Potential health benefits of sphingolipids in milk and dairy products

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Abstract

Sphingolipids are found in all eukaryotic and some prokaryotic cells. Milk and dairy products are one of the most important sources of sphingolipids. This compounds participate in a variety of indispensable metabolic, neurological, and intracellular signaling processes. Sphingolipids and their derivatives are highly bioactive compounds with anti-cancer, bacteriostatic and cholesterol-lowering properties. Therefore, this review focuses on the potential health benefits of the milk and dairy sphingolipids.

Key words: sphingolipids, sphingoid bases, gangliosides, milk, dairy products

Introduction

Milk and dairy products, which provide one third of the total daily intake, are one of the most important sources of sphingolipids. Sphingolipids are not "ordinary fats". They are better categorized as "functional ingredients" since they have not only structural, but also regulatory functions, and are effective already at low concentrations.

The health benefits of milk and fermented products have been known since medieval times. Milk is an important food in the human diet, not only as the sole source of nutrition for infants but also as a source of energy in the form of fat, protein and carbohydrate for children and adults. Directly transferred from the mother to the newborn, all the constituents of milk are in their natural state and adapted to bring energy and bioactive molecules. Milk from animals is also transformed into various dairy products for infant and adult human consumption. Aside from the nutritious value consisting of basic proteins, lipids and saccharides, milk contains also numerous biologically active substances, such as imunoglobulins, enzymes, antimicrobial peptides, oligosaccharides, hormones, cytokines and growth factors (Donovan, 2006; Pouliot and Gauthier, 2006; Tunick and Van Hekken, 2014).

Milk lipids

Milk contains about 3 to 5 % (w/w) fat, but the absolute percentage of fat in milk varies according to multiple factors that include species, maternal diet and stage of lactation. Lipids contained in milk fat of mammals vary widely in content and composition (Jensen, 2002). Lipids are present in milk in the form of spherical entities of about 4 to 5 μm diameter called milk fat globules, which are enveloped by a biological membrane known as the milk fat globule membrane (MFGM). This is highly complex biological membrane that surrounds the fat globule, hereby stabilizing it in the continuous phase of the milk, and preventing it from enzymatic degradation by lipases (Danthine et al., 2000). Many detailed investigations of the MFGM composition and structure

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(Figure 1) as well as it's technological, nutritional and health properties have been recently performed and reported in scientific reviews (Dewettinck et al., 2008; Gallier et al., 2012). Organization of the MFGM as a trilayer structure is now well accepted, with the inner layer composed of proteins and polar lipids from the endoplasmic reticulum and the outer bilayer of polar lipids originating from specialized secretory regions of the apical plasma membrane of the mammary epithelial cells (Heid and Keenan, 2005). The lipids of the MFGM are primarily polar lipids, although neutral lipids can also occur. The latter are triglycerides, diglycerides, monoglycerides, cholesterol and its esters. The polar lipids of the MFGM consist of phospho- and sphingolipids.

Nature and characteristics of phospholipids

Phospholipids are amphiphilic molecules with a hydrophobic tail and hydrophilic head group. The phospholipids found in most cell membranes are basically glycerophospholipids, which consists of fatty acids esterified to a glycerol backbone, a phosphate group and a hydrophilic residue (e.g. choline, ethanolamine, serine, and inositol). Thus, the MFGM is composed of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine. In addition to their structural roles, phospholipids participate in a variety of indispensable metabolic, neurological and intracellular signaling processes (Guo et al., 2005).

The backbone of phospholipids can also be the long chain amino-alcohol sphingosine instead of glycerol. These phospholipids are classified as sphingophospholipids, the most representative being sphingomyelin (Figure 2), consisting of sphingosine esterified to one fatty acid and phosphocholine.

Beside sphingophospholipids, the MFGM is composed of sphingolipids, such as gangliosides (Dewettinck et al., 2008; Lopez, 2011; Gallier et al., 2014).
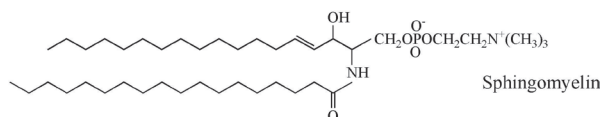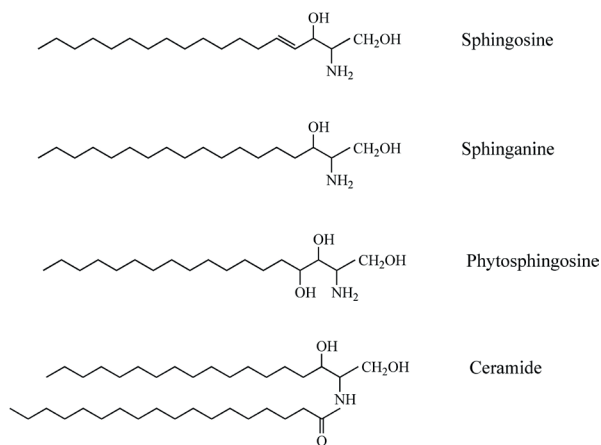

Figure 2. Structure of sphingomyelin

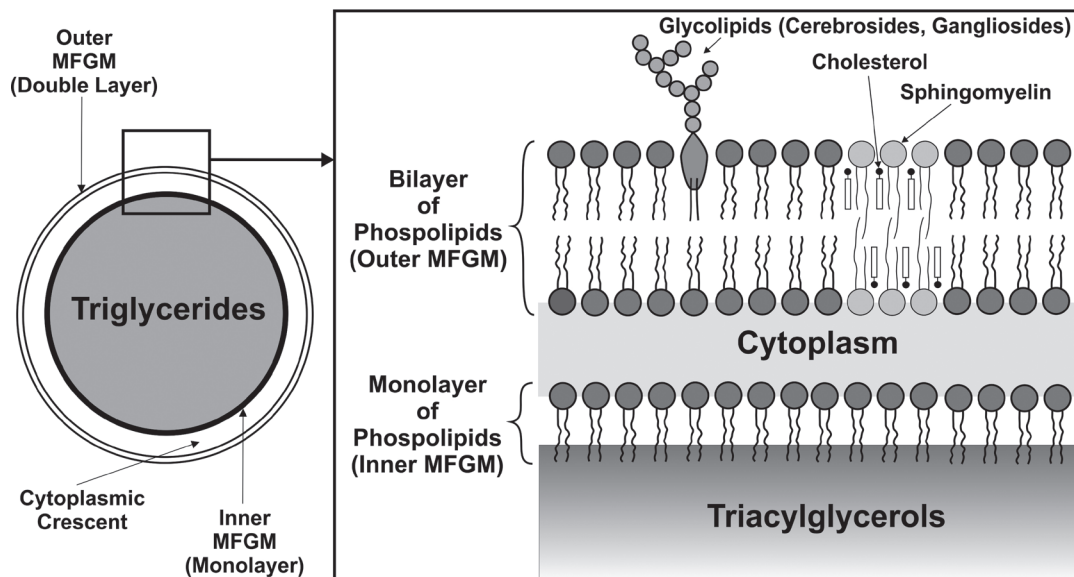Figure 3. Structures of sphingoid bases and ceramide

Figure 1. Structures of milk fat globule membrane

Sphingolipids

Sphingolipids are ubiquitous lipids that were evolutionarily conserved among all eukaryotes. At the most basic level, they can be defined as any lipid molecule that contains the sphingoid backbone, derived from condensation of an amino acid (predominantly serine) and a fatty acid (predominantly palmitate) (Merrill, 2011). For many years, these molecules were considered structural components of biologic membranes; however studies over more than two decades have disclosed important biologic activities of many sphingolipids.

Structure and classification

The core of an SPL is an organic aliphatic amino alcohol, termed a sphingoid base. There are above 60 different sphingoid base backbones that vary in alkyl chain lenghts (Merrill, 2011), the degree of saturation and position of double bonds (Pruett et al., 2008) and the presence of a hydroxyl group (Hama, 2010). Mammalian organisms contain (Figure 3), mainly sphingosine (trans-4-sphingenine, d18:1D4), sphinganine (dihydrosphingosine, d18:0) and phytosphingosine (t18:0, 4-hydroxysphinganine) (Dickson, 2008). The presence or the absence of an acyl chain distinguishes ceramide from sphingosine (Figure 3), while phosphorylation of the 1-hydroxy group generates ceramide-1-phosphate or sphingosine-1-phosphate. Other common sphingolipids contain different headgroups at this position. due to the differing acyl CoAs that can be used to produce it, ceramide is technically a class of molecules rather than a single molecule and therefore may have different biological functions depending on the acyl chain it is composed of.

The most structurally diverse sphingolipids are glycosphingolipids which are produced from ceramide precursors, why they may differ in their acyl chain composition, revealing an additional layer of variation. The head group of glycosphingolipids always consists of one or more sugar residues attached to the 1-hydroxyl group. Cerebrosides are simple glycosphingolipids which have a hexose linked to the 1-hydroxyl position through a β-glycosidic bond. Physiologically important representatives of the cerebrosides are glucosyl- and galactosyl-ceramide. One of the most differentiated series are gangliosides, acidic glycosphingolipids with at least three sugars. One of them is sialic acid, also called N-acetylneuraminic acid (Neu5Ac). Due to the large head group, the number of different gangliosides is correspondingly large.

Sphingolipids can be synthesized *de novo* by a single biosynthetic pathway, with the end product being ceramide (Bartke and Hannun, 2009).

Table 1. Sphingolipids content of different dairy products

Sample	Sphingolipids (mg 100 g^{-1} product)	Reference
Butter	71,0	Rombaut et al. (2006)
Buttermilk	19,0	Rombaut et al. (2005)
Cream	49,0	Rombaut et al. (2006)
Skimmed milk	6,0	Rombaut et al. (2006)
Swiss cheese	8,4 [a]	Ahn and Schroeder (2002b)
Whey (Cheddar)	5,0	Rombaut et al. (2005)
Whey (Emmenthal)	3,0 [b]	Theodet and Gandemer (1994)
Whey (Emmenthal)	4,0 [b]	Baumy et al. (1990)
Whole milk	4,9 [b]	Christie et al. (1987)
Yoghurt	2,8 [a]	Ahn and Schroeder (2002b)

[a]A conversion factor of 751 gmol^{-1} was used
[b]A conversion factor of 1 g mL^{-1} was used

Within the cells, sphingolipids are mainly found in the plasma membrane, the Golgi apparatus and in the lysosomes. Thus, sphingolipids can be expected in minor amounts in all food products.

Sphingolipids in food

Sphingolipids are present in nearly all foods and their amounts vary considerably. Milk and dairy products, which provide a third of the total intake, are one of the most important sources of sphingolipids (Table 1). Dairy products followed by meat and fish, eggs, and vegetables (Vesper et al., 1999). Based on a yearly per capita food consumption of 873 kg, sphingolipids constitute from 0.01 to 0.02 % of the diet (by weight). The quantities range from a few μg/kg in fruit and some vegetables up to 1 g/kg in dairy products, meat, eggs and soybeans (Vesper et al., 1999). As in many foods, sphingomyelin also accounts for the major portion of sphingolipids in dairy products. Sphingomyelin occupies a special place as a sphingophospholipid. Milk and dairy products contain around 0.3-1 % of total fat as phospholipids. Approximately 60 % of the total phospholipids in milk are found in the milk fat globule membrane. Some milk by products such as buttermilk and especially its MFGM fraction contain up to 40 % (in weight) of phospholipids, of which 30 % is phosphatidylethanolamine, 7 % is phosphatidylinositol, 5 % is phosphatidylserine, 31 % is phosphatidylcholine, and 20 % is sphingomyelin (Fong et al., 2007; Rodriguez-Alcala and Fontecha, 2010). The season and stage of lactation affect the sphingolipid content in the milk (Parodi, 1997). The content of membrane lipids, and consequently also sphingolipids, decreases along with lactation progress due to fewer presence of larger milk fat globules with thinner globule membrane (Bitman and Wood, 1990).

Structures of sphingolipids vary considerably with the type of food. In milk is mostly found sphingomyelin, but also a considerable amount of lactosylceramide, glucosylceramide, gangliosides (Jensen, 2002) and less amounts of sphingoid bases (Ribar et al., 2006; Ribar et al., 2007). Even more structural diversity is achieved by variations in the sphingoid base backbone and amide-bound fatty acids. These possible variations make sphingolipids structurally the most diverse class of membrane lipids.

Sphingolipids from food are most probably not essential for normal growth and development since most sphingolipids are synthesised de novo (Vesper et al., 1999). Nonethless both, complex sphingolipids and their digestion products (ceramides and sphingosines) as well are highly bioactive compounds with profaund effects on cell regulation. Sphingolipids from food cross the intestinal membrane and are biologically active, particularly in inhibiting colon carcinomas (Dillehy et al., 1994).

Digestion and degradation of sphingolipids

Before humans can actually utilise sphingolipids, they must be released from the food matrix by digestive enzymes. Dietary phospholipids and sphingolipids are not acted upon by lingual and gastric lipases, but the pancreatic one (occurring in in the intestinal lumen) does cleave fatty acids from their *sn*-2-position. Sphingomyelin leaves the stomach in a predominantly intact state and is first hydrolysed in the subsequent sections of the small and large intestine (Nyberg et al., 1997). The alkaline sphingomyelinase catalyses hydrolysis of sphingomyelin into ceramide and phosphocholine (Duan et al., 1995). Enzymatic activity of sphingomyelinase is low in the duodenum and reaches the highest level in the middle and lower parts of the small intestine (Nyberg et al., 1997; Duan et al., 1995). Subsequently, ceramides can be further hydrolyzed to sphingosine and fatty acid by ceramidase, which cleaves the amide bond. All of them then enter the enterocyte.

In many foodstuffs, glycosphingolipids occur in smaller quantities than sphingomyelin and are structurally more diverse. Degradation of glycosphingolipids occurs in the acidic compartments (lysosomes and endosomes) of the cell. Different hydrolases (depending on the sugar residue of the glycosphingolipid) cleave the sugar residues from the non-reducing end. The resulting mono saccharides, sialic acid, fatty acids and ceramides leave the lysosomes and are further degraded, absorbed or reintegrated into the sphingolipid cycle (Sandhoff and Kolter, 2003). Ceramide and sphingosine absorbed by the cells are either further broken down and become bioactive, or are again converted to complex sphingolipids (Merrill et al., 1995).

Sphingolipids activity

For many years, sphingolipids were considered as structural components of biologic membranes. However studies performed over more than two decades disclosed important biologic activities of many sphingolipids. Sphingolipids are abundant in the apical membrane and in the absorptive epithelium in the gut (Danielsen and Hansen, 2006), while their digestion products (ceramides and sphingosine) are considered as the most bioactive compounds, having important effects on the cell regulation. Sphingolipids such as sphingosine, ceramide, sphingosine-1-phosphate have been implicated as modulators of physiologic and pathophysiologic processes such as cell growth, cell death, autophagy, angiogenesis, cell adhesion, differentiation, migration, senescence, intracellular trafficking, stress (Nikolova-Karakashian and Rozenova, 2010; Stancevic and Kolesnick, 2010) and inflammatory responses (El Alwani et al., 2006). Ceramide and sphingosine-1-phosphate (S1P) often exert opposing functions in the cell; as ceramide has been shown to mediate cell cycle arrest and cell death in response to cell stress (Hannun and Obeid, 2011), sphingosine-1-phosphate has been shown to promote cell survival and proliferation (Maceyka et al., 2009). Alterations in bioactive sphingolipids and their metabolism have been linked to several human diseases including cancer (Zeidan and Hannun, 2007; Furuya et al., 2011).

Health benefits of dietary sphingolipids

Effects of sphingolipids on cancer

Alongside classical pharmaceutical interventions to reduce the progression or the risk of several diseases, there is an increasing interest in nutraceuticals, or functional foods, as part of encouraging lifestyle intervention. There is no known nutritional requirement for sphingolipids in the diet. However, it has been reported that dietary supplementation with sphingolipids has diverse physiological benefits. Many in vitro and in vivo studies focused on the health effects of sphingolipids. Most research is centered on some cancers and neurological pathologies. Ceramides and sphingosine, the digestion products of sphingolipids, affect cell growth, differentiation and apoptosis, suggesting that their release may

affect the behavior of normal or transformed cells, especially in the intestine (Duan and Nilsson, 2009). Dietary sphingolipids may be the key link connecting diet, inflammation and cancer (Canela et al., 2016). Sphingolipids may enhance or inhibit carcinogenesis, depending on their source and ability to be metabolized to sphingosine-1-phosphate (Degagne et al., 2014). The majority of "cancer and sphingolipids" studies concern colon cancer. The effect of dietary sphingolipids in colon cancer was actively studied both *in vitro* and *in vivo*. Evidence of the importance of dietary sphingolipids in fighting colorectal cancer was first described by Dudeja et al. (1986), who found differences in both sphingolipid composition and sphingolipid enzyme activities between tumor and normal tissue. Certain sphingolipids are also active against other cancer types, such as enigmol, a sphingoid base analog (Symolon et al., 2011). Sphingoid bases are cytotoxic for many cancer cell lines and are thought to contribute to suppression of intestinal tumorigenesis in vivo by ingested sphingolipids. Enigmol represents a novel category of sphingoid bases analogue that is orally bioavailable and has the potential to be effective against multiple types of cancer.

Sphingomyelin is one of the best-studied compounds in chemoprevention, ought to its roles as messenger in the development, growth, differentiation, and apoptosis of human cells. Therefore, several studies suggested its use in adjunct colon cancer treatment (Kuchta et al., 2012). In synthesis, sphingomyelin isolated from milk fat reduced the number of cryptic foci and prevented 1, 2-dimethylhydrazine-induced colon cancer in ICR rats and CF1 mice (Zhang et al, 2008; Dillehay et al., 1994). In colon cancer cells and mice models, sphingomyelin and glycosphingolipids from milk reduce the number of aberrant colonic crypt foci (Dillehay et al., 1994; Schmelz et al., 1996; Schmelz et al., 2000; Kurek et al., 2013). Milk-derived sphingomyelin also transformed adenocarcinomas to more benign adenomas (Schmelz et al., 1996) and exhibited chemopreventive and chemotherapic effects in CF1 mice (Lemonnier et al., 2003). Due to its content of long chain saturated fatty acids, sphingomyelin highly contributes to form, together with cholesterol, the rigid membrane domains, called "lipid rafts". Consequently, the regulation of the composition and density of lipid rafts could potentially alter cancer

cell viability and metastatic behavior (Küllenberg et al., 2012). Non-pharmacological amounts of sphingomyelin in the diet showed chemopreventive and chemotherapeutic effects on chemically induced colon cancer in mice (Lemonnier et al., 2003). The chemopreventive effects of sphingomyelin appear to be due to its principal metabolites, i.e. sphingosine, sphingosine-phosphate, and ceramide, all of which induce apoptosis (Patwardhan et al., 2016).

Sphingoid bases and ceramide have been shown to induce apoptosis in colon cancer cells and suggested as potential mediators of the protective role of more complex dietary sphingolipids in colorectal cancer development (Ahn and Schroeder, 2002a). Ceramide production and its biological function are highly dependent on the length of the covalently linked fatty acid. The interplay between ceramides of various chain lengths seems to be crucial for cancer progression. Specific sphingolipids (and bioactive sphingolipid metabolites) together with changes in the expression and/or function of sphingolipid-metabolizing enzymes, could impact therapeutic response.

Altered sphingolipid composition was shown in colon cancer cells (Van Blitterswijk et al, 1984). There is increasing evidence that alteration in sphingolipid metabolism can modulate susceptibility to intestinal tumorigenesis. Indeed, dietary sphingolipids have both, chemopreventive and chemotherapeutic effects in colon cancer animal models. In addition, modulation of the enzymes implicated in sphingolipid metabolism seem to change the susceptibility to colon cancer formation as shown in the SK1$^{-/-}$ model following AOM/DSS treatment (Kawamori et al., 2009).

Role of sphingolipids in binding and inactivation of toxins and bacteria

It is well known that appropriate consumption of gangliosides through milk and other dairy products may protect from infections by binding and inactivating certain bacteria, viruses and their toxins (Rueda, 2007). Gangliosides which are profusely present on the surface of the apical membrane of enterocytes function as binding sites for bacteria and their toxins to prevent translocation of pathogens from the gut to the internal environment. Bacteria, viruses and toxins are inactivated after binding with glycosphin-

golipids, and by exogenous sphingolipids (provided in diet) protect passage of the microorganisms through the intestinal mucosa. For example, bacterial toxins of *Shigella* and *Escherichia* or rotaviruses are bound and inactivated (Lafont et al., 2002; Lanne et al., 1995; Rolsma et al., 1998). Oral administration of gangliosides has resulted in the regresion of *H. pylori* infection (Miller-Podraza et al., 2004; Wada et al., 2010). Sprong et al. (2001) reported that milk fat sphingomyelin and lyso-sphingolipids are potent anti-bacterial agents that increase resistance toward intestinal pathogens (*E. coli*, *Salmonella enteritidis*, *Campylobacter jejuni*, *Listeria monocytogenes*) mostly due to the medium-chain (C10:0 and C12:0) fatty acids of the lyso-derivatives. Haug et al. (2007) observed that gangliosides contained in MFGM modify the gastrointestinal receptor for microbial toxins, thereby partially preventing some digestive disorders. Proper gangliosides supplementation, for example, by consumption of milk, eggs, and other dairy products may protect from infections through binding and inactivation of bacterial toxins (Birecki et al., 2006; Rueda, 2007). Therefore, it can be concluded that dietary sphingolipids, particularly milk and egg gangliosides, may protect gut against infections through binding and inactivation of microbes and their toxins.

Relation between dairy sphingolipids intake and cognitive function

Few observational findings suggest that dairy food intake may be positively related to cognitive functions (Eskelinen et al., 2008; Vercambre et al., 2009; Park and Fulgoni, 2013). Sphingomyelin and sphingolipid metabolites are fundamental components in the central nervous system of myelin sheat that surrounds the axons of some neurons. A research on developing rats demonstrated that sphingolipids, especially sphingomyelin from milk, can contribute to the myelination of the central nervous system (Oshida et al., 2003). Tanaka et al. (2012) carried out a study on premature infants and showed that the administration of sphingomyelin-fortified milk to the basis was positively associated with the neurobehavioral development. Recently, gangliosides have been associated with-enhancing spatial learning and affecting brain growth and composition in neonatal piglets (Liu et al., 2014). Studies focusing on dairy products-derived phospholipids

showed that consumption of one glass of milk per day does improve cognitive function and memory in adults (Crichton et al., 2012). Recently, Park and Fulgoni (2013) observed better global cognitive function in adults and aged individuals who consumed milk goods as compared with those who abstained from such products.

Sphingolipids are associated with age-related diseases and the development of Alzheimer's disease (Parodi, 2001), as sphingolipid signalling may play a role in the progressive loss of cell function during the aging process. There are studies supporting the hypothesis that dietary phospholipids could contribute to the therapeutic approach to Alzheimer's disease, even if this is still a controversial issue.

Effects of sphingolipids on lipid profile, cholesterol and cardiovascular risk

Lipids, although essential for humans, were historically thought to elevate blood cholesterol and were therefore considered as dangerous to health. That attitude has been changing in recent years. Sphingolipids are involved in the intestinal uptake of cholesterol. Dietary sphingolipids supplementation could limit cholesterol-related diseases by inhibiting their absorption in intestinal mucosal cells (Kurek et al., 2013). Sphingomyelin was found to dose-dependently lower the intestinal absorption of cholesterol and fats in rats (Eckhardt et al., 2002; Noh and Koo, 2003). Sphingomyelin affects different aspects of cholesterol transport and metabolism suggesting that it may influence atherosclerosis (Vesper et al., 1999). Noh and Koo (2004) observed that milk sphingomyelin is more potent inhibitor of the intestinal absorption of cholesterol than egg sphingomyelin and this behavior can be explained by the higher degree of saturation and longer chain length of the fatty acyl groups. Duivenvoorden et al. (2006) reported that dietary sphingolipids play an important role in lowering plasma cholesterol and triacylglycerol and protecting the liver from fat- and cholesterol-induced steatosis. In addition, through the promotion of adiponectin signaling, dietary sphingolipids inhibit fatty liver, hipercholesterolemia, and insulin resistance (Yunoki et al., 2010; Bamba et al., 2012). It may be concluded that dietary sphingolipids hold great potential to treat multiple aspects of the metabolic syndrome, such as dyslipidemia, insulin resistance and cardiovascular diseases.

Effects of sphingolipids on the maintenance of skin homeostasis

Some studies indicate that the ingestion of sphingolipids, especially DHCer (dihydroceramide), confers beneficial effects for the restoration of permeability barrier dysfunction caused by ultraviolet (UV) B irradiation (Fukami et al., 2014). Russell et al. (2010) suggested that milk phospholipids, and in particular sphingomyelin, act upon skin cells protecting them against the effect of ultraviolet radiation. Studies on some cell types indicated that sphingolipids may have a protective activity even against damage from γ-irradiation and chemical agents (Vesper et al., 1999). Some studies have shown that GlcCer metabolism is essential to the maintenance of skin homeostasis (Takagi et al., 2005).

Conclusions

The public should be taught about the benefits of milk fat and of milk in general. Despite advertising and educational efforts, many consumers are still not fully aware that dairy foods are a good source of high quality ingredients. These efforts will have to be supported by further research on the mechanisms whereby milk components benefit humans. Although sphingolipids are probably not essential dietary components, they can make a contribution to human health (Figure 4). Unfortunately nutritional science is at the time insufficiently familiar with their use in practice, effect on the plasma sphingolipids level and the effective quantity of the sphingolipids ingested. As scientists continue to investigate food as it relates to health, people will realize the importance of dairy products in the diet.
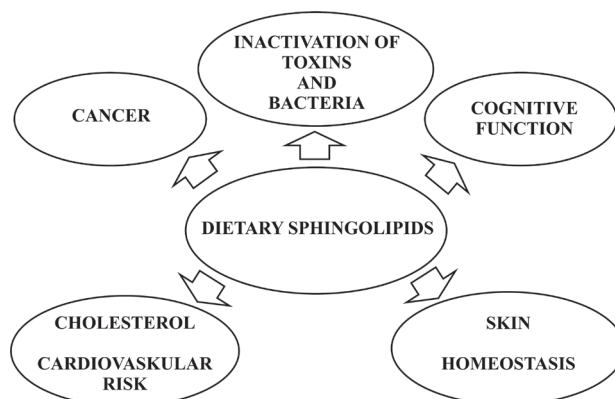

Figure 4. Health benefits of dietary sphingolipids

Potencijalne zdravstvene dobrobiti sfingolipida mlijeka i mliječnih proizvoda

Sažetak

Sfingolipidi su prisutni u svim eukariotskim i nekim prokariotskim stanicama. Mlijeko i mliječni proizvodi su jedan od najvažnijih izvora sfingolipida. Sfingolipidi sudjeluju u raznim prijeko potrebnim metaboličkim, neurološkim i unutarstaničnim signalnim procesima. Oni i njihovi derivati su vrlo bioaktivni spojevi s anti-tumorskim i bakteriostatskim svojstvima, a djeluju i na sniživanje kolesterola. Stoga je ovaj pregled usredotočen na potencijalne zdravstvene dobrobiti sfingolipida mlijeka i mliječnih proizvoda.

Ključne riječi: sfingolipidi, sphinzinske baze, gangliozidi, mlijeko, mliječni proizvodi

Explanation of abbreviation:
MFGM = milk fat globule membrane

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