Sphingolipids as Functional Food Components: Benefits in Skin-improvement and Disease Prevention

Tatsuya Sugawara

Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake Cho, Sakyo-ku, Kyoto, Kyoto 606-8502, Japan

Corresponding author, Tel: +81-75-753-6212; Fax: +81-75-753-6212; E-mail: sugawara@kais.kyoto-u.ac.jp; sugawara.tatsuya.6v@kyoto-u.ac.jp

ABSTRACT

Sphingolipids are ubiquitous components in eukaryotic organisms and have attracted attention as physiologically functional lipids. Sphingolipids with diverse structures are present in foodstuffs as these structures depend on the biological species they are derived from, such as mammals, plants, and fungi. The physiological functions of dietary sphingolipids, especially those that improve skin barrier function, have recently been noted. In addition, the roles of dietary sphingolipids in the prevention of diseases, including cancer and metabolic syndrome, have been studied. However, the mechanisms underlying the health-improving effects of dietary sphingolipids, especially their metabolic fates, have not been elucidated. Here, we review dietary sphingolipids, including their chemical structures and contents in foodstuff; digestion, intestinal absorption, and metabolism; and nutraceutical functions, based on the available evidence and hypotheses. Further research is warranted to clearly define how dietary sphingolipids can influence human health.

KEYWORDS: ceramide, cerebroside, food function, glucosylceramide, nutraceutical, skin, sphingomyelin, sphingosine, sphingoid base

1. INTRODUCTION

Sphingolipids are ubiquitous among eukaryotic organisms. They are rarely found in prokaryotes, archaea, and viruses. After their discovery in the brain in the 1880s, sphingolipids were considered to be membrane structural components. It has been subsequently clarified that sphingolipids associated with cholesterol, phospholipids, and proteins form microdomains of cellular membranes called "lipid rafts," which play an important role in cell signaling pathways[1]. In addition, numerous intermediates of sphingolipid metabolism, such as ceramide, ceramide-1-phosphate, sphingosine, and sphingosine-1-phosphate, are highly bioactive components that act as signaling molecules both within and between cells[2]. There is no doubt that endogenous sphingolipids play crucial roles in biological regulation, although they are not commonly considered essential nutrients. Sphingolipids are consumed daily as general food components. However, sphingolipids can be *de novo* synthesized by the condensation reaction between serine and palmitoyl CoA as the initial reaction and there are no symptoms related to their deficiency as nutrients. Recent emerging evidence has implicated dietary sphingolipids in human health[3–7]. The structures of sphingolipids in foodstuff are diverse. Their structures depend on biological species, such as mammals, plants, and fungi[8–11]. However, the details of the differences in the physiological activities of food-derived sphingolipids owing to differences in their structures, including their intestinal absorption and metabolic fate, remain to be determined.

The purpose of this review is to discuss the intestinal absorption and functions of sphingolipids as functional food components, focusing on their chemical structures.

2. DIVERSITY OF SPHINGOLIPID STRUCTURES IN FOODSTUFF

Sphingolipids are a family of compounds that have a sphingoid base (long-chain base) with an amide-linked fatty acid and a polar head group, such as phosphorylcholine (for sphingomyelin) or carbohydrate (for cerebrosides, gangliosides, and other complex glycolipids). The diverse structures of sphingoid bases depend on biological species (Fig. 1). In mammals, sphingosine (*trans*-4-sphingenine, d18:1^{4t}) is the most common sphingolipid, with smaller amounts of sphinganine (dihydrosphingosine, d18:0) and phytosphingosine (4-hydroxysphinganine, t18:0)[8]. The majority of sphingoid bases in mammals have 18 carbon chains. A dihydroxyl sphingoid base with 18 carbons and a double bond is abbreviated "d18:1," and a trihydroxyl sphingoid base with 18 carbons and no double bond is abbreviated "t18:0." Phytosphingosine (t18: 0) is abundant in the small intestine, kidney, and skin[12]. In bovine milk, sphingomyelin has a more varied distribution of sphingoid bases with different carbon chain lengths, ranging from C16–19, than egg sphingomyelin, which is primarily sphingosine (d18:1)[13]. Sphingoid bases in plants have a more complicated structure than those in mammals, because the sphingoid bases can be desaturated at the C8-position by 8-*cis*/*trans*-sphingolipid desaturase, producing *cis* and *trans* isomers of 8-unsaturated sphingoid bases (d18:1$^{8c/t}$, d18:2$^{4t,8c/t}$, t18:1$^{8c/t}$)[9]. A small amount of triene-type sphingoid base (sphingatrienine) has also been found in higher plants, such as rice and maize[19]. In humans, 4,14-sphingadienine (sphingadiene, d18:2^{4t,14c}) has also been found[14–16]. A typical feature of sphingoid bases in fungi is the presence of a C9-methylated sphingoid base (d19:2^{4t,8t} or 9-Me d18:2^{4t,8t}), an enzymatic reaction product of C9-methyltransferase[10,11]. Sphingolipids of marine invertebrates have atypical types of chain length and unsaturation in sphingoid bases, such as 2-amino-4,8,10-octatriene-1,3-diol (d18:3)

66 and 2-amino-9-methyl-4,8,10-octatriene-1,3-diol (d19:3)[17,18].

67 Sphingomyelin is the most abundant sphingolipid in animals and is not found in plants. In some
68 invertebrates, phosphoethanolamine and ceramide 2-aminoethyl phosphonate (CAEP) are present instead of
69 sphingomyelin (Fig.2)[20]. The polar head group of CAEP possesses a C-P bond that consists of a phosphorus
70 atom directly bound to a carbon atom, unlike the C–O–P linkage encountered in choline phosphate as the polar
71 head of sphingomyelin. Glycosphingolipids are structurally diverse and are formed by linking the sphingoid
72 base to a sugar head group through glycosidic bonds. Cerebroside (ceramide monohexoside) contains a hexose,
73 such as glucosylceramide and galactosylceramide. Gangliosides contain oligosaccharides, N-acetylglucosamine,
74 N-acetylgalactosamine, and one or more neuraminic (sialic acid) residues as the sugar chain. Although
75 glucosylceramide is common in eukaryotes, galactosylceramide and ganglioside are typical animal
76 sphingolipids that have not been found in higher plants. In contrast, plant acidic glycosphingolipids such as
77 inositol phosphorylceramide (IPC) and glycosyl IPC (GIPC) are more abundant than glucosylceramide
78 (Fig.2)[21,22]. In fungi, glucosylceramide, galactosylceramide, and lactosylceramide as neutral classes and IPC
79 and GIPC as acidic classes were found[11,21]. Plant GIPCs that have been identified include conservative
80 glucuronic acid residues, hexuronic acid, and hexose, pentose, and amino sugars.

81

82 ## 3. SPHINGOLIPIDS IN FOODSTUFF

83 The distribution and content of sphingolipids vary greatly among different foods, with fruits and vegetables
84 containing less and dairy products and eggs containing large amounts (see the worthwhile reviews[4,7,23]). Dairy

products are a significant source of sphingolipids, mainly sphingomyelin, lactosylceramide, glucosylceramide, and gangliosides. Raw milk contains 1.9–12.1 mg of sphingomyelin, 0.7–1.9 mg of lactosylceramide, and 0.8–1.2 mg of glucosylceramide per 100 g. The content of gangliosides varies between 0.14 and 1.10 mg/100 mL[24]. In dairy products, such as butter, cream, and cheese, sphingolipid ratios range from 20.5%–42.4% in their phospholipid fractions. Because the lipid content of dairy products is higher than that of raw milk, their sphingolipid concentrations are higher than those of raw milk. Eggs are also a rich source of sphingolipids (82 mg sphingomyelin/100 g)[25]. Egg yolk contains approximately 10% phospholipids and is rich in sphingomyelin. The content of egg yolk gangliosides was reported to be 15.9 mg/100 g[26]. Meat and fish also contribute to the dietary sources of sphingolipids; chicken, beef, and pork contain 270–557 nmol sphingomyelin/g and 32–67 nmol glycosphingolipids/g[27]. The sphingolipid content of fish is relatively lower than that of meats (118 ± 17 nmol total sphingolipids/g in cod up to 301 ± 43 nmol/g in salmon). However, the ratio of glycosphingolipids in total sphingolipids is higher (approximately 30%–50%). The contents of gangliosides in chicken, beef, pork, and fish are 0.95–1.44, 0.48–0.95, 0.49, and 0.76–6.48 mg/100 g, respectively[28].

In the case of plant-derived foods, such as rice, wheat, and soybean, the main types of sphingolipids are ceramide, glucosylceramide, and GIPC. The sphingolipid content in vegetables and fruits is relatively lower than that in other foods[4,7,23]. Five major glycolipid classes (acylated steryl glucoside, steryl glucoside, glucosylceramide, monogalactosyldiacylglycerol, and digalactosyldiacylglycerol) were identified in 48 edible plants available in Japan, and glucosylceramide was uniformly distributed in these plants[29]. Takakuwa *et al.* analyzed glucosylceramide contents in crop tissues and byproducts from their processing (0.01–0.94 mg/g dry

104 weight)[30]. GIPCs are considered to be significant sphingolipids in plants. Leaf vegetables (cabbage, komatsuna,
105 and lettuce) exhibit relatively higher GIPC content (10–20 mg/100 g) than other vegetables (less than 10 mg/100
106 g)[31].

107 Sphingolipids in edible marine invertebrates, such as squids, octopus, clams, and sea cucumbers, are also
108 relatively abundant, including sphingomyelin, cerebroside, and CAEP. Li *et al*. reported that the percentage of
109 total sphingolipids including ceramide, cerebroside, and CAEP in four edible shellfish accounted for 18.8%–
110 38.6% of the total lipids (more than 500 nmol/g), with sphingomyelin not being abundant in these shellfish[32].
111 Wang *et al*. reported that squid (*Loligo chinensis*) had the highest CAEP content (4.9 ± 0.4 mg/g dry weight)
112 and starfish (*Asterias amurenis*) had the lowest CAEP content (1.9 ± 0.6 mg/g dry weight) among five aquatic
113 products examined (squid, mussel, oyster, neptunea, and starfish)[33].

114 The intake of total sphingolipids in the United States is estimated to be approximately 300–400 mg/day
115 according to calculations of sphingolipid content in food materials[23]. The daily Japanese diet contains 130–300
116 mg of sphingolipids (80–220 mg of sphingomyelin and CAEP, 50–80 mg of glucosylceramide) in high-calorie
117 meals (3,000 kcal) and 50–80 mg of sphingolipids (10–60 mg of sphingomyelin and CAEP, 30 mg of
118 glucosylceramide) in low-calorie meals (1,600 kcal)[34]. In addition, the daily intake of glucosylceramide from
119 plant sources has been reported as 50 mg in the Japanese diet[29]. Human breast milk contains higher levels of
120 sphingomyelin than cow milk, and infants are estimated to consume up to 150 mg of sphingomyelin daily from
121 breast milk[35]. How GIPC contributes to sphingolipid intake in humans is unclear.

122

4. DIGESTION AND ABSORPTION OF DIETARY SPHINGOLIPIDS

Complex sphingolipids with a polar head, such as phosphocholine and a sugar chain, are commonly hydrolyzed by enzymes in the digestive tract before intestinal absorption (Fig. 3). However, the digestibility of sphingolipids is lower than that of glycerolipids, which appears to cause relatively lower intestinal absorption. For example, 32%–45% of sphingomyelin was recovered as intact sphingomyelin and digestive forms (ceramide and sphingosine) in feces during 24 h after ingestion in rats[36].

The first step in complex sphingolipid digestion is the hydrolysis of polar heads to generate ceramides. Digestive enzymes are found in the small intestine but not in the pancreatic fluid. Alkaline sphingomyelinase found on the brush border membrane of the intestinal tract with highest levels in jejunum can hydrolyze sphingomyelin to ceramide and phosphocholine[37,38,40]. This enzyme is located on the surface of the microvilli and is released into the lumen by bile salt and pancreatic trypsin[39]. In humans, but not in other species, alkaline sphingomyelinase is expressed in the liver and secreted in the bile[40,41]. Marine-derived CAEP can also be hydrolyzed by mouse intestinal mucosa. This hydrolysis is more rapid at pH 7.2 than at pH 9.0, which is the optimal condition for alkaline sphingomyelinase[42]. Although glycosphingolipid digestion is less well understood, lactase-phlorizin hydrolase contributes to the hydrolysis of glycosylceramides[43,44]. Glycosphingolipids are hydrolyzed in the digestive tract only marginally more than sphingomyelin[45,46]. Plant-derived glucosylceramide, which has more complicated sphingoid bases, is also hydrolyzed in the digestive tract, similar to animal-derived glucosylceramide[47].

In the second step, ceramide liberated from dietary complex sphingolipids is hydrolyzed to a sphingoid

142 base and a fatty acid by the neutral ceramidase located at the brush border in the intestine[48–50]. The enzyme is
143 released and is active in the presence of bile salts and is resistant to pancreatic proteases. Free sphingoid bases
144 can be found in the digestive tract after oral administration of plant- and marine-derived sphingolipids with
145 unique sphingoid bases[42,47]. In addition, the gut microbiota may contribute to sphingolipid digestion. Reduced
146 sphingomyelin hydrolytic activity in the colon of germ-free mice compared to conventional mice has been
147 reported in a previous study[40]. Another study reported that glucosylceramidase and ceramidase activities toward
148 plant-derived glucosylceramide in the cecal content of rats were comparable to those toward mammalian origin[47].
149 Sphingosine is a major sphingoid base in mammals. It is believed that sphingosine can pass through the
150 intestinal epithelial cells in an intact form by passive diffusion[51]. Recently, Narita *et al*. reported that acyl-CoA
151 synthetases promote the cellular uptake of sphingoid bases, including sphingosine, sphinganine, and
152 phytosphingosine[52]. After absorption, sphingosine is mainly metabolized in the mucosal cells to chylomicron
153 palmitic acid, and a smaller remaining portion is resynthesized into ceramide and more complex
154 sphingolipids[45,46]. In intestinal cells, exogenous sphingosine is rapidly converted to sphingosine-1-phosphate
155 (S1P) by sphingosine kinase. S1P is degraded to aldehyde (2-hexadecenal) and ethanolamine phosphate by S1P
156 lyase or dephosphorylated by S1P phosphatase and subsequently resynthesized to complex sphingolipids.
157 Sphingosine kinase and S1P lyase catalyzing sphingosine decomposition are highly expressed in the intestinal
158 mucosa[53,54]. Interestingly, phosphorylation of sphingosine is required for the incorporation of exogenous
159 sphingosine into complex sphingolipids[55]. Although hexadecanal generated from sphinganine can be
160 metabolized to palmitic acid by the same pathway of sphingosine catabolism, 2-hexadecenal from sphingosine

needs to be dehydrogenated by its Δ2-unsaturated bond to metabolize to palmitic acid. Nakahara *et al*. revealed that S1P is metabolized to glycerolipids via hexadecenal, hexadecenoic acid, hexadecenolyl-CoA, and palmitoyl-CoA[56]. In addition, phytosphingosine can be metabolized to pentadecanoic acid through 2-hydroxypalmitic acid as an intermediate[57,58].

The absorption ratio of plant- and marine-derived types of sphingoid base into lymph was reportedly lower than that of sphingosine in cannulated rats[59–61]. *In vitro* and *in vivo* studies have shown that P-glycoprotein, a member of the ATP-binding cassette transporters, contributes to the efflux of sphingoid bases other than sphingosine from intestinal cells[62,63]. The mechanism of selective efflux of sphingoid bases remains unclear because the substrate specificity of P-glycoprotein is broad, and a wide variety of hydrophobic compounds are eligible substrates. Despite the low ratio of intestinal absorption, polyunsaturated sphingoid bases derived from plants, fungi, and marine invertebrates can be absorbed and metabolized to complex sphingolipids. Mikami *et al.* reported that orally administered polyunsaturated sphingoid bases are absorbed and detected in the lymph fluid as intact forms and metabolites, such as ceramides, hexosylceramides (probably glucosylceramides), and sphingomyelins[64]. The authors reported an absorption ratio ranging from 0.1%–1.2%, depending on the structures of the bases. Regarding the catabolic fate of these sphingoid bases, there is no evidence that they can be metabolized to fatty acids, such as sphingosine.

As mentioned above, dietary complex sphingolipids need to be digested to free sphingoid bases before intestinal absorption. However, levels of molecular species of ceramide of d18:1 as well as d17:1 and d16:1 sphingosine (specific to milk sphingomyelin) are increased in rat lymph after ingestion of milk sphingomyelin,

and the composition of the molecular species is similar to ceramide moieties of dietary milk sphingomyelin[65]. Recently, dietary rice glucosylceramide (composed mainly of sphingadienine) and free ceramides prepared from soy sauce lees (mainly composed of phytosphingosine and its derivatives) were detected in mouse plasma after ingestion, through highly sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis[66]. In this case, levels of the ceramide molecules containing sphingadienine, which are hydrolysates of dietary plant glucosylceramide, were also significantly increased in the plasma after ingestion. Our findings strongly suggest that dietary ceramides and glucosylceramides can be partly absorbed as intact molecules. However, since it is difficult to distinguish between endogenous and exogenous sphingolipids using LC-MS/MS, a direct evaluation, for example, with isotope labels, is needed to fully understand the absorption and metabolism of dietary sphingolipids.

5. SKIN-IMPROVING EFFECT OF DIETARY SPHINGOLIPIDS

The skin is the largest organ that plays an important role as an effective barrier between the outside and inside of the body. The stratum corneum, the outermost layer of the skin, acts as the main barrier that protects against excessive transepidermal water loss (TEWL) and blocks irritants. Lipid lamellae in the extracellular space of corneocytes comprise 50% ceramides, 25% cholesterol, and 15% fatty acids and are vital in the barrier function and maintaining a hydrophobic environment[67–69]. The levels of epidermal ceramides decrease with skin diseases, including dry skin, atopic dermatitis, and psoriasis, as well as with aging, resulting in the deterioration of skin barrier function[70–74].

199 The beneficial effects of intake of various food-derived sphingolipids, including those derived from plants,
200 animals, and marine organisms, have been reported in several animal studies and human trials[75]. Although most
201 of these animal studies were conducted using models with disrupted skin caused by ultraviolet (UV) exposure,
202 sodium dodecyl sulfate (SDS) treatment, tape-stripping, and a special commercial diet (HR-AD), a few reports
203 on animals under normal conditions are available (Table 1). HR-AD is a magnesium-deficient diet that induces
204 skin damage and atopic dermatitis-like skin symptoms, although the causative mechanism is not clear[76,77]. After
205 causing skin perturbation by feeding hairless mice a HR-AD, dietary supplementation of plant-derived
206 glucosylceramide, sphingomyelin, and CAEP can accelerate the recovery of their skin damage, such as
207 increased TEWL and decreased stratum corneum hydration, by changing to a normal diet[78–80]. In addition, the
208 direct addition of sphingolipids, including plant-derived glucosylceramide and milk-derived sphingomyelin, to
209 the HR-AD diet drastically suppresses skin damage[81–83]. Exposure to UV radiation is a key factor in the initiation
210 of photoaging and can be characterized by dryness, wrinkling, and mottled pigmentation[84]. Mice with a single-
211 dose UVB-irradiated dorsal skin (20–200 mJ/cm^2) were used as the damaged skin model. Administration of
212 sphingolipids such as glucosylceramide and sphingomyelin for 1–2 weeks can ameliorate skin inflammation
213 and disruption of the epidermal barrier induced by UVB exposure[85–88]. In other reports, physical perturbation of
214 the skin barrier by SDS treatment and tape-stripping in hairless mice was protected by supplementation with
215 glucosylceramide for 2 weeks[60,78,79,89,90]. In contrast, even under normal conditions, dietary sphingolipids seem
216 to further improve skin conditions. Haruta-Ono *et al*. examined the effect of dietary milk sphingomyelin
217 concentrate on epidermal conditions in hairless mice[91–93]. In this case, stratum corneum hydration and TEWL

were significantly improved in the sphingomyelin-fed mice. Recently, we also found that TEWL in hairless mice was significantly suppressed by dietary supplementation with free ceramide prepared from soy sauce lees as or more effectively than maize glucosylceramide[94]. Overall, the differences in sphingolipid structure, including a polar head and sphingoid bases, are not important for skin protection and improving effects in skin-damaged models and normal animal skin. However, in most studies, sphingolipids were not included in the semi-purified diet as a control diet, and their deficiency may have affected the results.

Despite the structural differences between food-derived sphingolipids and epidermal ceramides, whether dietary sphingolipids improve skin barrier function remains unelucidated. Although the mechanism underlying skin barrier improvement remains largely unknown, some mechanisms have been proposed by foregoing studies. Epidermal ceramide levels were increased by dietary sphingolipid intake in various animal studies. However, it was unclear whether the increase in epidermal ceramide content was associated with reutilization of the metabolite of dietary sphingolipids. Ueda *et al*. reported that orally administered radiolabeled sphingosine can be distributed in the skin, and the cumulative recovery of radioactivity in the skin was 0.72% at 168 h after a single administration (4 mg/kg body weight)[95]. Haruta-Ono *et al*. evaluated the distribution and fate of radiolabeled metabolites in mice orally administered [4,5-^{3}H-sphinganyl] sphingomyelin and detected radiolabeled sphingomyelin and ceramide in the skin[93]. These results indicate that dietary sphingosine and sphinganine, the major sphingoid bases in mammals, can be partly reutilized in epidermal sphingolipids.

It is unlikely that directly reutilizing sphingolipids from food plays a major role in the skin improvement effect. As mentioned above, the absorption ratio of dietary sphingolipids, particularly plant-derived types, is

much lower than that of other lipids[59]. Ono *et al*. reported that 8-unsaturated and 9-methylated sphingoid bases were not detected in the skin after 7 weeks of feeding 0.1% maize or yeast glucosylceramide diet[96]. However, the beneficial effects of intake of various plant-derived sphingolipids having different sphingoid bases from mammals on the skin have been reported to be similar to those of mammalian sphingolipids, which are mainly composed of sphingosine. A new insight into the mechanism for the skin barrier-improving effect of dietary sphingolipids appears to be the activation of *de novo* ceramide synthesis in the epidermis. We reported that dietary glucosylceramide (from maize) upregulated ceramide synthase in the epidermis of HR-AD mouse models, similar to sphingomyelin (from porcine)[79]. Dietary milk sphingomyelin was found to significantly increase the content of covalently bound ω-hydroxyceramides in the skin of HR-AD mice[82]. The structure formed by the binding of ω-hydroxyceramides to cornified envelope proteins plays an important role in skin barrier function[97]. In addition, dietary CAEP (from squid) and glucosylceramide (from maize) significantly increased the content of covalently bound ω-hydroxyceramides, and the expression of their biosynthesis-related genes in the skin of HR-AD mice[80]. Considering this evidence, the difference in sphingolipid structures, especially sphingoid bases, is not crucial for the activation of *de novo* ceramide synthesis in the epidermis.

Several studies have shown that dietary sphingolipids affect the expression of genes involved in the maintenance and formation of the stratum corneum, which is associated with cornified envelope and tight junction protein formation. Ideta *et al.* demonstrated a significant increase in the mRNA expression of genes related to the cornified envelope and tight junction formation in glucosylceramide (from konjac)-fed/SDS-treated mouse skin, through microarray analysis[90]. Hasegawa *et al*. reported that the level of transglutaminase-

1 mRNA expression in UVB irradiation-induced barrier-perturbed hairless mouse skin was increased by dietary konjac glucosylceramide[85]. These results suggest that the enhanced tight junction formation could be induced by dietary plant-derived glucosylceramide and could at least partly contribute to the improvement of tight junction permeability function.

Inhibition of the inflammatory response in the skin may also be another mechanism for the skin barrier-improving effect of dietary sphingolipids. The production of the inflammatory cytokine interleukin (IL)-1α in SDS-treated skin of hairless mice was reduced by the oral administration of glucosylceramide (from konjac)[89]. In a chronic irritant contact dermatitis mouse model of inflammation, oral glucosylceramide administration suppressed the mRNA expression of proinflammatory cytokines such as IL-1β and IL-6[98]. In addition, we previously evaluated the effects of orally administered maize glucosylceramide on inflammation using a 2,4-dinitro-1-fluorobenzene-treated murine model[99]. Oral supplementation with glucosylceramide suppressed ear swelling and leukocyte infiltration to the inflammatory site and downregulated the activation of tumor necrosis factor-alpha, suggesting that dietary glucosylceramide has anti-inflammatory properties. Oba *et al*. indicated that oral administration of milk sphingomyelin significantly downregulated mRNA levels of acute inflammation-associated genes, including thymic stromal lymphopoietin (TSLP), IL-1β, and IL-6 in hairless mouse skin exposed to a single dose of UVB[87]. Similarly, dietary milk sphingomyelin significantly decreased both thymus and activation-regulated chemokine (TARC) and TSLP mRNA levels in the skin of HR-AD mice[82]. Clinical trial data have also supported the skin barrier-improving effect of dietary sphingolipids (Table 2). The oral intake of glucosylceramide (from konjac, 1.8 mg/day for 8 weeks) decreased the TEWL levels in atopic

dermatitis patients[100]. In another study, oral intake of glucosylceramide (from konjac, 1.8 mg/day for 2 weeks) improved skin symptoms (scoring atopic dermatitis [SCORAD] index) and reduced skin allergic responses induced by house dust mite in children with atopic dermatitis[101]. Uchiyama *et al*. described significantly lower cheek TEWL in the test product group (glucosylceramides from konjac, 1.8 mg/day) than in the control group in a randomized, double-blind, placebo-controlled study[89]. Other research groups have also shown that intake of food-derived sphingolipids, such as plant- and yeast-derived glucosylceramide and milk sphingomyelin, improved skin condition in healthy subjects[102–106]. The difference in sphingolipid structure, including a polar head and sphingoid bases, has not proven to be crucial in the skin-improving effect in clinical trials, similar to the results of animal studies. Surprisingly, the results of these clinical studies have indicated that extremely low doses are effective, considering the intake of total sphingolipids from daily meals and the results from the aforementioned animal model-based studies.

To elucidate the mechanism underlying the skin barrier-improving effect of dietary sphingolipids, several *in vitro* studies using cultured keratinocytes and a three-dimensional human skin model have been conducted. Most of the findings support the hypothesis that sphingolipid metabolites, especially sphingoid bases, are involved in barrier improvement[79,85,86,107]. For example, Shirakura *et al*. reported that sphingoid bases prepared from konjac glucosylceramide (d18:2 and t18:1) activated genes related to *de novo* ceramide synthesis and increased ceramide production, whereas glucosylceramide and sphingosine could not[107]. However, considering the digestion and absorption mechanisms, difference in chemical structures, and levels of sphingolipids in daily meals, it is difficult to explain the mechanism of the skin-improving effects of dietary sphingolipids using the

results of *in vitro* studies. To define how dietary sphingolipids can influence skin barrier function, it is necessary to fully understand the metabolic processes of sphingolipids.

6. BENEFICIAL EFFECT OF DIETARY SPHINGOLIPIDS IN DISEASE PREVENTION

Sphingolipids have the potential to prevent and alleviate several diseases, including cardiovascular disease, non-alcoholic fatty liver disease, type 2 diabetes mellitus, and colon cancer[3–7,108]. The functions of dietary sphingolipids in the prevention and alleviation of these diseases may be related to their resistance to digestion and absorption in the gastrointestinal tract.

Dietary sphingomyelin and its hydrolysates, including ceramide and sphingosine, inhibit the absorption of cholesterol and fatty acids in cultured cells and animal studies[109–116]. The possible mechanism is thought to be that the combination of sphingolipids and cholesterol causes molecules to accumulate in tighter micelles, suppressing the release of cholesterol in the digestive tract[109]. Indeed, several studies have shown the hypolipidemic effects of dietary sphingomyelin and sphingomyelin-rich fractions from food sources in animal studies and human trials[3]. Furthermore, marine-derived cerebroside (from sea cucumber) can decrease serum cholesterol levels in mice[117]. However, concerning the relationship between sphingolipids and cardiovascular disease, increased circulating ceramide levels are associated with a variety of metabolic and cardiovascular pathologies[118–121]. Thus, specific circulating ceramides can be used as biological predictors and markers of cardiovascular disease. However, unlike endogenous sphingolipids, dietary sphingolipids attenuated the development of atherosclerosis, rather than promoting cardiovascular disease, in animal studies[122–124].

313 Furthermore, supplementation with milk polar lipid enriched cream cheese (containing sphingomyelin and
314 ceramide) decreased the levels of atherogenic sphingomyelin (C16:1 and C18:1) and ceramide (C24:1) species
315 associated with the improvement of cardiovascular risk markers in a human trial[125].

316 Several studies have indicated the suppressive effects of dietary sphingolipids derived from foodstuffs
317 on colon cancer in chemically induced rodent models, such as 1,2-dimethylhydrazine and azoxymethane, as
318 well as in inheritance models[6]. Sphingolipids are not as easily digested in the small intestine as glycerolipids.
319 Thus, upon reaching the lower digestive tract, their intact forms and their hydrolysates, such as ceramide and
320 sphingoid bases, can increase the levels of bioactive ceramide and sphingoid bases in the colon; these effects
321 may be associated with the therapeutic function. Regardless of the difference in sphingolipid structure, including
322 a polar head and sphingoid bases, intake of various food-derived sphingolipids, including sphingomyelin (milk
323 and egg) and glucosylceramide (soybean, maize, and yeast), suppressed formation of aberrant crypt foci in a
324 rodent model of colon cancer. However, the efficiencies of the chemotherapeutic and chemopreventive effects
325 seem to be slightly dependent on their chemical structures[6]. These effects may partly result from anti-
326 proliferative activities via the anti-inflammatory effects of sphingolipids. Indeed, several reports have also
327 indicated the anti-inflammatory effects of dietary sphingolipids in a dextran sulfate sodium-induced colitis
328 model[126,127].

329 Significant amounts of dietary sphingolipids and their hydrolysates can reach the colon, where they may
330 modulate the gut microbiota and host response to microbial components. Norris *et al*. reported that
331 supplementation with milk sphingomyelin lowered circulating lipopolysaccharide content in mice fed a high-

fat diet and observed that fecal microbiota composition was modulated to increase *Bifidobacterium* and reduce *Bacteroidetes* abundance[128]. Chung *et al*. evaluated the effect of egg sphingomyelin on atherosclerosis in apolipoprotein E (*ApoE*)-knockout mice fed a high-fat diet. Dietary sphingomyelin reduced aortic arch lesion size; however, this effect was abolished by co-ingestion of antibiotics[122]. Millar *et al*. also found that dietary egg sphingomyelin attenuated aortic root plaque development and modulated the gut microbiota in *ApoE*-knockout mice[123]. These findings suggest that the anti-atherosclerotic effects of dietary sphingomyelin may act in part through its effects on the gut microbiota. In addition, we previously found that the contents of acetic acid, propionic acid, and total short-chain fatty acids in the cecum were significantly increased with supplementation of cerebroside from sea cucumber in hairless mice[60]. The increased production of short-chain fatty acids is indicative of an improved intestinal environment owing to the interaction between microbiota and non-digested dietary sphingolipids. Glucosylceramide prepared from koji, a Japanese traditional dietary fungus, has been reported to act as a prebiotic for *Blautia cocoides*[129]. Further studies on the potential effects of dietary sphingolipids on gut microbiota–host interactions are warranted.

7. PERSPECTIVES FOR RESEARCH ON DIETARY SPHINGOLIPIDS

As highlighted in this review, the food functionality of sphingolipids has been firmly established. There is no doubt that dietary supplementation with sphingolipids can contribute to human health. However, knowledge of this field is not comprehensive, compared to that of other nutrients. Sphingolipids have not been considered essential nutrients, especially since no symptoms related to their deficiency have been observed. Recently, it

has been suggested that dietary polar lipids such as phospholipids and sphingolipids, especially ganglioside, may have positive effect on cognitive development in infants[130,131]. The potential benefits of maternal dietary ganglioside supplementation on fetal and infant brain development have been also discussed[132]. On the contrary, nutritional intervention via administration of sphingomyelin-fortified milk has been positively associated with the cognition and neurodevelopment of low-birth-weight infants. Schneider *et al*. found that dietary sphingomyelin level positively correlates with cognitive behavior and brain myelin formation in healthy children[133]. The presence of sphingomyelin in breast milk may play a specific role in infant nutrition. The composition of milk sphingolipid is species specific. For example, human breast milk has a higher level of total sphingomyelin content than bovine milk[35]. Considering the effect of dietary sphingolipids in the improvement of the skin barrier, sphingomyelin in breast milk may also contribute to the development of the infant skin barrier.

The structural diversity of sphingolipids in food has impeded the understanding of their absorption, metabolism, and functionality. Recent advances in LC-MS technology have enabled detailed qualitative and quantitative analyses of sphingolipids in various biological samples[7,134,135]. However, knowledge remains insufficient to distinguish between endogenous and exogenous constituent sugars (e.g., glucose and galactose) and differences in the double bond positions. The supply of standard materials and stable isotope labels for a large number of specific sphingolipid molecules in food will greatly contribute to the progress of future research on dietary sphingolipids. Although several mechanisms of sphingolipid functionality have been proposed, many aspects remain unclear. The fact that sphingolipids exert their functions despite their low absorbability, which is not dependent on their structural differences, is one of their features, and elucidation of their intestinal

absorption mechanism and metabolic fate is necessary to understand the detailed role of dietary sphingolipids.

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Conflict of Interest Disclosure

The author declares no competing financial interests.

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709

710 Figure legends

711

712 Figure 1. Typical structures of sphingolipids and differences in sphingoid base among biological species.

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715 Figure 2. Structures of marine sphingophosphonolipids and plant sphingophospholipids. Cer, ceramide; R, a

716 sugar residue.

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719 Figure 3. Proposed scheme of digestion, absorption, and metabolic fate of dietary sphingolipids.

720

721 Table 1. Animal Studies Examining the Effects of Dietary Sphingolipids on Skin

Author	Experimental Design	Results
Tsuji *et al.*2006[78]	0.1% GlcCer from rice germ or bran in diet for 4 weeks after HR-AD diet for 4 weeks (n = 4 or 5)	↓ TEWL (−60%, $p < 0.05$); ↑ Stratum corneum flexibility (+130%, $p < 0.01$)
	Tape-stripping after 0.1% maize GlcCer in diet for 5 weeks (n = 8)	↓ TEWL (−40%, $p < 0.05$)
Uchiyama *et al.* 2008[89]	Oral administration of konjac GlcCer (30 μg/day) for 14 days with 10% SDS treatment from day 4 (n = 10)	↓ ΔTEWL (SDS-treated site - SDS-untreated site) (−40%, $p < 0.01$)
	Oral administration of konjac GlcCer (250 μg/day) for 14 days with 10% SDS treatment (n = 4 or 5)	↓ IL-1α production in skin (−60%, $p < 0.05$)
Haruta *et al.*2008[91]	0.8% SM (as phospholipid concentrate from milk) in diet for 6 weeks (male, n =12; female, n = 12)	↑ Stratum corneum hydration (male +30%; female +14%, $p < 0.05$); ↑ Contents of ceramides in stratum corneum (male +33%; female +7%, $p < 0.05$)

		↔TEWL
Ideta *et al.* 2011[90]	10% SDS treatment for 4 days after oral administration of konjac GlcCer (30, 165, 250 μg/day) for 2 weeks (n = 5)	↓ ΔTEWL (SDS-treated site - SDS-untreated site) (250 μg/day vs control −50%, $p < 0.05$)
Hasegawa *et al.* 2011[85]	Irradiation of UVB (150 mJ/cm^2) after oral administration of konjac GlcCer (250 μg/day) for 14 days (n = 6)	↓ TEWL (−17%, $p < 0.05$ at 3 days; −20%, $p < 0.01$ at 4 days); ↑ Transglutaminase-1 (+50%, $p < 0.05$)
Duan *et al.* 2012[79]	0.1% maize GlcCer or 0.1% porcine brain SM in diet for 6 days after HR-AD diet for 10 weeks (n = 6)	↓ TEWL (−54%, $p < 0.05$); ↑ mRNA expression of CerS 3 and CerS 4 in epidermis (+150%, $p < 0.05$)
	Tape-stripping after 0.1% maize GlcCer in diet or 0.1% porcine brain SM in diet for 2 weeks (n = 6)	↓ TEWL (−15%, $p < 0.05$)
Haruta-Ono *et al.* 2012[92]	0.03, 0.11, 0.67% SM concentrate from milk in diet for 6 weeks (n = 7)	↑ Stratum corneum hydration (+20%, $p < 0.05$); ↓ TEWL (−28-40%, $p < 0.05$);
Haruta-Ono *et al.* 2012[93]	0.1% milk SM (n = 7) or 0.7% SM concentrate from milk (n = 10) in diet for 8 weeks	↑ Stratum corneum hydration (+18-38%, $p < 0.05$)

Kawano *et al.* 2013[81]	0.1% GlcCer (as beet extract) in HR-AD diet for 8 weeks (n = 4)	↓ TEWL (− 60%, $p < 0.001$); ↓ Scratching (−40%, $p < 0.01$); ↓ Thickness of the epidermis (−45%, $p < 0.001$)
Oba *et al.* 2015[87]	Oral administration of milk SM (146 mg/kg body weight/day) for 10 days and then irradiation of UVB (20 mJ/cm^2) at day 8 (n = 8)	↓ TEWL (−38%, $p < 0.05$); ↑ Stratum corneum hydration (+75%, $p < 0.05$); ↓ mRNA expression of TSLP (−55%, $p < 0.05$), IL-1β (−75%, $p < 0.05$) and IL-6 (−75%, $p < 0.05$) in the skin one day after irradiation
Morifuji *et al.* 2015[82]	0.07% or 0.41% milk phospholipid concentrate (containing 16% SM) in HR-AD diet for 8 weeks (n = 10)	↓ TEWL (−25% at low dose; −75% at high dose, $p < 0.05$); ↑ Stratum corneum hydration (+80% at low dose; +190% at high dose, $p < 0.05$); ↑ Covalently bound ω-hydroxy ceramide in the epidermis (+20-70% at low dose; +125-1600% at high dose, $p < 0.05$); ↓ mRNA expression of TSLP (−97% at high dose,

		$p < 0.05$) and TARC in the skin (−40% at low dose; −70% at high dose, $p < 0.05$); ↓ Serum IgE (−90% at low dose; −95% at high dose, $p < 0.05$), TSLP (−35% at low dose; −98% at high dose, $p < 0.05$), TARC (−30% at low dose; −60% at high dose, $p < 0.05$), and sP-selectin (−30% at low dose; −60% at high dose, $p < 0.05$)
Duan *et al.* 2016[60]	Tape-stripping after 0.1% sea cucumber cerebroside in diet for 2 weeks ($n = 5$)	↓ TEWL (−15%, $p < 0.05$)
Kuwata *et al.* 2017[83]	0.1% pineapple GlcCer in HR-AD diet for 4 weeks ($n = 8$)	↓ TEWL (−35%, $p < 0.05$)
Tokudome *et al.* 2017[88]	Oral administration of beet GlcCer (300 μg/day) for 2 weeks and UVB irradiation (200 mJ/cm^2) at day 7 ($n = 6$)	↓ Thickness of the epidermis (−45%, $p < 0.01$)
Tomonaga *et al.* 2020[80]	0.1% maize GlcCer or 0.1% squid skin CAEP in diet for 6 days after HR-AD diet for 11 weeks ($n = 6$)	↓ TEWL (−20%, $p < 0.05$, CAEP vs control 3 days after treatment); ↑ Stratum corneum hydration (+15%, p

		< 0.05); ↑ Covalently bound ω-hydroxy ceramide in the epidermis (+110-400%, p < 0.05); ↓ Thickness of the epidermis (−60%, p < 0.05)
Ohta *et al.* 2021[94]	0.1% maize GlcCer or 0.1% soy sauce lees Cer in diet for 2 weeks (n = 6)	↓ TEWL (−25%, p < 0.05, Cer vs control)

Abbreviations: CAEP, ceramide 2-aminoethyl phosphonate; Cer, ceramide; GlcCer, glucosylceramide; SM, sphingomyelin; TARC, thymus and activation-regulated chemokine; TEWL, transepidermal water loss; TSLP, thymic stromal lymphopoietin; ↓, statistically significant decrease; ↑, statistically significant decrease; ↔, no change.

727 Table 2. Human Trials Examining the Effects of Dietary Sphingolipids on Skin

Author	Subjects	Treatment and duration	Results
Miyanishi *et al.* 2005[100]	Patients with atopic eczema (nine female and five male)	Konjac GlcCer (1.8 mg/day) for 8 weeks	↓ TEWL (−30%, $p < 0.05$); ↓ SCORAD index (−15%, $p < 0.01$)
Kimata 2006[101]	Children with moderate atopic dermatitis (the control group consisted of 12 boys and 13 girls and the ceramide group consisted of 12 boys and 13 girls)	Konjac GlcCer (1.8 mg/day) for 2 weeks	↓ SCORAD index (−30%, $p < 0.01$); ↓ Allergic skin responses to house dust mite (−15%, $p < 0.01$); ↓ House dust mite-specific IgE (−40%, $p < 0.01$)
Uchiyama *et al.* 2008[89]	Healthy individuals who	Konjac GlcCer (1.8 mg/day) for 12 weeks	↓ TEWL (−20% cheek at 10 w, $p < 0.01$; −15% arm at 4 w, $p <$

	self-assessed their skin roughness and itching (n = 41 for GlcCer group)		0.01; −5% cheek at 8 w, $p < 0.05$; −15% arm at 12 w, $p < 0.05$)
Guillou *et al.* 2011[102]	Healthy women with dry to very dry skin (n = 25)	Wheat extract oil (containing ceramides, glycosylceramides, digalactosyldiglycerols, phospholipids, triacylglycerols, and sterols; 350 mg/day) for 3 months	↑ Skin hydration (+35%, $p <$ 0.001); skin dryness and redness tended to be reduced; visual analogue scale index tended to increase
Higurashi *et al.* 2015[103]	Healthy subjects with low skin hydration (n = 29 for 5 mg/day; n = 30 for 10 mg/day)	Milk SM (10 or 5 mg/day) for 12 weeks	↑ Skin hydration (+400%, $p <$ 0.05, 5 mg/day vs placebo)
Koikeda *et al.* 2017[104]	Healthy adult volunteers (n = 11	Peach GlcCer (0.6 and 1.2 mg/day) for 20 days	↓ TEWL (−30%, $p < 0.01$, left calves); ↑ Water content of skin

	for 0.6 mg/day; n = 12 for 1.2 mg/day)		(+30%, $p < 0.05$, 1.2 mg/day vs placebo)
Fukunaga et al. 2018[105]	Subjects who experienced subjectively assessed skin chapping and dryness in winter ($n = 11$)	Yeast GlcCer (1.8 mg/day) for 4 weeks	↓ TEWL (−15%, $p < 0.05$, forearm)

728 Abbreviations: GlcCer, glucosylceramide; SM, sphingomyelin; TEWL, transepidermal water loss; SCORAD,
729 scoring atopic dermatitis↓, statistically significant decrease; ↑, statistically significant decrease.

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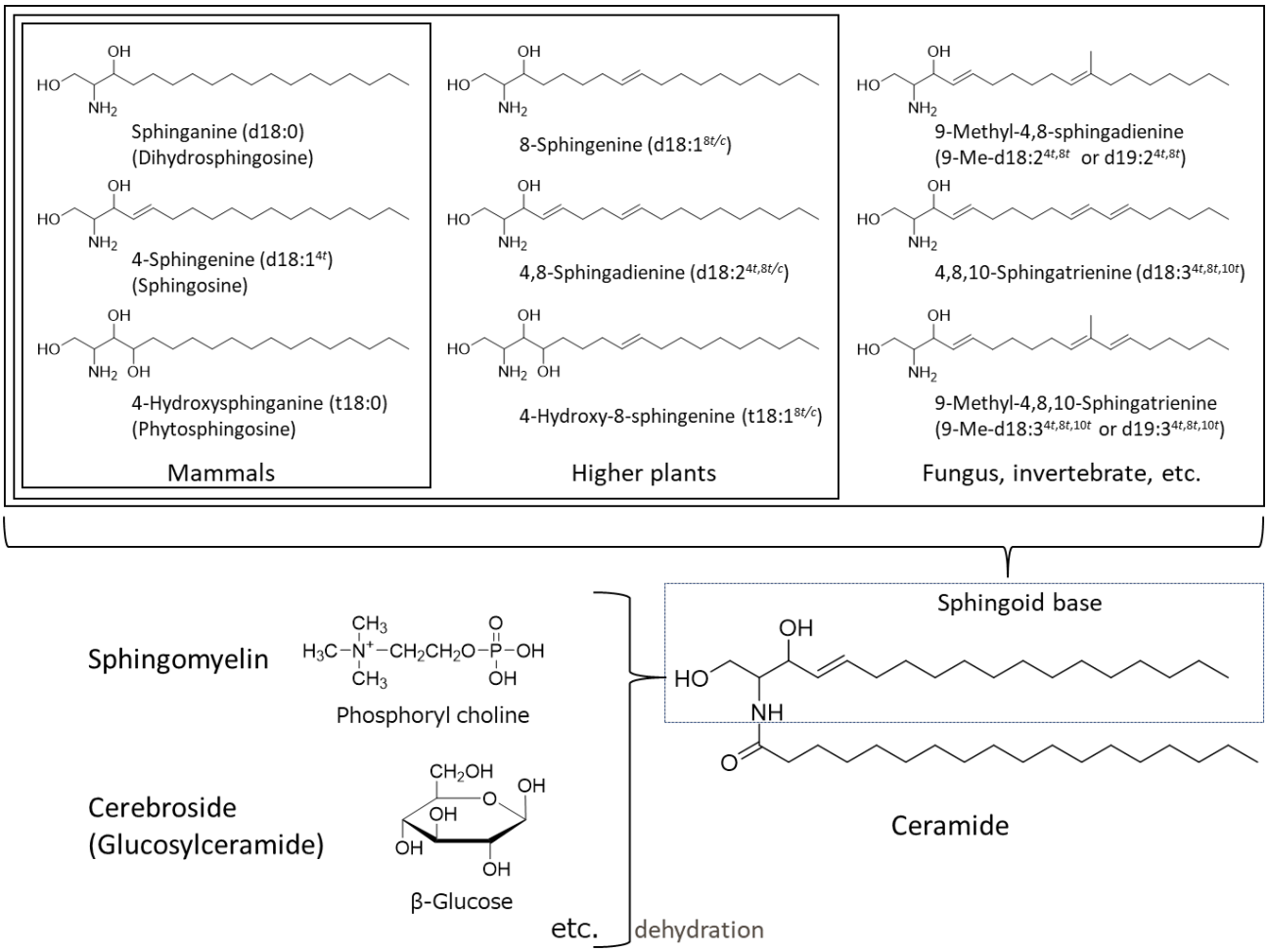

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735 Figure 1

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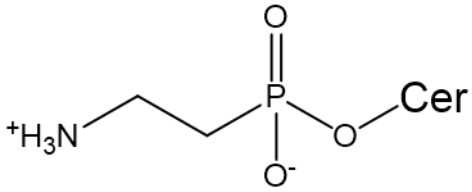
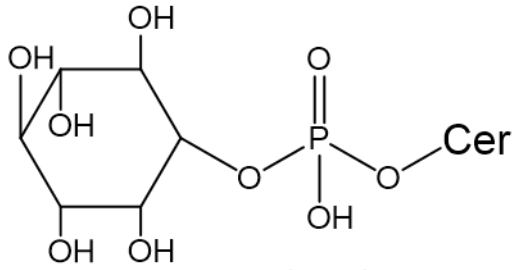


Ceramide 2-aminoethyl phosphonate (CAEP)



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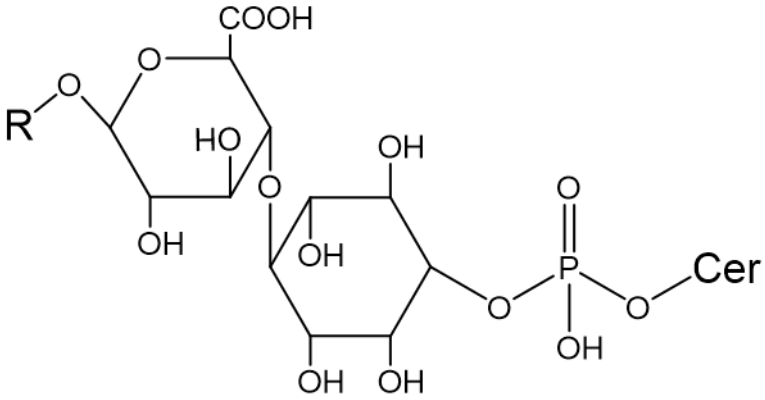

Glycosyl inositol phosphorylceramide (GIPC)

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740 Figure 2

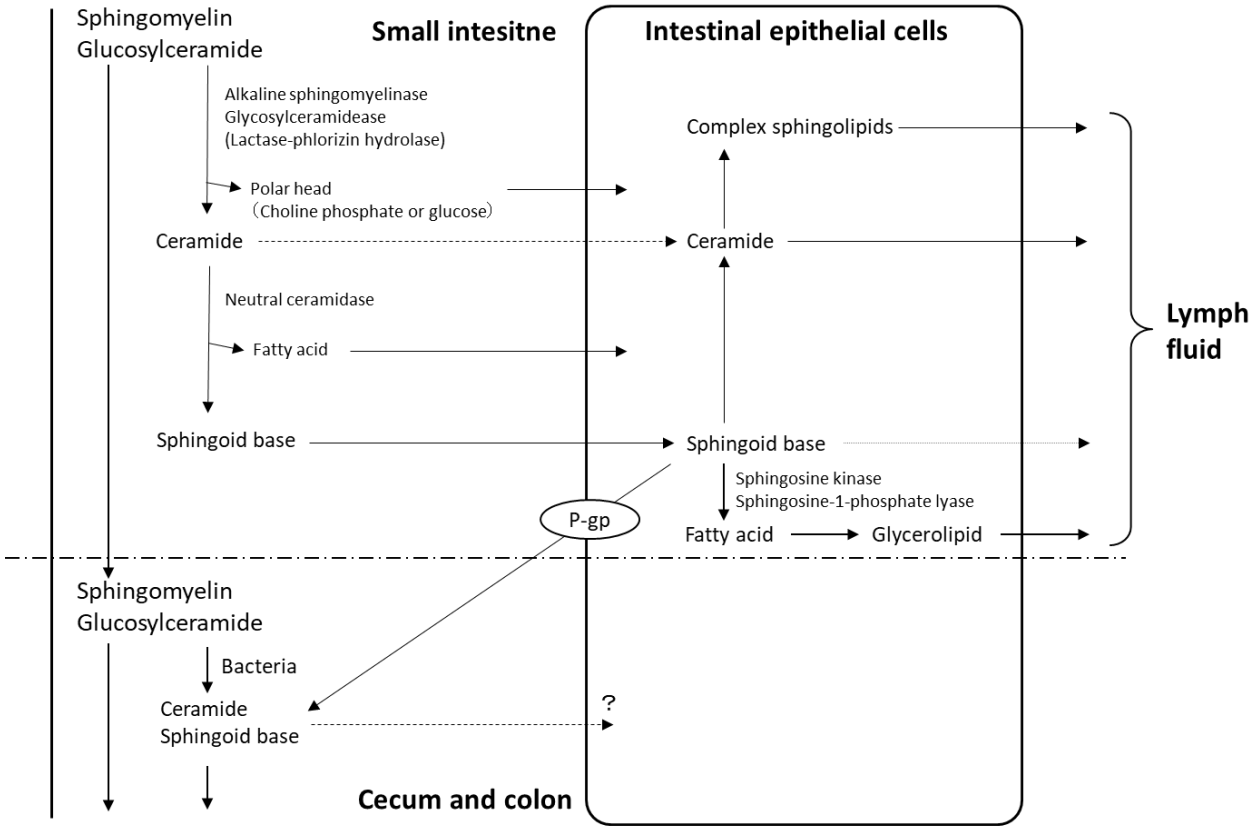
Sphingomyelin
Glucosylceramide
Small intesitne
Intestinal epithelial cells
Alkaline sphingomyelinase
Glycosylceramidease
(Lactase-phlorizin hydrolase)
Complex sphingolipids
Polar head
(Choline phosphate or glucose)
Ceramide
Ceramide
Neutral ceramidase
Fatty acid
Lymph fluid
Sphingoid base
Sphingoid base
Sphingosine kinase
Sphingosine-1-phosphate lyase
P-gp
Fatty acid
Glycerolipid
Sphingomyelin
Glucosylceramide
Bacteria
Ceramide
Sphingoid base
?
Cecum and colon

Figure 3

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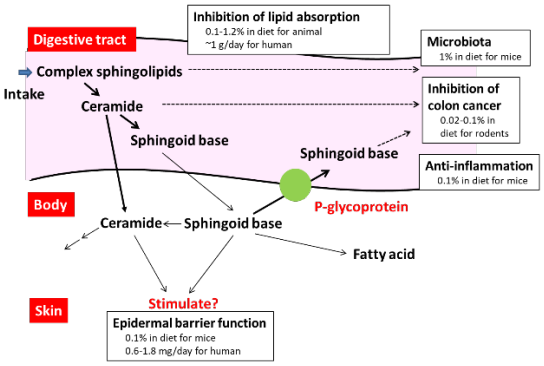

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751 Table of contents graphic