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REVIEWS: CURRENT TOPICS

Protective properties of milk sphingomyelin against dysfunctional lipid metabolism, gut dysbiosis, and inflammation

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Abstract

The identification of natural bioactive compounds aimed at promoting optimal gut health and improving lipid metabolism is paramount in the prevention of chronic disease. In this review, we summarize basic science and clinical research examining the protective properties of milk sphingomyelin (SM) against dysfunctional lipid metabolism, gut dysbiosis, and inflammation. Dietary SM dose-dependently reduces the intestinal absorption of cholesterol, triglycerides, and fatty acids in cell culture and rodent studies. Overall, rodent feeding studies show dietary milk SM, milk polar lipid mixtures, and milk fat globule membrane reduce serum and hepatic lipid concentrations. Furthermore, these hypolipidemic effects are observed in some supplementation studies in humans, although the extent of reductions in serum cholesterol is typically smaller and only one trial was conducted with purified SM. Dietary milk SM has been reported to affect the gut microbiota in rodent studies and its hydrolytic product, sphingosine, displays bactericidal activity *in vitro*. Milk SM may also improve gut barrier function to prevent the translocation of inflammatory gut bacteria-derived molecules. Current evidence from pre-clinical studies indicates that dietary milk SM has protective properties against dysfunctional lipid metabolism, gut dysbiosis, and inflammation. The hypolipidemic effects of milk SM observed in animal studies have been reported in some human studies, although the magnitude of such effects is typically smaller. More research is warranted to clearly define how dietary milk SM influences lipid metabolism, gut microbiota, and inflammation in humans.
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1. Introduction

Overnutrition can promote hyperlipidemia, ectopic lipid accumulation, and inflammatory responses in adipose tissue, liver, and intestine, which can manifest as chronic, low-grade inflammation. The intestinal absorption of cholesterol and saturated fat are widely recognized to influence both fasting plasma lipids [1–3] and inflammation [4–7], the major drivers of cardiometabolic disease. Dietary patterns rich in saturated fat, cholesterol, and sugar, so called "Western" diets, can drive inflammation through the accumulation of toxic lipid species in metabolic tissues, also known as lipotoxicity [8]. High-fat diets (HFDs) have also been reported to increase intestinal inflammation and gut barrier permeability to lipopolysaccharides (LPS), a component of Gram-negative bacteria [9–12]. This appears to be partially dependent on chylomicron secretion subsequent to lipid absorption, changes in gut microbiota, and infiltration of pro-inflammatory macrophages in the colon [10,12–14]. LPS is thought to contribute to systemic inflammation and metabolic disease by activating Toll-like receptor 4 (TLR4) signaling pathways throughout the body [15]. Additionally, other classes of bacterial products, such as lipopeptides, have become associated with inflammation and chronic diseases via TLR2 activation [16–18]. Compelling evidence now links the development of systemic inflammation and metabolic disease with alterations in gut health. Thus, there is a critical need to identify therapeutic strategies aimed at improving dysfunctional lipid metabolism, gut health, and host-microbiota interactions in the management and prevention of disease.

Polar lipids (PLs) are major components of all biological membranes and found primarily as glycerophospholipids and sphingolipids in the human diet. Consumption of dietary sphingolipids in Western diets is relatively common and estimated to be in the range of 300-400 mg/day [19]. In Western countries, dietary sphingolipids mainly consist of sphingomyelin (SM) derived from dairy (~25% of total milk PLs), meat (~5-10% of total meat PLs), and egg (~1.5% of total egg PLs) [19–22]. As other animal sources, fish meat also contains SM in the order of 10 mg/100g [19,22] (in the range 2-10% of total fish PLs [22,23]). Appreciable quantities of dietary sphingolipids reach the colon intact or partially digested where they can interact with the gut microbiota. Indeed, Nilsson et al. [24] have established that 25% of SM was found in feces of rats in the undegraded form (10%), ceramide form (30–90%) and free sphingosine form (3–6%). More recently, human ileostomy content have shown that ~19 to 25% of dietary SM went unabsorbed [25,26]. Dietary SM has demonstrated potential to influence inflammation through inhibiting intestinal lipid absorption [27,28], altering gut microbiota [29], and neutralizing LPS [30]. However, currently little is known about an optimal intake of SM; as the impact of dietary SM on health has been largely overlooked. Significant research efforts have been dedicated to the study of phytonutrients and their impact on health, whereas less is known about zoonutrients, such as SM. The purpose of this review is to summarize the basic science and clinical research examining the effects of milk SM intake, the most important source of dietary SM, on dysfunctional lipid metabolism, gut dysbiosis, and inflammation.

2. Milk SM as a dietary component

2.1. Milk SM structure and dietary sources

Dietary SM consists of a ceramide with a phosphorylcholine head group. Ceramide is composed of a fatty acid of varying properties that is connected to a long-chain sphingoid base through an amide linkage. SM is considered a zoonutrient, as it is abundant in animal cell membranes but is not found in plants, bacteria, or fungi [31]. SM can thus be found in animal-derived food products, such as fishes, meats, eggs, and milk. In milk, SM is located within the milk fat globule membrane (MFGM), which is a specific trilayered biological membrane derived from both the endoplasmic reticulum membrane and the cell membrane of lactating cells [32,33]. Like in all animal cell membranes, PLs are the major structural lipids and SM are located therein within lipid rafts [34]. PLs account for about 1% of total lipids in milk; the SM content comprises approximately 25% of total milk PLs, and SM is found at ~3:1 ratio to cholesterol by mass [35]. Notably, dairy products vary quite considerably when comparing PL to total lipid content because of the various dairy processes that remove more or less of the fat globules or drain more or less whey (Table 1). Milk processing (churning, centrifuging, homogenization, spray-drying) is also known to affect the molecular profile of milk PLs [36]. SM is also a major PL in human milk, and infants are estimated to consume up to 150 mg of SM per day [37]. Feeding bovine milk SM to rat pups has been shown to improve markers of gut maturation [38], providing evidence of the importance of dietary SM for newborn gut development. Rich sources of bovine milk SM in the human diet include cottage cheese (139 mg SM/100 g food), buttermilk quark (74 mg SM/100 g food), and butter serum (379 mg SM/ 100 g food) [39,40]. Butter serum is a dairy processing co-product which consists of the aqueous phase removed during anhydrous milk fat production, and may be an inexpensive, yet rich source of milk SM [40]. Whole milk contains about 2-fold greater SM content than fat-free milk [39]. Bovine milk SM contains a natural mixture of SM species which vary in their fatty acid and sphingoid base composition [40–42]. The fatty acid composition of milk SM contains significant amounts of very-long chain fatty acids (C22:0-C24:0); a characteristic which is different from egg SM, which is primarily composed of palmitic acid (C16:0). Milk SM also has a more varied distribution of sphingoid bases (d16:0 to d19:0) than egg SM, which is primarily sphingosine (d18:1). These differences may contribute to differences in biological activity observed between milk SM and egg SM [28,29].

2.2. Dietary SM digestion and absorption

Dietary SM does not appear to be absorbed by the intestine intact, but through a delayed and incomplete digestion that can yield the bioactive lipids, ceramide and sphingosine, in the distal small intestine [43]. Alkaline sphingomyelinase (Alk-SMase) found on the brush border membrane and in human bile hydrolyzes SM to ceramide and phosphocholine. Phosphocholine is hydrolyzed by phosphatases in the gastrointestinal (GI) tract and is absorbed as choline. Long-chain ceramides generated by Alk-SMase are likely not absorbed intact, but are further hydrolyzed by brush border neutral ceramidase (N-CDase) to yield sphingosine and a fatty acid, which are both readily absorbed by enterocytes. Sphingosine may then be reincorporated into complex sphingolipids, although it is mainly converted to palmitic acid, which is then incorporated into chylomicron triglycerides (TG) and secreted into the lymphatic duct [24,44]. The presence of gut microbiota may be important for SM digestion, as germ-free mice have reduced SM

Table 1
Milk polar lipids content in different dairy products. Adapted from [36,136] in [137].

Polar lipids (g/100g) *							
Raw milk	Skimmed milk	Cream	Cream pasteurized	Butter (batch)	Buttermilk	Butter serum	Cheddar
g/100 g of product							
0.03-0.04	0.02	0.19	0.14	0.14-0.23	0.16	1.25	0.15
g/100 g of dry matter							
0.23-0.32	0.28	0.40	0.31	0.17-0.26	2.03	11.6	0.25
g/100 g of fat							
0.7-0.98	19.1	0.45	0.4	0.2-0.27	33.1	48.4	0.5

* Among which 20-28%, on average 25%, is sphingomyelin.

hydrolysis in the colon compared to conventionally-raised mice [45]. Very-long chain ceramide species found in milk SM appear in the lymph of rats after feeding milk PLs, suggesting that some milk-derived sphingosine and fatty acids are reincorporated into complex sphingolipids and can enter circulation [46]. However, how ingestion of dietary SM potentially impacts sphingolipids in the bloodstream, notably in chylomicrons, during the intestinal absorption of lipids remains to be established in humans.

3. Hypolipidemic effects of dietary SM

3.1. Overview of cholesterol and fat absorption

In order to understand potential SM action, we will provide a brief summary of dietary lipid and cholesterol absorption mechanisms. Because cholesterol is a fat-soluble molecule with limited solubility in aqueous domains, it must first be hydrolyzed to a non-esterified form and solubilized in bile for proper absorption by the intestine. A sufficient amount of bile is compulsory for both exogenous (i.e., dietary) and endogenous (e.g., biliary) cholesterol absorption in the intestinal lumen [47]. After incorporation into bile salt mixed micelles, dietary (~1/3 of cholesterol intake) and biliary free cholesterol (~2/3 of cholesterol intake) are able to diffuse through the unstirred water layer for absorption by the proximal intestine via passive-mediated processes [48]. A number of facilitative transport proteins are involved in cholesterol uptake by enterocytes, however, the major protein involved seems to be Niemann-Pick C1–like 1 (NPC1L1) [48]. After endocytosis of cholesterol-NPC1L1 complexes by enterocytes, cholesterol is trafficked to the endoplasmic reticulum (ER) where it is esterified by acyl-coenzyme A:cholesterol acyltransferase 2 (ACAT2) [49]. Subsequently, cholesteryl esters are incorporated into nascent chylomicrons by microsomal triglyceride transfer protein (MTTP) and apoB-48 at the ER, with final processing in the Golgi complex before secretion into the lymph [48].

The digestion of dietary fat (mostly TG) in humans begins in the stomach via the action of gastric lipase, which works particularly well on medium- and short-chain TG [50,51]. The hydrolysis of dietary fat in the stomach produces partial glycerides and free fatty acids, which helps to emulsify the TG for lipases found in the intestinal lumen [50,51]. The bulk of dietary fat digestion occurs in the proximal small intestine through the actions of the pancreatic lipase-colipase complex. Pancreatic lipase works only at the emulsion-water interface; colipase first binds to the fat emulsion interface, which subsequently promotes its binding to pancreatic lipase [51]. Pancreatic lipase produces free fatty acids and 2-monoglycerides from fat emulsions, and these hydrolytic products are further emulsified into mixed micelles through the actions of bile. The lipolytic products of fat digestion are then taken up by the enterocyte by passive diffusion and carrier-mediated pathways (e.g., CD36) before fatty acids are re-esterified and assembled into chylomicrons along with cholesterol and apolipoproteins [50].

3.2. Evidence of inhibition of cholesterol absorption by SM

The biophysics of SM and cholesterol interactions have been studied quite extensively, due to their preferred assocation in cellular membranes [52]. Dietary SM and its hydrolytic products (ceramide, sphingosine) have been shown to dose-dependently reduce the cellular uptake of cholesterol in intestinal cell culture systems [53–56] and intestinal cholesterol absorption in rodent studies [27,28,53,57–59]. Several proposed mechanisms have been gleaned from these studies for the sphingolipid-mediated inhibition of cholesterol absorption, which comprise both cell-dependent and -independent effects, and includes: (1) strong hydrogen bonding/interactions of SM with cholesterol leads to tighter molecular packing in micelles which slows cholesterol release for cellular uptake [28,57]; (2) reduced micellar solubility for cholesterol results in decreased thermodynamic activity, or active concentration, of cholesterol monomers available for cellular uptake [53], and (3) SM decreases the expression of enterocyte NPC1L1 and/or its capacity for cholesterol uptake [54,56].

With regard to SM-cholesterol interactions, the interfacial region of SM is more polar than in other phospholipids, and the strong hydrogen bonding capacity of SM is known to be critical for its interactions with other lipids in cell membranes [52]. Important SM intermolecular hydrogen bonds are thought to be formed by the 2–NH group of SM with the hydroxyl group of cholesterol, while interactions between the choline nitrogen and hydroxyl group of cholesterol could also occur [52]. Furthermore, intermolecular hydrogen bonding between the phosphate oxygen and 3–OH group of SM molecules may create a network that will interfere with cholesterol desorption from these lipid complexes [52]. Evidence suggesting a mutual inhibition of the intestinal absorption of SM and cholesterol was first reported by Nyberg et al. [57]. In a series of experiments, rats were intragastrically-administered milk SM-containing lipid emulsions containing either radiolabeled cholesterol or radiolabeled dihydro-SM. Lipid emulsions that contained SM dose-dependently lowered radiolabeled cholesterol absorption, as measured with the dual-isotope plasma ratio method. The maximum effect was seen with a SM:cholesterol molar ratio of 1:1 (14% cholesterol absorption at this ratio vs. 68% cholesterol absorption in control). Interestingly, when tracking radiolabeled dihydro-SM absorption, increasing the sterol (cholesterol and sitostanol) content of lipid emulsions had a reciprocal inhibition of SM absorption, demonstrated by increased recovery of radioactivity in feces of rats (38% SM radioactivity recovered in feces with SM + cholesterol in 1:1 molar ratio vs. 16% SM radioactivity recovered with only SM). Thus, the sterol content of diets is expected to be an important factor in determining the bioavailability of dietary SM. Notably, SM is known to be incompletely digested in the proximal GI tract, possibly due to the fact that both cholesterol and SM are often found together in membrane structures of foods, such as in MFGMs.

Another factor that may influence SM-cholesterol interactions is the amide-linked fatty acid chain characteristics. It has been reported that cholesterol has greater affinity for phospholipids containing saturated fatty acids chains [60]. Furthermore, the length of the amide-linked fatty acid chains also appears to be important, as naturally-derived SM species with varying fatty acid composition have shown differential effects on intestinal cholesterol absorption. Noh et al. [28] intragastrically-administered cholesterol-containing lipid emulsions with either milk SM or egg SM to lymph cannulated rats, and found that radiolabeled cholesterol absorption was inhibited greater with the milk SM compared to egg SM. This may be due to the fact that milk SM consists of longer-chain saturated fatty acids than those in egg SM, which may allow the SM acyl chains and cholesterol to pack more tightly, slowing the release of micellar lipids to the enterocyte.

Garmy et al. [54] reported that sphingosine also formed condensed lipid complexes with cholesterol similar to SM. A significant dose-dependent inhibition of cholesterol uptake in both Caco-2 and HT-29-D4 human intestinal cell lines was observed with sphingosine. The authors suggested that sphingolipid-cholesterol complexes may not be able to enter cells via NPC1L1 due to their larger size compared to lipid monomers. Through use of different structurally-related compounds, sphingosine-cholesterol interactions were revealed to be dependent on the terminal 1–OH and amino ($-NH_2$) groups of sphingosine, and not dependent on non-specific hydrophobic binding. The protonated form of the amino group ($-NH_3^+$) may allow for hydrogen bonding with the hydroxyl group of cholesterol, as the greatest interactions were seen between pH 2-7, with a gradual decreasing up to pH 12 (50% of maximal interaction). The terminal 1–OH and amino groups of sphingosine are thought to form

intramolecular bonds that allow for optimal interaction with cholesterol. However, Feng et al. [55] found that sphingosine was the least effective based on molar ratio to cholesterol of several sphingolipids tested (SM, ceramide, sphingosine). Micelles containing SM:cholesterol at a 1:1 molar ratio caused a ~40% reduction in cholesterol uptake in Caco-2 cells, while the inclusion of C16:0-ceramide at a 1:1 molar ratio to cholesterol caused a ~50% reduction. Interestingly, sphingosine also inhibited cholesterol uptake albeit much weaker (~40% reduction at 8:1 molar ratio with cholesterol), likely due to the rapid absorption of sphingosine into cells. Therefore, SM digestion to ceramide in the intestinal lumen would be expected to enhance the inhibition of cholesterol uptake, while digestion of ceramide to sphingosine would be expected to decrease this effect. Notwithstanding, Duivenvoorden et al. [58] showed that a 1% (w/w) dose of phytosphingosine was still able to significantly reduce dietary cholesterol absorption by ~50% in *ApoE*3*Leiden mice, as measured by the fecal dual-isotope method.

It has also been suggested that the tight interactions between SM and cholesterol in vesicles render these complexes more resistant to bile salt detergents than lecithin-cholesterol vesicles, resulting in reduced micellar solubilization of cholesterol [53]. This reduction of micellar cholesterol solubility would be expected to decrease monomeric cholesterol concentrations, and therefore, lower its intestinal absorption. Eckhardt et al. [53] determined that milk SM reduced the thermodynamic activity of cholesterol, i.e., the active concentration of cholesterol monomers. Furthermore, they reported a significant dose-dependent inhibition of cholesterol uptake in Caco-2 cells by milk SM (~6-50-fold molar excess of SM:cholesterol). In this study, the thermodynamic activity of model micelles containing SM were highly correlated with cholesterol uptake in Caco-2 cells, suggesting effects on cholesterol absorption were largely independent of any changes in cell function. Finally, *in vivo* studies using mice showed incorporation of milk SM into chow diets could dose-dependently inhibit cholesterol absorption, as assessed by the fecal dual-isotope ratio method. Diets containing 5%, 0.5%, and 0.1% of milk SM (w/w) resulted in reductions in cholesterol absorption of 86%, 54%, and 20%, respectively. Although early experiments mainly supported a cell-independent effect of SM due to lipid-lipid interactions, Yang et al. [56] showed that egg SM-containing micelles (0.3-1.2 mM SM concentrations) markedly reduced NPC1L1 mRNA and protein expression in polarized Caco-2 cells. These effects were found alongside approximate 72% reduction in apical-basolateral uptake of cholesterol (SM:cholesterol molar ratio of 6), while no effect was observed on basolateral-apical uptake. The authors suggested this effect of SM was due to a downregulation of NCP1L1 transcription, concomitant with reduced expression of its transcriptional regulator, SREBP-2. They also reported SM/cholesterol lipid complexes increased the apparent molecular weight of mixed micelles, which they speculated would result in lower cholesterol uptake via NPC1L1. Egg SM was also reported in this study to have increased taurocholate binding capacity compared to glycerophospholipids, and was shown to lower the micellar solubilities of both cholesterol and taurocholate.

In contrast to pre-clinical models, only limited studies have been conducted on SM:cholesterol interactions in humans. A small-scale human crossover study conducted in healthy adults did not find evidence of 1 g/day of milk SM supplementation affecting intestinal cholesterol absorption, by a dual-stable isotope method [61]. In this study, a cholesterol absorption coefficient was calculated using the

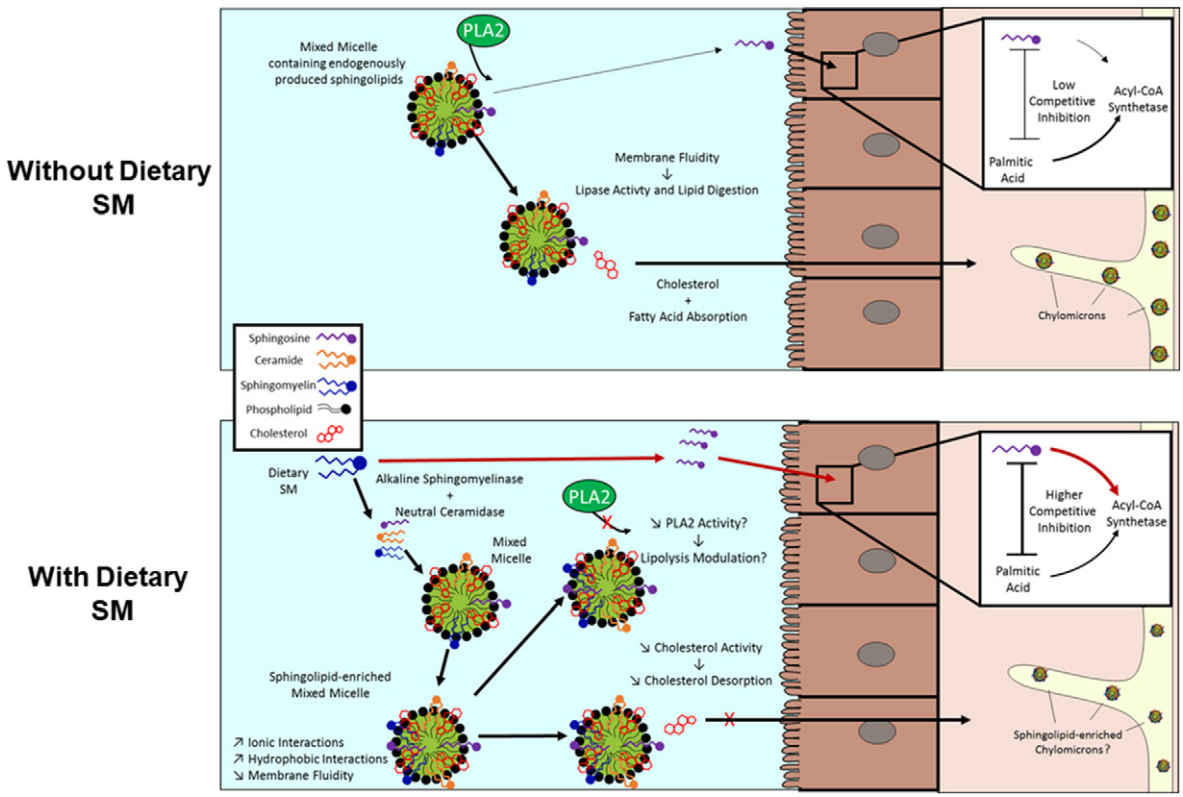

Fig. 1. **Potential mechanisms to explain dietary milk SM's inhibition of intestinal lipid absorption.** Evidence from pre-clinical studies suggests that dietary SM and its hydrolytic products can inhibit lipid absorption via both intestinal cell-dependent and -independent mechanisms. Sphingolipid-mediated inhibition of cholesterol absorption may be due to their ability to decrease the thermodynamic activity, or active concentration, of cholesterol monomers available for cellular uptake. For fat absorption, the presence of sphingomyelin in mixed bile salt micelles has been reported to inhibit human pancreatic lipase-colipase activity *in vitro*, potentially affecting TG hydrolysis. Long-chain bases (e.g., sphingosine) have also been reported to compete with palmitic acid for cellular uptake *in vitro* through a process dependent on acyl-CoA synthetases. Acyl-CoA synthetases are involved in long-chain fatty acid uptake into cells, via their catalytic conversion of fatty acids to acyl-CoAs as well as putative transport activity. PLA2, pancreatic phospholipase A2. SM, sphingomyelin.

ratio of orally ingested ^{13}C-cholesterol to intravenously administered D_7-cholesterol as red blood cell free cholesterol. Purified milk SM was added at a daily dose of 1 g to prepared diets, which also included 240 mg cholesterol per day. Milk SM-containing diets were consumed for 2 weeks prior to undergoing intestinal cholesterol absorption tests. Furthermore, Ohlsson et al. [25] did not observe any changes in total cholesterol content of ileostomy outputs of subjects consuming different amounts of milk SM (50-200 mg) within a test meal. Although these short-term, human studies of limited sample size showed no effect, overall evidence from pre-clinical studies suggests that SM and its hydrolytic products can inhibit cholesterol absorption via both intestinal cell-dependent and -independent mechanisms (Fig. 1). This was very recently confirmed in humans in a crossover clinical trial, where 4 ileostomized subjects consumed different cream cheeses with varying enrichment of milk PLs (including milk SM) via a butter serum concentrate: either control (no milk PL/no milk SM), 3 g milk PLs (0.8 g milk SM), or 5 g milk PLs (1.3 g milk SM) [26]. An increased ileal output of both total cholesterol (of dietary + endogenous origin) and of milk SM was observed in ileal efflux after both milk PL-rich meals. This could have been enhanced by the fact that SM was consumed here as part of a complex milk PL mixture within the cheese (from MFGM fragments of the butter serum ingredient), rather than pure. Thus, more research should be conducted in humans examining effects of milk SM on lipid absorption, since it is likely that a higher SM dose is required than in mice, due to alk-SMase also being present in human bile. Furthermore, we cannot rule out that milk SM ingestion would be more effective in humans when provided within intact MFGM or MFGM fragments in dairy, as this may impact SM bioavailability. Indeed, a study conducted in rats showed that co-ingestion of milk SM with glycerophospholipids improved the bioavailability of SM [46]. The authors speculated that the inclusion of phosphatidylcholine (PC) in the milk PL emulsion improved solubility and inhibited SM crystallization, resulting in increased bioavailability of dietary SM.

3.3. Evidence of inhibition of fat absorption by SM

Early experiments reported an inhibition of human pancreatic lipase-colipase activity by SM when present in mixed bile salt micelles [62]. A lower *in vitro* lipolysis of lipid droplets by pancreatic lipase was then observed by Armand et al. [63] when droplets were covered by SM compared with PC. Because hydrolysis of SM is slow and incomplete throughout the GI tract, it is expected that this effect may impede fat absorption. In a similar way to cholesterol, Noh et al. [28] showed that the inclusion of SM in lipid emulsions reduced the total fatty acid output in lymph of cannulated rats, with milk SM having a greater effect than egg SM. Mathiassen et al. [64] reported that the *in vitro* lipolysis of ^{3}H-triolein-containing emulsions by pancreatic lipase was lower when they contained milk PLs, a source of SM, compared to soy lecithin. In contrast, gastric lipase activity towards lipid emulsions containing milk PLs was enhanced compared to soy lecithin, with a greater combined lipolysis rate for milk PLs when both enzymes were tested. These effects were speculated to be due to the higher saturation of fatty acids in milk PC compared to soy lecithin, as increasing the concentration of di-stearoyl PC increased the gastric lipase activity towards soy lecithin emulsions. In vitro, Lecomte et al. [65] demonstrated that milk PLs could increase intestinal TG hydrolysis compared with soy lecithin. The opposing effects of milk PLs on TG hydrolysis by pancreatic lipase (inhibition), gastric lipase (stimulation), and gastric lipase + pancreatic lipase (stimulation) are intriguing. Rodents are nearly lacking in gastric lipase activity, while humans have considerable activity throughout their lifespan [66]; thus, this notable difference in lipases may lead to species differences in the effects of milk PLs on TG digestion. Notably, the addition of purified milk SM to soy lecithin emulsions inhibited pancreatic lipase activity, and a combined gastric lipase and pancreatic lipase hydrolysis [64]. However, regardless of concentration, the milk SM did not impact gastric lipase activity towards radiolabeled lipid emulsions. Interestingly, the absorption of radiolabeled fatty acids from milk PLs stabilized emulsions was slower than from soy lecithin emulsions in mice, but ultimately result in similar fatty acid absorption overall. This suggests a greater proportion of fatty acids reach the distal gut before absorption in lipid emulsions containing milk PLs, with potential consequences on the gut microbiota.

Sphingoid bases may be responsible for some of the reported effects of SM on fat absorption, as they are known to compete with palmitic acid for intestinal cell uptake via acyl-CoA synthetase transporters [67]. Acyl-CoA synthetases are thought to be involved in long-chain fatty acid (LCFA) uptake into cells, via their catalytic conversion of fatty acids to acyl-CoAs as well as putative transport activity. In both yeast (BY4741) and mammalian intestinal cell (IEC) lines, dihydrosphingosine treatment dose-dependently inhibited the uptake of radiolabeled palmitic acid (50% at 1:1 and 80% at 4:1 molar ratios). Reciprocally, palmitic acid treatment dose-dependently inhibited the uptake of radiolabeled dihydrosphingosine, revealing important competitive interactions that presumably would affect LCFA and sphingoid base bioavailability in Western diets. However, the palmitic acid content of typical Western diets far outweighs the sphingoid base content, and thus likely exceeds the capacity of sphingoid bases to inhibit palmitic acid to any significant extent. Nevertheless, Duivenvoorden et al. [58] reported an inhibitory effect of phytosphingosine on monounsaturated fat absorption. Mice that were orally gavaged with olive oil had significantly lower ^{3}H-triolein (-33%) and ^{14}C-oleic acid (-43%) absorption when the dispersion contained 1% (w/w) phytosphingosine. Furthermore, since phytosphingosine was effective against the absorption of both radiolabeled lipids, it suggested that fatty acid absorption was affected rather than TG lipolysis. It was postulated by the authors that these effects could have been due to ionic interactions between positively-charged sphingoid backbones and negatively-charged fatty acids in lipid complexes. However, further research in this area is needed to confirm this notion. Fig. 1 summarizes the potential mechanisms that milk SM may inhibit fat absorption as reported in the literature. It will be important to examine if dietary fat absorption is impacted by milk SM in humans, since there are known species differences in digestive lipase activities between rodents and humans [66]. Recent human results by Vors et al. [26] report a decreased high-fat, high-sucrose meal-induced chylomicronemia after 4 weeks of daily consumption of cream cheese enriched with 5 g milk PLs (includes 1.3 g milk SM). These initial findings encourage further investigations in the field.

3.4. Effects of dietary milk SM consumption on serum and hepatic lipids in animals

Several *in vivo* studies demonstrate hypolipidemic effects of purified dietary SM [29,58,68,69] and SM-rich fractions from different food sources [70–72]; however, it is important to note that some studies report no effects [59,72,73] or even hyperlipidemic effects of some dietary SM sources [29,74]. Furthermore, in mice, milk PLs (1.2% w/w) increased postprandial lipemia compared to soybean PLs, consistent with enhanced digestive lipolysis (as per residual lipid digestion products in small intestinal lumen and *in vitro* digestion experiments) [65]. Several research groups have investigated the effects of dairy PL extracts rich in SM on hyperlipidemia in rodents (Table 2) [70–72]. Several studies have found that milk PL extracts reduce both serum and hepatic lipids in mice fed HFD [71,72], while one study found no effect when fed with a low-fat diet [72]. In contrast, feeding obese KK-A^y mice either lipid concentrated butter serum or a milk-derived ceramide-rich fraction, but not a SM-rich fraction, was shown to significantly lower serum and hepatic lipids [70]. Norris et al.

Table 2
Animal studies examining effects of milk sphingomyelin on serum and hepatic lipid concentrations

Author	Year	Animal Model	Diet and Duration	Results
Sphingomyelin				
Nyberg et al. [57]	2000	Male Sprague-Dawley rats ($n = 5$-8)	Mixture of Cholesterol:SM molar ratio 2.6:1 or 1:1 *vs* 0.5:1.	Mixture of SM and cholesterol ↓ intestinal cholesterol absorption
Norris et al. [29]	2016	Male C57BL/6J mice ($n = 10$)	4 weeks on HFD (21% AMF by weight) with 0.25% (w/w) milk SM	Serum Lipids: Milk SM: ↓ TC (-15%), ↔ TG, ↔ PL; Hepatic Lipids: Milk SM: ↓ TG (-47%), ↔ TC
Norris et al. [138]	2017	Male C57BL/6J mice ($n = 14$)	10 weeks on HFD (31% lard; 0.15% cholesterol by weight), 0.1% (w/w) milk SM or egg SM	Serum Lipids: Milk SM: ↔ TC, ↔ TG; Hepatic Lipids: Milk SM: ↓ TC (-23%), ↓ TG (-30%)
Yamauchi et al. [141]	2016	Obese/diabetic KK-A^y ($n = 7$) and Male C57BL/6J ($n = 6$)	4 weeks on HFD (lard, soybean, linseed or fish) + 1% (w/w) milk SM (purified from butter serum)	KK-A^y mice/Serum Lipids: Soybean: ↓ non HDL-cholesterol, ↓ LDL-cholesterol Linseed: ↓ LDL-cholesterol KK-A^y mice/Hepatic lipids: Lard and Linseed: ↓ total FA KK-A^y mice/Fecal lipids: Lard and soybean: ↑ total lipid, ↑ cholesterol, ↑ bile acid Linseed: ↑ total lipid, ↑ cholesterol No effect in wild type mice
SM-rich ingredients				
Noh et al. [28]	2004	Male Sprague-Dawley rats ($n = 5$)	Infusion in stomach with catheter: Lipid emulsion + 80 μmol milk SM	Lymphatics Lipids: ↓ NEFA, ↓ phospholipids ↓ intestinal cholesterol absorption
Eckhardt et al. [53]	2002	Male C57L/J mice ($n = 6$)	4 days of PL-enriched diet in chow (0.1%, 0.5% or 5% of milk SM by weight)	↓ intestinal cholesterol absorption
Wat et al. [72]	2009	Male C57BL/6 mice ($n = 10$)	8 weeks of either LFD or Western-type diet (21% AMF; 0.15% cholesterol by weight) with and without 1.2% (w/w) PL from phospholipid-rich dairy milk extract (PLRDME) (0.26% of diet by weight as SM)	Serum Lipids: PLRDME with LFD: ↔ TC, ↔ TG, ↔ phospholipids, ↔ HDL-C; PLRDME with Western-type diet: ↓ TC (-23%), ↓ TG (-20%), ↓ phospholipids (-21%), ↓ HDL-C (-19%) Hepatic Lipids: PLRDME with LFD: ↔ total lipid, ↔ TG, ↔TC, ↔ phospholipids; PLRDME with Western-type diet: ↓ total lipid (-33%), ↓ TG (-44%), ↓ TC (-48%), ↓ phospholipids (-16%)
Kamili et al. [71]	2010	Male C57BL/6 mice ($n = 10$)	3, 5 or 8 weeks of Western-type diet (21% AMF; 0.15% cholesterol by weight) with and without 1.2% (w/w) PL from phospholipid-rich dairy milk extract (PLRDME) (0.26% SM w/w) or 0.2% (w/w) milk phospholipid concentrate (PC-700) (0.34% SM w/w)	Plasma Lipids: PLRDME after 8 weeks: ↓ TC (-23%), ↔ TG PC-700: not reported Hepatic Lipids: PLRDME after 5 weeks: ↓ total lipid (-41%), ↓ TG (-47%), ↓ TC (-39%) PLRDME after 8 weeks: ↓ total lipid (-18%), ↓ TG (-28%) PC-700 after 5 weeks: ↓ total lipid (-45%), ↓ TG (-63%), ↓ TC (-57%)
Watanabe et al. [70]	2011	Female KK-A^y mice ($n = 7$)	4 weeks of AIN-93G diet with 1.7% (w/w) of lipid-concentrated butter serum (LC-BS) (0.13% SM, 0.18% ceramides by weight in diet) or 0.5% (w/w) of ceramide-rich fraction (Cer-fr) (0.34% ceramide by weight in diet) or 0.5% (w/w) of SM-rich fraction (SM-fr) (0.25% SM by weight in diet)	Plasma Lipids: LC-BS: ↓ TC (-18%), ↓ LDL-C (-45%)↔ TG, ↔ phospholipids, ↔ HDL-C Cer-fr: ↓ TC (-25%), ↔ LDL-C, ↔ TG, ↔ phospholipids, ↔ HDL-C SM-fr: ↔ TC, ↔ TG, ↔ phospholipids, ↔ HDL-C; Hepatic Lipids: LC-BS: ↓ TG (-27%), ↔ TC Cer-fr: ↓ TG (-38%), ↓TC (-47%) SM-fr: ↔ TG, ↔ TC
Zhou et al. [139]	2012	Fischer-344 rats ($n = 3$-4)	12 weeks of AIN-76A diet with 2.5% (w/w) AMF, 2.5% (w/w) MFGM (including 0.11% of SM) *vs* corn oil or AMF (0.5% w/w)	Hepatic Lipids: ↓ esterified cholesterol ↑ TG
Reis et al. [98]	2013	Male C57BL/6J ($n = 13$)	5 weeks on a HFD control + 5 weeks on a HFD (~20% lard by weight) with 1.7% (w/w) total polar lipids extracts or 1.4% (w/w) PL-rich extract or 0.4% (w/w) SM-rich extract	Hepatic Lipids: Total PL extract and PL-rich extract : ↓ FA synthesis SM-rich extract: trend for ↓ TG synthesis and ↓ 16:1n-7/16:0
Lecomte et al. [65]	2015	Female Swiss mice ($n = 7$)	Gavaged with emulsion + 5.7 mg of milk PLs (control = emulsion with SPL)	Plasma Lipids: Milk PL *vs* SPL: trend for ↑ TG, ↑ NEFA after 1h ↓ TG and NEFA after 4h Duodenum gene expression; Milk PL *vs* SPL: plasma TG and NEFA associated

Table 2 (*continued*)

Author	Year	Animal Model	Diet and Duration	Results
				with ApoB48 and Sar1b *In vitro* study : ↑ TG hydrolysis
Lecomte et al. [140]	2016	Male C57BL/6J (n = 10-12)	8 weeks on HFD (17% w/w palm oil) + 1.2 % (w/w) milk PLs or SPL	Hepatic lipids : ↔ total lipid, ↔ TG, ↔ phospholipids Plasma Lipids: ↔ no differences Fecal lipids : ↑ VLCFA such as C22:0, C24:0 and C22:4(n-6)
Zhou et al. [120]	2019	Male ob/ob mice (n = 11-18)	2 weeks on moderately high-fat AIN-93G diet (34% kcal as fat) (CO) with and without 0.2% (w/w) milk gangliosides (GG) or 1% (w/w) milk PLs (PL)	↑ NEFA, ↑ DAG, ↔ TC Hepatic Lipids: GG: ↔ CE, ↔ TG, PL: ↓ CE, ↔ TG Plasma Lipids: GG: ↔ TC, ↔ TG, ↔ PL, ↔ SM, ↔ NEFA, ↔ DAG PL: ↑ NEFA, ↑ PL, ↑ SM,

AMF, anhydrous milk fat; ApoB, apolipoprotein B; CE, cholesteryl ester; DAG, Diacylglycerol; FA, fatty acid; FC, free cholesterol; GG, gangliosides; HDL, high-density lipoprotein; HFD, high-fat diet; LDL, low-density lipoprotein; LFD, low-fat diet; NEFA, nonesterified fatty acids; PL, polar lipid; PLRDME, phospholipid-rich dairy milk extract; Sar1B, Secretion Associated: SM, sphingomyelin; SPL, soybean polar lipids, TC, total cholesterol; TG, triglyceride; VLCFA, very long-chain fatty acids; ↑, increase; ↓ decrease; ↔, no change

found that feeding purified milk SM to HFD-fed mice reduces hepatic lipids when fed at both 0.1% (w/w) and 0.25% (w/w) of diet [29,68]; however, only 0.25% (w/w) milk SM significantly lowered serum cholesterol [29], whereas 0.1% (w/w) milk SM had no effect [68]. One area of further study would be to determine whether milk SM affects endogenous sphingolipid metabolism in tissues such as intestine and liver. One mouse study showed only a minimal effect of dietary egg SM (0.6% w/w of diet) on liver SM or ceramide content, with the exception that 24:1-ceramide was lower in mice fed egg SM [59].

3.5. Effects of dietary SM consumption on serum lipids in humans

To date, only one small clinical trial has addressed dietary supplementation with purified milk SM [61]. Although there was no effect on non-HDL-C, the authors found a significant increase in HDL-C in healthy adults supplemented with 1 g/ day of milk SM for 2 weeks. In addition to this study, there are clinical trials examining the use of other purified sphingolipids. Although this means the components are not identical, the structural similarities among sphingolipids suggest milk SM may have similar hypolipidemic effects, which will deserve confirmation. For instance, providing adult men with metabolic syndrome (MetS) 1 g/day of phytosphingosine for 4 weeks led to a significant reduction in plasma LDL-C [75]. Several research groups have reported findings from clinical trials evaluating milk PLs using either whole foods (*e.g.*, buttermilk, whipping cream) [76–79] or sphingolipid-rich extracts from dairy products [80,81]. Rosqvist et al. [79] supplemented overweight adults with 40 g of milk fat/day in the form of whipping cream (i.e., containing the MFGM, ~200 mg milk PL per day, of which ~50 mg milk SM) as ingredient in a muffin during an 8-week single-blind, randomized, controlled, isocaloric parallel study. When compared to 40 g of milk fat/day as butter oil in muffin, which has had most of the PLs removed (~1 mg left), the whipping cream significantly lowered plasma LDL-C, non-HDL-C, and apoB:apoA-I ratio. Another 4-week study examined the ingestion of 45 g/day of buttermilk during a double-blind randomized crossover study with healthy adults [78]. Consuming the buttermilk-containing cocoa-flavored drink (including ~190 mg of milk PLs/d, with~24 mg of milk SM/d) resulted in significant reductions in both plasma cholesterol (-3.1%) and TG (-10.7%) compared to a calorically-matched placebo. The authors suggested this could be related to a reduction in cholesterol absorption, measured indirectly through plasma β-sitosterol concentrations. In contrast, Baumgartner et al. [76] reported that the addition of traditional buttermilk (90 mg PLs per day, i.e. we can estimate ~25 mg milk SM/d) for 12 weeks to a beverage containing one egg yolk per day did not significantly affect serum lipids or inflammation markers in healthy adults. Furthermore, Severins et al. [77] showed that the addition of traditional buttermilk (70 mg PLs per day, i.e. we can estimate ~18 mg milk SM/d) for 12 weeks to one egg yolk per day did not affect LDL-C, hs-CRP, and liver enzymes, but tended to lower serum TC and TG in hypercholesterolemic adults. Ohlsson et al. [80] observed no effect of a MFGM-enriched buttermilk drink (supplying 700 mg SM daily) on blood lipids during a 4-week intervention. Although, when the authors stratified by sex, plasma LDL-C tended to be lower (P = .056) in women who consumed the buttermilk drink. Weiland et al. [81] conducted two intervention trials investigating the effects of milk PL isolates on cardiometabolic markers in overweight and obese men. In the first trial, the addition of 2 g per day milk PLs (including ~0.46 g SM) to a diet for 8 weeks was shown to lower waist circumference and serum gamma-glutamyl transferase (GGT), and tended to lower alanine transaminase (ALT) (P = .057), but was not shown to affect other markers (serum lipids, glucose, inflammation markers) compared to control (2 g milk fat). In the second trial, 2 g per day of milk PLs (including ~0.5 g SM) for 7 weeks was only shown to affect serum GGT compared to soy PLs control, but not other markers (serum lipids, glucose, inflammation markers). The first human trial reporting a significant impact of milk PL supplementation on reducing an array of cardiometabolic risk factors was recently published by Vors et al. [26]. A higher dose of 3 to 5 g/day of milk PLs (0.8 - 1.3 g/day milk SM) via a butter serum concentrate rich in MFGM fragments incorporated in cream cheese resulted in reduced total and LDL-C, as well as decreased total/HDL-cholesterol and decreased ApoB/ApoA1 ratio, compared to the control cream cheese devoid of milk PL (in which fat ingredient was butteroil only).

Overall, the hypolipidemic effects of milk SM observed in animal studies have been reported in some human studies, although the magnitude of such effects is typically smaller (often insignificant), except in the recent trial using a real dairy food matrix and 3 to 5 g/day of milk PLs [26] which resulted in significant favorable effects (Table 3). However, we cannot rule out that the small or neutral effects of lower intakes observed in 4- to 12-week trials could contribute to maintaining a relative homeostasis of blood cholesterol profiles in the long term. The few clinical studies conducted vary in source of SM, dosage, duration, and are often relatively small in size. Most studies were conducted in healthy adults, while those that have found significant effects on serum lipids and other cardiometabolic markers were conducted in overweight or hyperlipidemic conditions. The effects of milk SM appeared to be more pronounced when provided as a component of buttermilk, rather than as an isolate. Dosages of sphingolipids are much lower in human studies than in rodent studies, even after differences in size are considered. This may explain the lack of effect in some human studies compared to rodent studies. For example, the recent study by Vors et al. [26] observed significant benefits at the higher dosage of milk PLs at 5 g/day, but less at 3 g/day. More studies should now be conducted in humans to explore the

Table 3
Clinical studies examining effects of milk sphingomyelin on serum lipids and other cardiometabolic risk factors

Author	Year	Population	Study Design	Treatment and Duration	Results of SM impact
Milk sphingomyelin					
Ohlsson et al. [25]	2010	Human ileostomy content ($n = 6$ ♂ + 6 ♀)	Overnight fast	First : Milk SM (250 mg) mixed in water and skimmed Second: milk SM (50, 100 or 100 mg) mixed in milk-like oat drink	↑ output of VLCFA specific of milk SM (22:0, 23:0 and 24:0).
Ramprasath et al. [61]	2013	Healthy adults ($n = 10$)	Controlled diets, crossover	2 weeks of prepared diets (30% kcal from fat, 240 mg cholesterol/day) with or without 1 g/day added milk SM	↑ HDL-C (+13%), ↔ total cholesterol, ↔ non-HDL-C, ↔ TG with milk SM
SM-rich ingredients					
Ohlsson et al. [80]	2009	Healthy adults ($n = 48$)	Parallel, placebo	4 weeks of 2 drinks/day totaling 975 mg SL (700 mg SM, 180 mg GC, 95 mg GS) of test drink or 119 mg total SL in isocaloric placebo drink	↔ Plasma lipids between groups, trend for ↓ plasma LDL-C ($P = .056$) in women compared to placebo
Ohlsson et al. [142]	2010	Healthy adults (n = 18)	HF breakfast with milk-like formulation (5.8% fat) + 2/3 egg PLs + 1/3 butteroil (SL : 119 mg)	Milk-like formulation supplemented with milk PL-enriched buttermilk (SL : 975 mg)	Plasma lipid: ↔ TG, ↔ cholesterol, ↔ TG ↔ HDL, ↔ LDL after 1h, trend for ↓ cholesterol in large TG-rich lipoproteins
Keller et al. [143]	2013	Healthy adults ($n = 14$ ♀)	Longitudinal with increasing PL doses (no control group)	2 supplementation periods (each for 10 days): 3 g milk PLs/d (including 0.70 g/d of SM) and 6 g of PLs/d (including 1.4 g/d of SM)	Plasma lipid: with 3 g: ↓ total cholesterol, ↓ HDL-cholesterol (*vs* first period with 0 g) then with 6 g: ↑ total-cholesterol and ↑ LDL-cholesterol (*vs* 3 g)
Conway et al. [78]	2013	Healthy adults ($n = 34$)	Double-blind, placebo crossover	4 weeks of beverage with buttermilk powder (187.5 mg total PLs, 23.6 mg SM per day) or placebo (34.6 mg total PLs) matched for calories and minerals	↓ Serum cholesterol (-3.1%), ↓ TG (-10.7%), trend for ↓ LDL-C ($P = .057$) compared to placebo, ↓ LDL-C (-5.6%) in participants with highest (top 50%) baseline LDL-C
Baumgartner et al. [76]	2013	Healthy adults ($n = 97$)	Single-blind, parallel	12 weeks of intervention either 1) control (n = 20), 2) one egg per day (n = 57), or 3) 100 mL traditional buttermilk (90 mg PLs) + one egg per day (n = 20)	↔ hs-CRP, sICAM, ALT, AST, serum lipids, apoA-1, apoB100, campesterol, or lathosterol between one egg per day and buttermilk groups
Rosqvist et al. [79]	2015	Overweight adults ($n = 57$)	Single-blind, parallel	8 weeks of intervention meals with either 40 g milk fat/day as whipping cream (including 198 mg milk PLs) or control diet with butter oil (1.3 mg milk PLs), matched for calories, macronutrients, and calcium	↓ Plasma cholesterol, ↓ LDL-C, ↓ non-HDL-C, ↓ apoB:apoA-I ratio with whipping cream vs. control group
Severins et al. [77]	2015	Mildly hypercholesterolemic adults ($n = 92$)	Single-blind, parallel	12 weeks of intervention beverages with either 1) 80 mL skim milk (n = 25), 2) 80 mL skim milk + 1.5 egg yolks (n = 23), 3) traditional buttermilk (72 mg PLs) (n = 23), 4) traditional buttermilk (72 mg PLs) + 1.5 egg yolks (n = 21)	Buttermilk effect not significant for change in serum LDL-C, trend for changes in serum TC ($P = .077$) and TG ($P = .063$), ↔ serum hs-CRP, ALT, AST, apolipoprotein A1, apoB
Weiland et al. [81]	2016	Study 1: Overweight and obese men ($n = 62$) Study 2: Overweight and obese men ($n = 57$)	Double-blind, parallel	Study 1: 8-wk intervention of daily 200 mL of PL-enriched drink (containing 2 g milk PLs from milk PL isolate) (n = 31) or 200 mL of control drink (containing 2 g milk fat) (n = 31) Study 2: 7-wk intervention of daily 250 mL of test drink that provided either 3 g milk-PLs (from milk PL isolate) or 2.8 g soy PLs (soy lecithin)	Study 1: ↓ GGT, waist circumference, ALT ($P = .057$), ↔ TC, HDL-C, LDL-C, TG, phospholipids, TC:HDL-C ratio, apoA1, apoB, glucose, insulin, HOMA-IR, hs-CRP, IL-6, sICAM, AST Study 2: ↓ GGT, ↔ TC, HDL-C, LDL-C, TG, phospholipids, TC:HDL-C ratio, apoA1, apoB, glucose, insulin, HOMA-IR, hs-CRP, IL-6, sICAM, ALT, AST
Grip et al. [144]	2018	Children ($n = 160$)	Experimental formula without MFGM (Breast-fed reference group)	Experimental formula with bovine MFGM (at 4% MFGM protein of total formula protein content) Plasma and serum lipidome at 4, 6 and 12 months of age	Plasma lipids: ↑ PC, ↓ SM
Vors et al. [26]	2019	Study 1: Overweight postmenopausal women ($n = 58$)	Double-blind, parallel, placebo	4 weeks with 100 g experimental cream cheese/day at 13% (w/w) of milk lipids totaling 0.8 g SM or 1.3 g SM (3 or 5 g milk PLs via butter serum concentrate) or no SM (triglycerides only) in control cream cheese	Study 1: Plasma lipids: ↓ fasting total cholesterol, ↓LDL-C, ↓total C/HDL-C ratio, ↓ ApoB/ApoA1 ratio ↓ postprandial total cholesterol, ↓ ApoB/ApoA1 ratio, ↓ chylomicron lipids
		Study 2: Ileostomized subjects ($n = 4$)	Double-blind, crossover	Acute postprandial tests during the digestion of the same cream cheeses like above-described, including ^{2}H-cholesterol tracer	Feces: ↑ coprostanol/cholesterol ratio Study 2: Plasma lipids: ↓ ^{2}H-cholesterol tracer in postprandial plasma and chylomicrons ↑ ileal output of total cholesterol and of milk SM

ALT, alanine transaminase; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; AST, aspartate transaminase; GC, glucosylceramide; GGT, gamma-glutamyl transferase; GS, gangliosides; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin -6; MetS, metabolic syndrome; MFGM, milk fat globule membrane; PL, polar lipid; sICAM, soluble intercellular adhesion molecule; SL, sphingolipids; SM, sphingomyelin; TC, total cholesterol; TG, triglyceride; VLCFA, very long chain fatty acid; ↑, increase; ↓ decrease; ↔, no change.

Table 4
Studies examining effects of milk sphingomyelin on intestinal barrier, inflammation, and gut microbiota. Adapted from [137].

	Author	Year	Model	Control	Treatment and Duration	Results on SM impact
				Milk sphingomyelin		
In vitro	Norris et al. [30]	2017	Raw264,7 Macrophages (n = 3-7 independent experiments)	Control: EtOH + LPS	Milk SM (0.8 or 8 μg/mL), or C16-Cer, C24-Cer, C16-dihydroceramide, C24-dihydroceramide (10 μM), or sphingosine (1 or 10 μM). Pre-incubation + 4h co-incubation with LPS	Inflammation: Milk SM : ↓ TNF-α, ↓ CCL2 mRNA Similar effects with C16-Cer, C24-Cer and sphingosine (10 μM); Anti-inflammatory effects appear to be due to sphingosine
	Milard et al. [119]	2019	Caco-2/TC7 (n = 2-3 independent experiments)	Mixed micelles	Mixed micelles + milk SM (0.2, 0.4, or 0.6 mM)	Gut barrier: ↑ Occludin, ↑ JAM-1, ↑ ZO-1 ↔ permeability Inflammation: ↑IL-8 (and IL-8 induced ↑ expression of tight junctions)
Mouse	Dillehay et al. [124]	1994	CF1 mice. Cancer induced by DMH (n = 10-14)	Semi-purified diet (AIN76A, 5% w/w corn oil)	One week after the final injection of DMH + 6 weeks on a diet with 0.025, 0.05, or 0.1% (w/w) of milk SM	↓ colonic aberrant foci, ↓ colon tumors (incidence)
	Schmelz et al. [145]	1996	CF1 mice. Cancer induced by DMH (n = 15-50)	Semi-purified diet (AIN76A, 5% w/w corn oil)	One week after the final injection of DMH + 34 weeks on a diet with 0.025, 0.05, or 0.1% (w/w) of milk SM	↓ colonic aberrant foci, All of the tumors of the mice fed the control diet = adenocarcinomas. Mice fed SM diet = a combination of adenomas and adenocarcinomas.
	Mazzei et al. [121]	2011	PPARγ null and PPARγ$^{+/+}$ mice (n = 10)	Semi-purified diet (AIN76A, 5% w/w corn oil). Cancer induced by AOM + DSS	68 days on a diet with 0.1% (w/w) milk SM	↓ inflammatory response to DSS ↓ tumor in PPARγ$^{-/-}$ mice
	Norris et al. [29]	2016	Male C57BL/6J mice (n = 3-10)	4 weeks on a HFD (21% AMF by weight)	4 weeks on a HFD (21% AMF by weight) with 0.25% (w/w) milk SM	↓ body weight gain Inflammation: ↓ IL-6 (muscle) and ↓ LPS activity (plasma) Gut barrier: ↔ Occludin, ↔ ZO-1, ↔ permeability Gut microbiota: ↑ Firmicutes, ↓ *Bacteroidetes*, ↑ *Actinobacteria*, ↑ *Bifidobacteria*, ↓ *Tenericutes*. ↑ Gram positives, ↓ Gram negatives
	Norris et al. [68]	2017	Male C57BL/6J mice (n = 13-14)	HFD (31% lard; 0.15% cholesterol by weight)	10 weeks on HFD (31% lard; 0.15% cholesterol by weight), 0.1% (w/w) milk SM	Inflammation (EAT): ↓ F4/80, ↓ TNF-α Inflammation (serum): ↓ CCL2
	Norris et al. [30]	2017	Male C57BL/6J mice (n = 13-14)	HFD (31% lard; 0.15% cholesterol by weight)	10 weeks on HFD (31% lard; 0.15% cholesterol by weight), 0.1% (w/w) milk SM	Inflammation (serum): ↓ IFN-γ, ↓ TNF-α, ↓ IL-6, ↓ MIP-1. Trend for ↓ LPS. Inflammation (colon): ↑ CCL2 Gut barrier: ↔ tight junctions and permeability Gut microbiota: ↔ *Fimicutes*, *Bacteroidetes*, *Akkermansia* ↑ *Acetatifactor*
	Milard et al. [119]	2019	Male C57BL/6J mice (n = 6-7)	Gavage: NaCl	Gavage: ~5 or 10 mg of milk SM	4h after Gut barrier: ↔ Occludin, ↔ JAM-1, ↔ ZO-1 in duodenum, jejunum and ileum
Rat	Motouri et al. [38]	2003	Sprague-Dawley rat pups (n = 5-6)	Reared artificially with milk containing 0.5% (w/w) phosphatidylcholine	Reared artificially with milk containing 0.5% (w/w) milk SM	↓ intestinal lactase activity Vacuolated cells in the tip of villi ↑ Auerbach nervus plexus area (ileum)
				SM-rich ingredients		
Mouse	Snow et al. [122]	2011	BALB/c mice (n = 6)	5 weeks on semi-purified diet (AIN76A, 5% fat corn oil w/w) + challenge (24h or 48h) with NaCl or LPS	5 weeks on diet supplemented with 10% (w/w) MFGM and challenge (24h or 48h) with NaCl or LPS	Inflammation: ↓ IL-6, ↓ IL-10, ↓ IL-17, ↓ MCP-1, ↓ IFNγ, ↓ TNF-α, ↓ IL2p70, below detection limit for IL-3

(continued on next page)

Table 4 (*continued*)

	Author	Year	Model	Control	Treatment and Duration	Results on SM impact
						Gut barrier: ↑ permeability with control diet and LPS (24h and all mice dead at 48h). However, diet supplemented with MFGM + LPS: ↔ permeability (24h and 48h)
	Lecomte et al. [140]	2016	Male C57BL/6J mice ($n = 10$-12)	HFD (17% w/w palm oil)	8 weeks on HFD (17% w/w palm oil) + 1.2% w/w milk PLs (0.3% milk SM w/w diet) or SPL	Inflammation: *vs* HFD, unlike SPL, milk PL: ↔ MCP-1, ↔ TNF-α, ↔ LBP, ↔ Leptin, ↓ CD68 Gut barrier: ↑ goblet cells in colon
	Milard et al. [91]	2019	Male C57BL/6J mice ($n = 7$-15)	HFD (21% w/w palm oil in chow)	8 weeks on HFD with 1.1% (w/w) milk PLs (0.25% milk SM w/w diet) or 1.6% (w/w) of milk PLs (0.4% milk SM w/w diet)	1.6% of milk PL: ↓ body weight gain and trend to ↓ epididymal adipose tissue Gut barrier: 1.6% of milk PL: ↑ colonic crypt depth. ↔ permeability Gut microbiota: 1.1% of milk PL: ↑ *Bifidobacterium*, ↑ *B.animalis* 1.6% of milk PL: ↓ *Lactobacillus* Correlation between *Bifidobacterium* and *Akkermansia.*
	Zhou et al. [120]	2019	Male ob/ob mice ($n = 11$-18)	Moderately high-fat AIN-93G diet (34% kcal as fat) (CO)	2 weeks on CO + 0.2% (w/w) milk gangliosides (GG) or CO + 1% (w/w) milk PLs (PL)	↔ plasma and local inflammation PL : ↑ serum IL-6, ↔ TNF-α, ↔ tPAI-1, ↔ resistin, ↔ MCP-1, ↔ LPS, ↔ gut permeability to FITC dextran, ↔ sucralose:mannitol ratio, ↓ jejunal ZO-1 GG : ↔ serum IL-6, ↔ TNF-α, ↔ tPAI-1, ↔ resistin, ↔ MCP-1, ↔ LPS, ↔ gut permeability to FITC dextran, ↔ sucralose:mannitol ratio, ↓ jejunal ZO-1
Rat	Sánchez Negrette et al. [146]	2007	Wistar male rats ($n = 20$)	Adequate diet. Cancer induced by DMH	4 weeks on adequate diet and drinking milk (cow or buffalo milk)	↓ number of intestinal tumors with milk diet
	Snow et al. [125]	2010	Fischer-344 rat + Injection DMH ($n = 16$)	13 weeks on semi-purified diet. AIN76A, 5% (w/w) as corn oil or 5% (w/w) as AMF	13 weeks on diet with 2.5% (w/w) MFGM (0.11% SM) and 2.5% (w/w) AMF	↓ aberrant crypt foci (colon) ↔ candidate colon cancer genes
Human	Rogers et al. [147]	2017	Overweight and obese men ($n = 36$)	Test meals included whipping cream or palm oil	Test meals included whipping cream or palm oil with MFGM (13.7% PLs w/w)	Inflammation (serum concentration): ↔ IL-6, TNF-α, CRP

AMF, anhydrous milk fat; AOM, azoxymethane; CRP, C-reactive protein; DMH, 1,2-dimethylhydrazine; DSS, Dextran sodium sulfate; DMEM, Dulbecco's Modified Eagle's Medium; EtOH, ethanol; FITC, fluorescein isothiocyanate; GG, ganglioside; HFD, high-fat; IL, interleukin; IFNγ, Interferon Gamma; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1 ; MFGM, milk fat globule membrane; PL, polar lipids; SM, sphingomyelin; SPL, soybean polar lipid; TNF-α, tumor necrosis factor-alpha ; tPAI-1, tissue plasminogen activator inhibitor-1 ; ZO-1, zonula occludens-1.

effects of milk SM and milk PLs on serum lipids and other cardiometabolic risk factors with varying intakes and in different populations.

4. Dietary SM and gut microbiota-host interactions

4.1. Link between Western diets, metabolic endotoxemia and inflammation

Consuming high-fat, high-cholesterol Western-type diets have been linked to chronic disease through increasing inflammation [82,83]. Detrimental effects of Western diets are influenced by the presence of microbes in the GI tract, since depletion of gut microbiota with oral antibiotics reduces the inflammatory response [10]. This is partly related to the effect that Western diets have on increasing LPS in circulation, termed "metabolic endotoxemia" [9,10]. Diets high in fat and cholesterol were reported to promote gut dysbiosis, intestinal inflammation, and gut barrier permeability to LPS. Such LPS translocation can occur through both (i) paracellular route (e.g., breakage in tight junctions) [9,12,84] and (ii) transcellular route (by co-absorption with fat and cholesterol via chylomicrons) [13], which was confirmed in humans [85–87]. Recently, a study highlighted that such impairment of the gut barrier also occurs in obese human. Furthermore, this situation is exacerbated following a dietary lipid challenge that enhances labelled LPS translocation *ex-vivo* [88]. In this study, obese subjects presented an increased serum concentration of the lipopolysaccharides binding protein (LBP) compared to normal-weight controls. Metabolic endotoxemia and gut dysbiosis have been linked to inflammation in animal models [9,11,12,84] and humans [89,90]. Chronic feeding of HFD to mice increased colonic pro-inflammatory macrophages, which promoted LPS translocation, insulin resistance, and inflammation [12]. Thus, compelling evidence links the development of systemic inflammation with impairments in gut barrier function caused by Western diets. Adiposity and metabolic inflammation are major features of obesity associated with altered gut microbiota and intestinal barrier. In this context, beneficial effects of milk SM (0.25% w/w in a HFD 45% kcal) have been recently reported on weight gain, inflammation, plasma endotoxemia (LPS activity) and intestinal microbiota during a 4-week study in mice [29]. Dietary milk SM (0.1% w/w in a HFD 60% kcal) significantly reduced both serum inflammatory cytokines and adipose inflammation in HFD-induced obese mice after 10 weeks [30,68]. However, there were no differences in food intake, adiposity, or liver weight at this dosage of milk SM [68]. Recently supplementation with 1.6% (w/w) of milk PLs (including 0.4% of SM) in a HFD (~45% kcal as fat, chow-based diet) induced a significantly lower weight gain (-37.8%) and a trend towards less epididymal adipose tissue (-23.3%) [91]. In this study, no impact of milk PL was observed on inflammatory markers, however the mixed chow-based control HFD did not induce significant inflammation. Effects on rodent adiposity and inflammation seem to depend on the background composition of the diet (semi-purified or non-purified chow-type diet) and the dose of milk PLs and/or SM added. Indeed, feeding mice a purified diet compared to a non-purified chow-diet induces alterations in gut microbiota [92] and in adiposity and adipose tissue inflammation [93]. Studies showed interesting effects of milk PLs and milk SM on HFD-induced body weight gain, suggesting impacts on the gut microbiota.

4.2. Evidence of dietary SM influencing gut microbiota

Dietary milk SM may also modulate the gut microbiota and the host response to microbial components like LPS (Table 4). Alk-SMase activity is highest in the middle and distal parts of the small intestine and SM digestion and absorption predominates in these portions [97]. However, significant quantities (~25%) of dietary milk SM and hydrolytic products can reach the colon [25], where they may affect

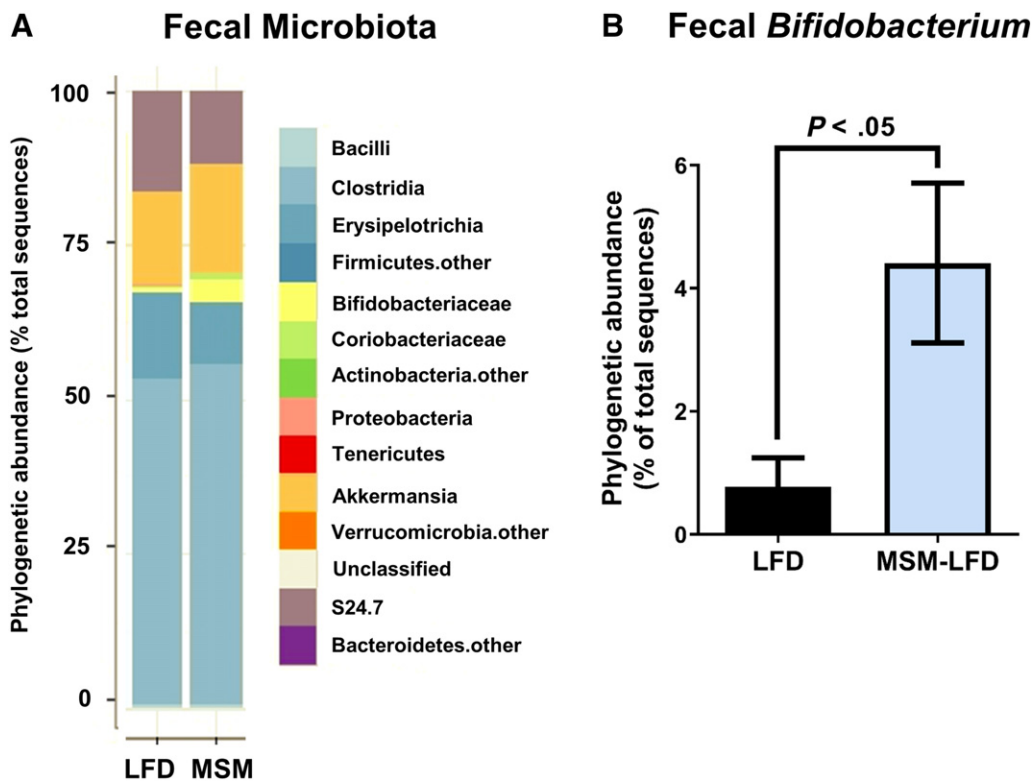

Fig. 2. **Milk SM modulates gut microbiota in mice fed low-fat diet.** Fecal microbiota composition (16S rRNA) of separately caged 8-week old male mice fed for 4 weeks with either a semi-purified, casein-based low-fat diet (LFD; 3.2% [w/w] milk fat, 15% kcal protein, 10% kcal fat, 75% kcal carbohydrate), or the same diet with 0.25% (w/w) milk SM added (MSM-LFD) to replace an equal amount of anhydrous milk fat. Phylogenetic abundance of bacterial taxa (A) and comparison of *Bifidobacterium* (B) are shown. Mean ± SEM, $n = 6$/group.

the gut microbiota. Sphingosine, a hydrolytic product of SM, has bactericidal effects [94]. Additionally, it has been reported that many sphingolipids can serve as binding sites for enterovirulent bacteria (e.g., Helicobacter pylori and SM) and their toxins [95,96], suggesting that consumption of milk SM may impact pathogenic bacteria residence in the GI tract.

Norris et al. [29] were the first to show that supplementing with purified dietary milk SM could lower circulating LPS (-35%) in mice fed a Western-type HFD. Notably, it was also observed that milk SM modulated the fecal microbiota composition of separately caged mice, by increasing *Bifidobacterium* and reducing Bacteroidetes relative abundances [29]. These bifidogenic effects of supplementing with purified milk SM appear to occur in a semi-purified low-fat diet condition as well (Fig. 2). However, Reis et al. [98] did not detect any differences in cecal microbiota from female mice fed various milk PL-rich diets for 5 weeks; in this particular study, mice were fed HFD for 5 weeks prior to being fed the HFD supplemented with butter serum-derived extracts of either total PLs, phospholipids, or sphingolipids for an additional 5 weeks. Recently, Milard et al. [91] fed mice a 1.6% milk PLs-enriched mixed non-purified HFD (0.4% SM) which showed a specific decrease in the abundance of *Lactobacteria reuteri* in cecal microbiota, which was negatively correlated with a higher proportion of very long chain fatty acids specific of milk PLs in fecal lipids (C23:0, C24:0 and C24:1, found in milk SM). This suggests a further impact of residual lipids reaching the colon in the gut and on metabolism. A supplementation with 1.1% of milk PLs (0.25% w/w of milk SM) induced a significant increase in *Bifidobacterium* spp. in particular *B. animalis* [91]. These results are consistent with results of Norris et al. on milk SM supplementation, Fig. 2 and [29]. Furthermore, in the Milard et al. study [91], *Bifidobacterium* spp. are correlated with *Akkermansia muciniphila*, which is described for its favorable metabolic effects [99,100] and can be impacted by the diet [101]. The impact of another source of dietary SM on microbiota and lipid metabolism was led by Chung et al. [73] who conducted a comprehensive study on the effects of egg SM on atherosclerosis in apoE$^{-/-}$ mice fed chow and high fat, high cholesterol diets. Male apoE$^{-/-}$ mice fed 1.2% (w/w diet) egg SM supplemented diets for 19 weeks had a reduction in aortic arch lesion size compared to control mice, which was abolished when mice were co-administered broad-spectrum antibiotics to deplete gut microbiota. More recently, feeding a much lower dosage of 0.1% (w/w diet) egg SM to male apoE$^{-/-}$ mice for 8 weeks was shown to attenuate aortic root plaque development, lower systemic inflammation, and modulate the gut microbiota [102]; whether milk SM also presents such anti-atherosclerotic effects deserves to be elucidated. Altogether, these findings suggest that anti-atherosclerotic effects of dietary SM may work in part via effects on gut microbiota.

Sphingosine, dihydrosphingosine, and phytosphingosine have been shown to have broad-spectrum bactericidal properties [94,103], and induce ultrastructural damage to bacteria such as *Escherichia coli* and *Staphylococcus aureus* [104]. However, one study found that the bactericidal effect of sphingosine was lost when it was subject to simulated GI conditions [105]. Nevertheless, it has been suggested that sphingoid bases can insert into the outer layers of bacteria and disrupt the normal function of their cytoplasmic membranes, or alternatively, accumulate inside the cells and interfere with normal metabolism [104].

Interestingly, sphingolipid production is restricted to only a small fraction of bacteria, which includes most members of the Bacteroidetes phylum [106]. *Porphorymonas gingivalis*, an oral bacterium of the Bacteroidetes phylum and producer of sphingolipids, is sensitive to antibacterial effects of sphingoid bases [107]. Furthermore, the authors noted that *P. gingivalis* seemed to be more sensitive than other bacteria tested previously by the authors (e.g., *E. coli, S. aureus*) [103,107]. Sphingolipid production in the form of dihydroceramide is important for survival during oxidative stress in *P. gingivalis* [108] as well as *Bacteroides* [109]. Bacteria that produce sphingolipids may be more susceptible an excess of sphingoid bases, as exogenous sphingosine may interfere with normal sphingolipid metabolism or trigger some signaling response that leads to cell death (similar to mammalian cells). If true, antibacterial effects of milk SM may select against Bacteroidetes if they are more sensitive than other gut microbes when consumed chronically – this is supported by a reduction in fecal Bacteroidetes in mice fed 0.25% (w/w) milk SM-supplemented HFD for 4 weeks [29]. Cecal Bacteroidetes of the S24-7 family have been linked with atherosclerosis development in apoE$^{-/-}$ [110]. However, it is possible the reported effects of dietary milk SM on lowering fecal Bacteroidetes and increasing Firmicutes [29] are secondary to the inhibition of lipid absorption by SM. Consumption of HFD has been linked to increases in Firmicutes and reductions in Bacteroidetes in rodents [111]. Mice that have a knockout in NPC1L1, and thus defective intestinal cholesterol absorption, have altered gut microbiota composition with reduced Bacteroidetes and increased Firmicutes [112]. However, LDL-receptor knockout mice that were fed a low-fat diet with extremely high cholesterol content (1.25% w/w) had no significant alterations in gut microbiota composition despite a 68-fold increase in fecal neutral sterol excretion [113]. Results from this last study suggest that effects of milk SM on gut microbiota may be independent of effects on intestinal cholesterol absorption. With respect to the observed bifidogenic effects of milk SM, while LCFA mixtures alone had no effect, a combination of LCFA and sphingosine (LCFA + sphingosine) was shown to increase the relative abundance of *Bifidobacterium* and decrease the relative abundance of the Gram-negative Proteobacteria in infant stool samples subjected to 24 h anaerobic *in vitro* fermentation [114]. Taken together, these data suggest that an interaction between milk SM and fatty acids, rather than interactions with cholesterol, may explain some of the reported effects on gut microbiota. Such effects remain to be elucidated in humans. In a recent clinical trial in postmenopausal women, up to 5 g of milk PLs (1.3g of milk SM) per day during 4 weeks via butter serum-enriched cream cheese did not modify the major bacterial phyla of gut microbiota nor the profile of short-chain fatty acids [26]. However, this intervention with milk SM (via milk PLs) increased the coprostanol/cholesterol ratio in feces, suggesting an increased conversion of cholesterol to coprostanol by the gut microbiota [26]. Studies should now verify the impact of different milk SM intakes on the gut microbiota in different populations.

4.3. Evidence of dietary SM modulating gut barrier and microbe-host interactions

Recent evidence suggests that milk SM shows potential to neutralize LPS-induced inflammation and Western diet-induced changes in gut barrier function (Table 4). Due to putative effects on intestinal lipid absorption, dietary SM may be useful in preventing translocation of gut bacteria-derived LPS into mesenteric lymph nodes and circulation, based on chylomicron-dependent translocation of LPS [13]. The effect of SM on lipid absorption would be expected to reduce the transcellular or paracellular transport of LPS across the gut barrier. Recently, sphingolipid *de novo* biosynthesis was found to be essential for intestinal cell survival and barrier function [115], suggesting the intracellular pool of sphingolipids is also important for maintaining gut barrier function. Additionally, interactions between SM and bile acids may be important in preventing gut permeability to microbial factors. In the above-mentioned study by Milard et al. [91], milk PLs (1.6% including 0.4% of milk SM) induced less hydrophobic bile acids and a significant positive correlation was observed between bile acid hydrophobicity index and *Lactobacillus* spp. and *L.reuteri*. The hydrophobic bile acid, deoxycholic acid, has been implicated in HFD-impairments in gut barrier function in mice via inducing intestinal epithelial damage [116]. Egg SM was shown to dose-dependently

reduced deoxycholate-induced apoptosis and hyperproliferation in Caco-2 cells [117]. Taurocholate-induced cytotoxicity in Caco-2 cells was shown to be reduced by the addition of SM to micelles [118]. Moreover regarding gut barrier markers in Caco-2/TC7 cells, mixed micelles supplemented with milk SM induced a significant increase in the gene expression of tight junction proteins (Occludin, Junctional adhesion protein-1 and Zonula occludens-1), implicated in the paracellular permeability. However, in this model that was not a model of altered gut barrier, SM in micelles did not further decrease permeability to macromolecules. This study also reveals that milk SM in micelles induces a specific secretion of IL-8 by Caco-2/TC7 cells, but not of other cytokines [119]. Results showed that IL-8 could be involved in the increased gene expression of tight junction protein, suggesting a new mechanism of action of dietary SM in the gut [119]. In contrast, Zhou et al. [120] recently found that 2 week feeding of milk PLs (1% w/w) to moderately (34% kcal as fat) high-fat diet-fed ob/ob mice had no impact on markers of gut permeability, with the exception of a decrease in jejunal ZO-1 protein abundance compared to control mice. Therefore, the severity of obesity should be taken into account. Milk SM might provide benefits in a preventive context rather than in already established severe obesity, although this would have to be verified.

Milk SM may also protect the gut from inflammatory damage, preventing leakage of LPS across the gut barrier. Milk SM (0.1% weight of diet) reduced large intestinal inflammation in a mouse model of chemically-induced colitis via effects mediated by peroxisome proliferator-activated receptor gamma (PPARγ) [121]. Furthermore, mice fed a diet supplemented with MFGM (~0.13% milk SM w/w) showed a better response to an i.p. LPS challenge, which was attributed to a reduction in gut permeability [122]. MFGM also attenuated GI complications associated with the administration of a diarrheagenic strain of *E. coli* in humans [123]. An impact on inhibition of colonic tumor has been also observed with milk SM [124] and milk PLs (rich in SM) [125].

Lipoproteins, which contain sphingolipids such as SM, neutralize LPS activity via phospholipid complexes [126,127]. Furthermore, previous studies have shown that LPS injection increases hepatic sphingolipid biosynthesis and enrichment in circulating lipoproteins, possibly to help neutralize LPS [128]. Milk SM and its hydrolytic products show potential to protect against the inflammatory effects of LPS. We recently examined if exogenous milk SM could influence LPS activation of RAW264.7 macrophages [30]. Milk SM dramatically attenuated inflammatory gene expression induced by LPS, and subsequent experiments showed that sphingosine appeared to be responsible for this activity. Consistently in Caco-2/TC7 cells, incubation with mixed micelles supplemented with milk SM or egg SM showed similar effects on tight junction protein expressions, also suggestion a potential impact of sphingosine base [119]. Furthermore, these effects did not appear to be due to cytotoxicity or induction of apoptosis. The lipid-A moiety of LPS, composed of saturated fatty acyl chains, is critical for its biological activity. Milk SM may influence LPS via hydrophobic interactions with its toxic lipid-A moiety. SM and LPS may associate and form lamellar structures, as they can in lipid rafts [129,130]. Incubation with bovine brain SM was shown to neutralize LPS stimulation of granulocytes via binding to phospholipid vesicles [126]. Hydrolytic products of SM also appear to impact LPS signaling. Ceramide synthase-2 null mice, which lack very-long chain ceramides, are more susceptible to LPS-mediated septic shock [131]. Additionally, exogenous C8-ceramide and sphingosine were reported to inhibit TNF-α secretion from LPS-stimulated macrophages [132]. Ceramides may compete with LPS and block TLR4 signaling, as they bind to CD14 in monocytes [133]. Sphingosine has been shown to stimulate PPARγ activity in macrophages, which may confer anti-inflammatory effects [121]. Furthermore, choline, found in SM and PC, also displays anti-inflammatory activity through activation of the nicotinic acetylcholine receptor subunit α7 [134]. Choline administration lowered plasma TNF-α and improved survival rate in mice given a lethal i.p. dose of LPS [134].

Besides the well-known role of LPS in TLR activation, the host response to other bacterial-derived lipids, such as lipopeptides that are TLR2 agonists, may be affected by milk SM. It seems plausible that dietary SM may impact the absorption or activity of bacterial lipids, in addition to its known inhibition of cholesterol and fat derived from foods. Preliminary investigations by Milard et al. support this concept. A lower gene expression of the above-mentioned TLR2 receptor was observed in Caco-2/TC7 cells after incubation with milk-SM enriched lipid micelles *vs* micelles devoid of SM (Fig. 3, additional analyses from the *in vitro* experiments published in [119]). Currently, there are no published data describing dietary factors (*e.g.*, milk SM) that influence host exposure to TLR2 activators, and this will be an important area to address in the future.

5. Conclusion

Although not considered a dietary essential, milk SM could play important roles in inhibiting lipid absorption, modulating adiposity and inflammation, and altering the host response to gut microbiota; thus, preventing the development of chronic diseases. Fig. 4 summarizes the potential effects of dietary milk SM on gut microbiota-host interactions as discussed above. There is still a large gap of knowledge in this area and many questions remain. For example, are the beneficial effects of milk SM on disease outcomes primarily due to hypolipidemic effects or gut microbiota-host interaction effects, and are these effects via un-/partially digested residues in the gut? Some studies with dietary SM show evidence of an attenuation of metabolic effects with the absence of a functional gut microbiota [73]. The interesting effects observed on weight gain in rodents need to be explored. What is the impact of a milk SM-enriched diet on energy expenditure and thermogenesis? Furthermore, how does milk SM intake affect endogenous sphingolipid levels in highly metabolic tissues such as liver? Combining dietary studies with lipidomics approaches will undoubtedly shed light on this area. Finally, it will be critical to determine if the effects of milk SM and sphingolipids reported in cell and animal models translates to humans. Alk-SMase is found in human bile in addition to the brush border membrane, so it is thought that SM digestion is more efficient in humans. The use of higher dosages of milk SM in human studies,

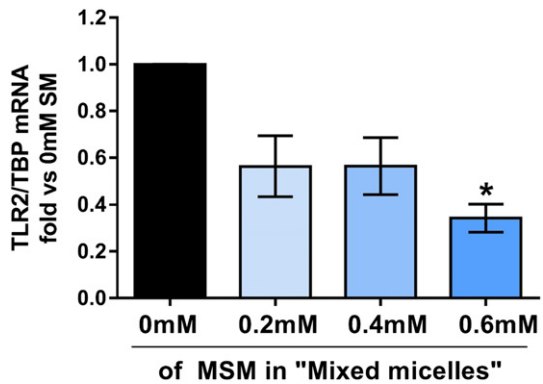

Fig. 3. **Milk sphingomyelin decreased mRNA expression of Toll-like receptor 2.** Caco-2/TC7 cells received a treatment with mixed lipid micelles supplemented with or without MSM (See Material and Methods in Milard et al. [119]). MSM decreased the gene expression of TLR2 in cells (three independent experiments in triplicate for 0, 0.2, and 0.4 mM and two independent experiments in triplicate for 0.6 mM). Gene expression values were normalized to TBP mRNA. Data are reported as mean fold vs 0 mM MSM ± SEM. Data were analyzed by one-way ANOVA (* $P<0.05$). Of note, 0.2 mM of MSM corresponds to 0.16 mg/mL in cell culture medium. MSM, milk sphingomyelin; TBP, TATA-binding protein; TLR, Toll-like receptor.

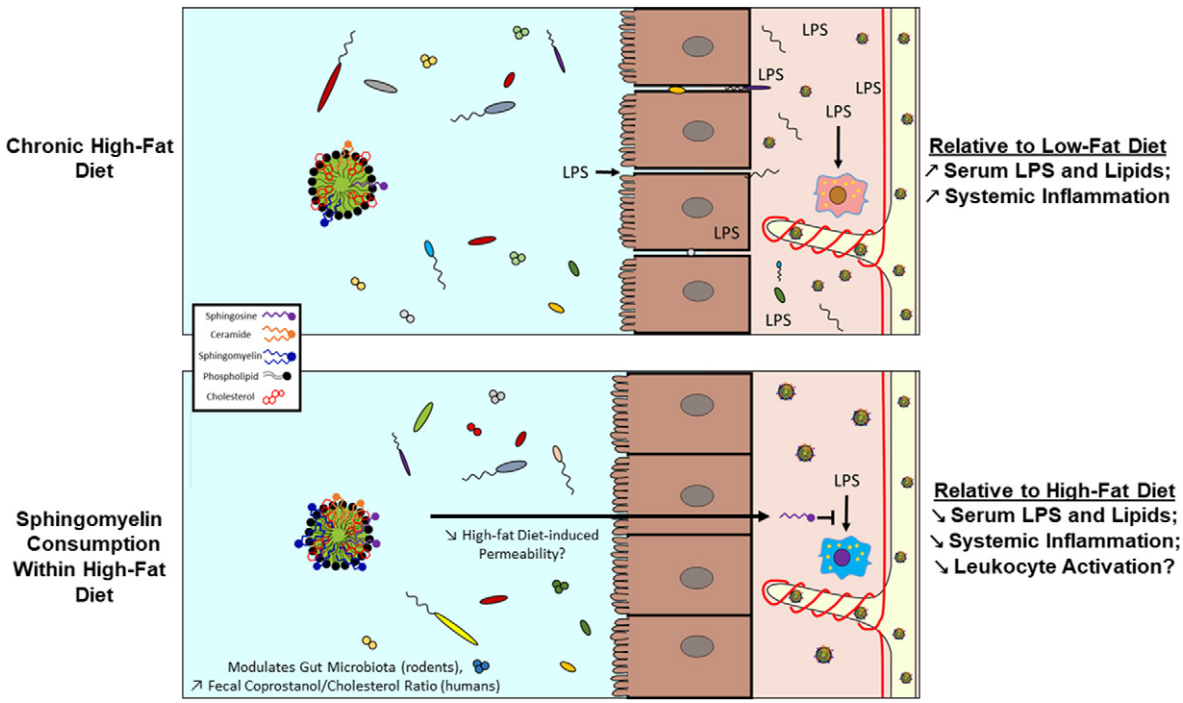

Fig. 4. **Working model of potential effects of dietary milk SM in modulating gut microbiota-host interactions during Western diet conditions from pre-clinical studies.** With a chronic high-fat, high-cholesterol, high-sucrose, Western diet (top panel), development of gut dysbiosis and increased gut permeability may lead to hyperlipidemia, low-grade inflammation, and metabolic endotoxemia. Evidence from pre-clinical studies in Western diet-fed mice shows that incorporation of milk SM results in an attenuation of hyperlipidemia, a modulation of gut microbiota (e.g., increased *Bifidobacterium* in feces), and a reduction in circulating lipopolysaccharide (LPS) activity (endotoxemia) (bottom panel). Impacts on gut microbiota composition and endotoxemia still need to be studied in humans. Furthermore, *in vitro* studies with macrophage and intestinal cell lines have reported attenuations in LPS-induced inflammation and increased tight junction expression, respectively, which may attenuate inflammation resulting from defects in gut barrier function. However, these observations need to be validated *in vivo*. In a recent clinical study [26], consumption of 0.8 to 1.3g/d of milk SM (as part of a milk PL complex in cream cheese) increased the ratio of coprostanol/cholesterol in the stools of volunteers after 4 weeks, suggesting increased conversion of cholesterol to coprostanol by gut microbiota. LP, lamina propria; LPS, lipopolysaccharide.

and/or in combination with mutually inhibitory lipids (e.g. cholesterol, palmitic acid from the MFGM, other milk lipids and/or other foods) may be necessary for sufficient sphingolipids to reach the distal gut for microbiota-related effects. If beneficial effects are demonstrated in further clinical trials, milk SM could be developed as a novel dietary component to enhance the functionality of food products marketed for health benefits. Moreover, this could lead to dietary recommendations that encourage the consumption of dairy products rich in SM. The recent dietary practice in the U.S. of eliminating full-fat dairy may have unwanted health consequences, as fat-free dairy has lower SM content than full-fat dairy [39,135]. Thus, Americans are likely not consuming optimal levels of dairy-derived SM and the biological activity of milk SM may not be fully realized. In 2015, The International Dairy Federation (IDF) estimated an important world milk production of ~800 million tons. The largest producers were Asia (29% of world production), Europe (24%) and U.S (18%). Co-products rich in milk PLs that have historically been undervalued to date, such as buttermilk and butter serum, may see wider nutritional applications due to the value of their high milk SM content. However, more research is warranted, notably in humans, to clearly define how milk SM influences lipid metabolism, gut barrier and microbiota, and inflammation.

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Conflicts of Interest

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