/130.3.522
56. Vesper, H., et al., Sphingolipids in food and the emerging importance of sphingolipids to nutrition. *The Journal of nutrition*, **1999**. *129*(7): p. 1239-1250. http//dx.doi.org/10.1093/jn/129.7.1239
57. Zanabria, R., et al., Milk fat globule membrane isolate induces apoptosis in HT-29 human colon cancer cells. *Food & Function*, **2013**. *4*. http//dx.doi.org/10.1039/C2FO30189J
58. Castro-Gomez, P., et al., Antiproliferative activity of buttermilk lipid fractions isolated using food grade and non-food grade solvents on human cancer cell lines. *Food Chemistry*, **2016**. *212*(dec.1): p. 695-702. http//dx.doi.org/10.3168/jds.2014-8128
59. Castro-Gomez, M.P., et al., Total milk fat extraction and quantification of polar and neutral lipids of cow, goat, and ewe milk by using a pressurized liquid system and chromatographic techniques. *Journal of Dairy Science*, **2014**. *97*(11): p. 6719-6728.
60. Vickers, M.H., et al., Supplementation with a mixture of complex lipids derived from milk to growing rats results in improvements in parameters related to growth and cognition. *Nutrition research*, **2009**. *29*(6): p. 426-435. http//dx.doi.org/10.1016/j.nutres.2009.06.001
61. Gurnida, D.A., et al., Association of complex lipids containing gangliosides with cognitive development of 6-month-old infants. *Early human development*, **2012**. *88*(8): p. 595-601. http//dx.doi.org/10.1016/j.earlhumdev.2012.01.003
62. Ryan, J.M., G.E. Rice, and M.D. Mitchell, The role of gangliosides in brain development and the potential benefits of perinatal supplementation. *Nutrition research*, **2013**. *33*(11): p. 877-887. http//dx.doi.org/10.1016/j.nutres.2013.07.021
63. Guillermo, R.B., et al., Supplementation with complex milk lipids during brain development promotes neuroplasticity without altering myelination or vascular density. *Food & nutrition research*, **2015**. *59*(1): p. 25765. http//dx.doi.org/10.3402/fnr.v59.25765
64. Rohrhofer, J., et al., The impact of dietary sphingolipids on intestinal microbiota and gastrointestinal immune homeostasis. *Frontiers in Immunology*, **2021**. *12*: p. 1690. http//dx.doi.org/10.3389/fimmu.2021.635704
65. Otnaess, A., A. Laegreid, and K. Ertresvåg, Inhibition of enterotoxin from Escherichia coli and Vibrio cholerae by gangliosides from human milk. *Infection and Immunity*, **1983**. *40*(2): p. 563-569. http//dx.doi.org/10.1128/iai.40.2.563-569.1983
66. Idota, T., H. Kawakami, and I. Nakajima, Growth-promoting effects of N-acetylneuraminic acid-containing substances on bifidobacteria. *Bioscience, biotechnology, and biochemistry*, **1994**. *58*(9): p. 1720-1722. http//dx.doi.org/10.1271/bbb.58.1720
67. Rueda, R., et al., Addition of gangliosides to an adapted milk formula modifies levels of fecal Escherichia coli in preterm newborn infants. *The Journal of pediatrics*, **1998**. *133*(1): p. 90-94. http//dx.doi.org/10.1016/S0022-3476(98)70184-2
68. Idota, T., et al., Inhibition of cholera toxin by human milk fractions and sialyllactose. *Bioscience, biotechnology, and biochemistry*, **1995**. *59*(3): p. 417-419. http//dx.doi.org/10.1271/bbb.59.417

 doi:10.20944/preprints202308.1184.v1

69. Yoshinaka, Y., et al., Light rhythmic exercise with dietary milk fat globule membrane improves physical fitness in an elderly Japanese population: a double-blind randomized placebo-controlled trial. *Bioscience, Biotechnology, and Biochemistry*, **2018**. *82*(4): p. 677-682. http//dx.doi.org/10.1080/09168451.2017.1412248
70. Snow, D.R., et al., Dietary milk fat globule membrane reduces the incidence of aberrant crypt foci in Fischer-344 rats. *Journal of Agricultural and Food Chemistry*, **2010**. *58*(4): p. 2157-2163. http//dx.doi.org/10.1021/jf903617q