Critical Review

Sphingolipids in Food and the Emerging Importance of Sphingolipids to Nutrition[1]

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ABSTRACT Eukaryotic organisms as well as some prokaryotes and viruses contain sphingolipids, which are defined by a common structural feature, i.e., a "sphingoid base" backbone such as D-*erythro*-1,3-dihydroxy, 2-aminooctadec-4-ene (sphingosine). The sphingolipids of mammalian tissues, lipoproteins, and milk include ceramides, sphingomyelins, cerebrosides, gangliosides and sulfatides; plants, fungi and yeast have mainly cerebrosides and phosphoinositides. The total amounts of sphingolipids in food vary considerably, from a few micromoles per kilogram (fruits) to several millimoles per kilogram in rich sources such as dairy products, eggs and soybeans. With the use of the limited data available, per capita sphingolipid consumption in the United States can be estimated to be on the order of 150–180 mmol (~115–140 g) per year, or 0.3–0.4 g/d. There is no known nutritional requirement for sphingolipids; nonetheless, they are hydrolyzed throughout the gastrointestinal tract to the same categories of metabolites (ceramides and sphingoid bases) that are used by cells to regulate growth, differentiation, apoptosis and other cellular functions. Studies with experimental animals have shown that feeding sphingolipids inhibits colon carcinogenesis, reduces serum LDL cholesterol and elevates HDL, suggesting that sphingolipids represent a "functional" constituent of food. Sphingolipid metabolism can also be modified by constituents of the diet, such as cholesterol, fatty acids and mycotoxins (fumonisins), with consequences for cell regulation and disease. Additional associations among diet, sphingolipids and health are certain to emerge as more is learned about these compounds. J. Nutr. 129: 1239–1250, 1999.

KEY WORDS: • *sphingolipids* • *diet* • *disease* • *cancer* • *functional foods*

Sphingolipids are constituents of most foods, but the amounts are relatively small, and there is no evidence that dietary sphingolipids are required for growth or survival. Nonetheless, both complex sphingolipids and their digestion products (ceramides and sphingosines) are highly bioactive compounds that have profound effects on cell regulation. This article reviews the structures of sphingolipids, their occurrence in food, digestion and metabolism, biochemical functions and apparent roles in both the etiology and prevention of disease.

Structures of sphingolipids

Sphingolipids were first characterized by J.L.W. Thudichum while studying the chemical constituents of brain (1884), whereupon, he named their novel and characteristic "sphingosin" backbone for "the many enigmas it has presented to the inquirer." D-*erythro*-sphingosine[5] is the prevalent sphingoid base of most mammalian sphingolipids, but there are >60 different sphingoid base backbones (Karlsson 1970) that vary in alkyl chain lengths (from 14 to 22 carbon atoms), degree of saturation and position of the double bonds, presence of a hydroxyl group at position 4 and branching of the alkyl chain (**Fig. 1**) (for a more in-depth overview of sphingolipids, see Merrill and Sweeley 1996).

The amino group of the sphingoid base is usually substituted with a long-chain fatty acid to produce "ceramides" (Fig. 1). The fatty acids vary in chain length (14–30 carbon atoms; sphingolipids account for a substantial portion of the very long-chain fatty acids of mammals), degree of unsaturation (but are usually saturated), and presence or absence of a hydroxyl group on the α- (or, in the case of the ceramides of skin, the ω-) carbon atom. More complex sphingolipids have a polar headgroup at position 1, as illustrated by a few examples in Figure 1. In yeast, and potentially in other organisms, sphingolipids are covalently attached to membrane proteins (Conzelmann et al. 1992). When variation in the sphingoid bases, fatty acids and headgroups are considered together, the individual molecular species of sphingolipids numbers in the thousands, making them the most structurally diverse, as well as complex, category of lipids.

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[5] Sphingosine is sometimes used as a generic term for all sphingoid bases, but most often refers specifically to D-*erythro*-1,3-dihydroxy, 2-aminooctadec-4-ene or *trans*-4-sphingenine (d18:1).

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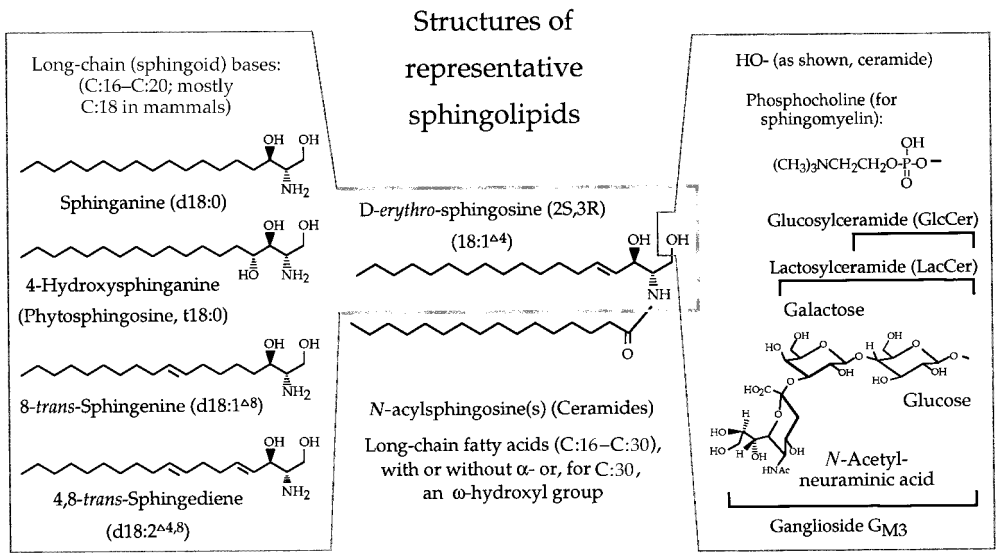

FIGURE 1 General structures of sphingolipids. Complex sphingolipids are elaborations of long-chain (sphingoid) bases by the addition of long-chain fatty acids in amide linkage and polar head groups. Sphingoid bases are abbreviated by citing (in order of appearance in the abbreviation) the number of hydroxyl groups (d and t for di- and tri-hydroxy, respectively), chain length and number of double bonds, as shown in the figure. Five common sphingolipids are shown: ceramide, sphingomyelin, glucosylceramide (GlcCer), lactosylceramide (LacCer) and ganglioside G_{M3}. Simple glycosphingolipids, such as GlcCer and LacCer, are often termed "cerebrosides," whereas gangliosides specifically contain one or more *N*-acetylneuraminic acids (sialic acids).

Occurrence and functions

Sphingolipids are located in cellular membranes, lipoproteins (especially LDL) and other lipid-rich structures, such as skin. The cellular functions of sphingolipids are summarized in **Figure 2**. Sphingolipids are critical for the maintenance of membrane structure, especially that of "microdomains" (such as caveolae) (Harder and Simons 1997); they modulate the behavior of growth factor receptors and extracellular matrix proteins (Hakomori 1991) and serve as binding sites for some microorganisms, microbial toxins and viruses (Bennun et al. 1989, Fantini et al. 1993, Karlsson 1986).

Sphingolipids function as "second messengers" for growth factors, cytokines, differentiation factors, 1α,25-dihydroxycholecalciferol and a growing list of agonists and toxins (and toxic insults, such as γ-radiation) (for reviews see Kolesnick 1998, Merrill et al. 1997, Riboni et al. 1997, Spiegel and Merrill 1996). As illustrated schematically in Figure 2, platelet-derived growth factor (PDGF)[6] induces sphingomyelin hydrolysis to ceramide (by sphingomyelinase), which is further metabolized (by ceramidase and sphingosine kinase) to sphingosine and sphingosine 1-phosphate. In contrast, tumor necrosis factor-α (TNF-α) usually activates only sphingomyelinase, which results in ceramide accumulation. These differences have profound effects on the behavior of the cells because sphingosine 1-phosphate is a potent mitogen and an inhibitor of apoptosis (Cuvillier et al. 1998, Olivera and Spiegel 1993), whereas sphingosine and ceramide inhibit growth and/or induce apoptosis (Hannun 1994, Jayadev et al. 1995, Sweeney et al. 1998). A given agonist can produce a different profile of these metabolites over time or at varying concentrations of the agonist; for example, interleukin-1β treatment of hepatocytes activates or inhibits ceramidase in a bimodal manner to elevate sphingoid bases at the expense of ceramide (and vice versa) (Nikolova-Karakashian et al. 1997). There is much yet to be learned about how these pathways are regulated; nonetheless, this model provides a starting point for exploration of the cellular behaviors that might be affected by provision of these bioactive molecules in the diet.

Sphingolipids in food

Sphingolipid content. **Table 1** summarizes the amounts of sphingolipids in food, estimated as closely as possible from the available literature. The amounts vary considerably, from the

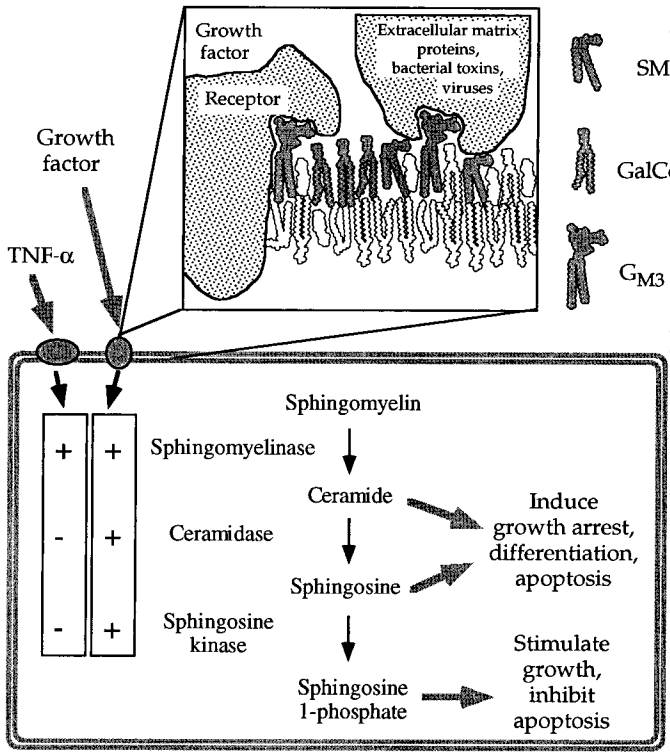

FIGURE 2 Depiction of cellular functions of sphingolipids. The exploded diagram highlights the predominantly extracellular orientation of sphingolipids in the plasma membrane (sphingolipids are depicted by shading), interactions of sphingomyelin (SM) with cholesterol (in black), the aggregation of galactosylcermide (GalCer) in "microdomains" (other cerebrosides and SM can also form microdomains), and interactions between gangliosides (such as G_{M3}) with cell receptors as well as extracellular proteins. Also shown is the pathway for turnover of sphingomyelin in response to platelet-derived growth factor (PDGF) or tumor necrosis factor-α (TNF-α) to produce different bioactive metabolites and intracellular responses.

[6] Abbreviations used: Cer, ceramide; DMH, 1,2-dimethylhydrazine; Gal, galactose; GC, gas chromatography; Glc, glucose; GPI, glycosylphosphatidylinositol; HMG, β-hydroxyl-β-methyl glutarate; Man, manose; MS, mass spectrometry; PDGF, platelet-derived growth factor; PUFA, polyunsaturated fatty acids; TNF-α, tumor necrosis factor-α; ZDF rats, Zucker diabetic fatty rats.

TABLE 1

Sphingolipids in food and yearly sphingolipid consumption per capita

Food sources	Sphingolipid content[1]	Food consumed per capita[2]	Sphingolipids consumed per capita	Reference
	μmol/kg	*kg/y*	*μmol/y*	
Dairy products[3]			38,464	
Milk (3.5%)	160	36	5764	Zeisel et al. 1986; Newburg and Chaturvedi 1992; Jensen 1995
Lowfat Milk (<2%)	92	60	5486	
Cream (37%)	1692	1	1692	
Cheese (29%)	1326	12	15,912	
Frozen dairy (11%)	503	14	7042	
Evaporated and condensed (9%)	412	4	1648	
Butter	460	2	920	Zeisel 1994
Meat products and fish			34,270	Blank et al. 1992
Beef and veal	390	29	11,310	
Pork	350	23	8050	
Chicken	530	22	11,660	
Turkey	390	6	2340	
Fish	130	7	910	
Eggs	2250	14	31,500	Zeisel 1994
Vegetables			8122 [33,782][9]	
Bell peppers	36	3	108	Whitaker 1996
Tomato	42[4]	41	1722	Whitaker 1996, Zeisel 1994
Potato	69[4]	64	4116	Galliard 1968a, Zeisel 1994
Sweet potato	669[5]	2	1338	Walter et al. 1971
Spinach	67[5]	0.3	20	Ohnishi et al. 1983
Soybeans	2410[5]	NA		Ohnishi and Fujino 1982
Cauliflower	183[6]	1	183	Zeisel 1994
Cucumber	27[6]	5	135	Zeisel 1994
Lettuce	50[6]	10	500	Zeisel 1994
Other vegetables			[25,660][7]	[7]
Fruits			3179 [5387][9]	
Apples	69	25	1725	Gaillard 1968b
Orange	24[6]	40	960	Zeisel 1994
Peanuts	78[6]	3	234	Zeisel 1994
Banana	20[6]	13	260	Zeisel 1994
Other fruits			[2208][8]	[8]
Cereals				
Wheat flour	576[5]	66	38,016	Laine & Renkomen 1974
Total sphingolipid intake (μmol/y)			153,551	[181,419][9]
Total sphingolipid intake (g, calculated as sphingomyelin)			116	[139][9]

[1] Sum of sphingomyelin and glycosphingolipids. Where the amounts have been published in grams, the conversion to moles was calculated using an average molecular weight for sphingomyelin of 751 g/mol or an average molecular weight for glycosylceramide of 747 g/mol.

[2] Putman and Allhouse 1995

[3] Dairy calculated with a density of milk of 1.03 g/mL. Milk (<2%) includes lowfat milk, skim milk, buttermilk and dry milk products. Sphingolipid estimate for whole milk based on the sum of ca 120 μmol sphingomyelin/kg (Zeisel et al. 1986), 26 μmol cerebrosides/kg (Newburg & Chaturvedi, 1992) and 14 μmol gangliosides/kg (Jensen 1995). For other sources, the estimates have been based on the average milk fat content of the product (shown in brackets; data obtained from USDA Nutrition Database for Standard Reference, Release 12, March 1998) and the sphingolipid content of the fat component of whole milk.

[4] Sum of reported contents of sphingomyelin and glycolipids.

[5] Estimation based on glycolipid content only.

[6] Estimation based only on reported sphingomyelin content (glycosphingolipid contents have not been reported).

[7] Estimated from the average sphingolipid content of vegetables listed above (including soybeans) of 394 μmol/kg multiplied by the remaining vegetable consumption (65 kg).

[8] Estimated from the average sphingolipid content of the fruits listed above (48 μmol/kg) multiplied by the remaining fruit consumption (46 kg).

[9] Total including estimates in brackets.

low micromoles per kilogram in fruits and some vegetables to ~2 mmol/kg (1–2 g/kg) in dairy products, egg and soybeans. It should be noted that the studies from which we calculated these amounts were designed in large part to elucidate the chemical structures of specific classes of sphingolipids rather than to quantify the sphingolipid content. Many utilized indirect measurements such as the phosphorous content of sphingomyelin (Blank et al. 1992, Zeisel et al. 1986), the hexose content of cerebrosides (Walter et al. 1971, Whitaker 1996) or the total lipid nitrogen content (Gaillard 1968a and 1968b); a few employed HPLC or gas chromatography (GC) to characterize individual molecular species (see Cahoon and Lynch 1991, Whitaker 1996, Zeisel 1994, for examples). Thus, depending on the procedures that were employed, the studies provided information about the content of an individual sphingolipid class (usually selected because it was the major species) or the sum for a group of compounds. Except for milk (Jensen 1995, Keenen and Patton 1995), little is known about variation in sphingolipid amounts over season (day of lactation, in the case of milk), losses during food preparation and

other aspects of food chemistry. As far as we are aware, this is the first collation of data on the sphingolipid content and types in food, and there is clearly a need for further analyses.

Consumption of sphingolipids per capita. The items in Table 1 cover almost 80% (by weight) of the foods consumed in the United States; the remainder is comprised of caloric sweeteners (9%) (which do not contain sphingolipids), other vegetables and fruits (12%) and miscellaneous (3%). Therefore, using these data, an approximation of the yearly consumption of sphingolipids from each source was prepared. Dairy products appear to be major sources, followed by meat and fish, eggs, and vegetables; the contribution from vegetables was the most difficult to estimate from available data. Yearly per capita intake of sphingolipids from the foods in Table 1 is 154 mmol, which is equivalent to ~116 g. If fruits and vegetables contribute the higher estimates for these categories (based on the average content of known fruits and vegetables, as described in footnotes 7 and 8 of Table 1), this adds another 28 mmol, for a total of 181 mmol (139 g) per year. Based on a yearly per capita food consumption of 873 kg, sphingolipids constitute from 0.01 to 0.02% of the diet (by weight). This amount (0.3–0.4 g/d) provides few "fat calories" but is comparable, instead, to lipids such as cholesterol and tocopherols (Ensminger et al. 1994). Consumption could be higher than this estimate, for the reasons enumerated above, and could vary considerably among individuals who consume foods that are particularly rich in sphingolipids.

Structural variation of sphingolipids in food. The sphingolipid backbones, fatty acids and headgroups vary considerably with the type of food. Most foods of mammalian origin (beef, milk or poultry, for example) have a wide spectrum of complex sphingolipids (sphingomyelins, cerebrosides, globosides, gangliosides or sulfatides) that are comprised of many different headgroup components (phosphocholine, glucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine, *N*-acetylneuraminic acid, fucose and other carbohydrates) and ceramide backbones (d18:1$^{\Delta 4}$, d18:0 and t18:0, with amide-linked fatty acids of 16–30 carbon atoms in length, some of which have an α-hydroxyl group) (Merrill and Sweeley 1996). For example, milk contains (per L) 39–119 mg of sphingomyelin, 6–11 mg of glucosylceramide, 6.5–15 mg of lactosylceramide and ~11 mg of gangliosides (~9–13 mg G_{D3}, 1.2 mg G_{D1b1}, 0.7 mg G_{M2}, 0.3 mg G_{M3} and 0.001 mg G_{M1}) (see Jensen 1995 for a review); the lipid backbones of milk sphingomyelin have mainly sphingosine (d18:1^{t4}, with smaller amounts of sphinganine and other chain length homologs) and 16:0, 22:0, 23:0 and 24:0 as the major fatty acids (Morrison 1969, Schmelz et al. 1996, Zeisel et al. 1986).

In contrast, the complex sphingolipids of plants are mainly cerebrosides (mono- and oligohexosylceramides) with glucose (Glc, the most common hexose), galactose (Gal), mannose (Man) and inositol.[7] Examples are as follows: wheat grain has glycosphingolipids with primarily Glc, but also, Man-Glc, $[Man]_2$-Glc and $[Man]_3$-Glc headgroups, and has the sphingoid base backbones d18:1$^{\Delta 4}$, d18:1$^{\Delta 8}$, d18:2 $^{\Delta 4,8}$, t18:0 and t18:1$^{\Delta 8}$ with 14:0–26:0 fatty acids (most as α-hydroxy fatty acids) (Fujino et al. 1983); rice grain has Glc, Man-Glc, Glc-Glc, $[Man]_2$-Glc, Glc-Man-, $[Man]_3$-Glc with d18:0, d18:1$^{\Delta 4}$ and d18:2$^{\Delta 4,8}$ sphingoid bases and 16:0–24:0 fatty acids (including some α-hydroxy fatty acids) (Fujino et al. 1985); spinach leaves sphingolipids are comprised of Glc, Cellobiose and Glc-$[Man]_2$-Glc with d18:0, d18:1$^{\Delta 8}$, d18:2$^{\Delta 4,8}$, t18:0, t18:1$^{\Delta 8}$ with 16:0–24:0 fatty acids (Ohnishi et al. 1983); soybean has a single cerebroside, Glc ceramide (Cer), with d18:0, d18:1$^{\Delta 4}$, d18:1$^{\Delta 8}$, d18:2$^{\Delta 4,8}$, t18:0, t18:1$^{\Delta 8}$ and 16:0–26:0 fatty acids (including α-hydroxy and α,β-dihydroxy fatty acids) (Ohnishi and Fujino 1982);[8] bell pepper and tomato also have mainly GlcCer with d18:2$^{\Delta 4,8}$, d18:1$^{\Delta 8}$, t18:1$^{\Delta 8}$ sphingoid bases and 16:0–24:0 (including α-hydroxy-) fatty acids (Whitaker 1996).

This structural variability and lack of reference material pose special problems for analyses of sphingolipids in food. Recent developments in the analysis of sphingolipids by GC/HPLC/mass spectroscopy (MS) and tandem mass spectrometry are making it feasible to accurately identify and quantify complex sphingolipids (for additional information, see Adams and Ann 1993, Murphy 1993).

Sphingolipid digestion and utilization

Hydrolysis of sphingolipids in the gastrointestinal tract. Sphingomyelin and cerebrosides undergo little cleavage in the stomach, but are hydrolyzed in all subsequent regions of the small intestine and colon of rats and mice (Nilsson 1968 and 1969b, Schmelz et al. 1994). The luminal contents of rat small (and large) intestine contain substantial sphingomyelinase, glucoceramidase and ceramidase activities (Nilsson 1969a and 1969b). Not all of the ingested sphingolipids are hydrolyzed and absorbed, however. Nilsson (1968) reported that ~25% of an administered dose of sphingomyelin was excreted in feces, of which 10% was the intact molecule, 80–90% was ceramide and 3–6% was free sphingosine. There is a direct correlation between the amount of sphingomyelin that is fed vs. the amount found in the colon (Nyberg et al. 1997). Germ-free mice show a drastically reduced hydrolysis of sphingomyelin, which suggests that intestinal microflora are major contributors to sphingolipid turnover in the lower bowel (Duan et al. 1995 and 1996). Similar studies with cerebrosides (Nilsson 1968) found that 43% was excreted, with 40–70% as the intact molecule and 25–60% as ceramide. Less is known about human metabolism of sphingolipids, but human pancreatic juices contain a taurocholate-dependent neutral sphingomyelinase (Chen et al. 1992), and an alkaline sphingomyelinase has been detected in human bile (Nyberg et al. 1996).

Uptake of sphingolipids. Much of the sphingosine (and, perhaps, ceramide) that is derived from hydrolysis of complex sphingolipids is rapidly taken up by intestinal cells and degraded to fatty acids (via fatty aldehydes) or reincorporated into complex sphingolipids that remain associated primarily with the intestine (Nilsson 1968, Schmelz et al. 1994). When sphingoid-base–labeled sphingolipids are fed to rats, a small amount of the radiolabeled sphingoid base is found in lymph, blood and liver, which implies that some component(s) of dietary sphingolipids are transported through the mucosa and appear in systemic circulation (Nilsson 1968, Schmelz et al. 1994). Chylomicrons may be involved in sphingolipid transport because intestinal lymph contains ~1 nmol/mL of sphingolipid (~40% of which is ceramide) (Merrill et al. 1995).

Transport of sphingolipids via serum lipoproteins. Sphingolipids are components of serum lipoproteins, with the greatest amounts in LDL followed by VLDL > HDL (Merrill et al. 1995). Sphingomyelin is the major sphingolipid of LDL and

[7] In this regard, some of the estimates in Table 1 are puzzling because plants are generally not thought to contain substantial amounts of sphingomyelin (Lynch 1993).

[8] The composition may depend on the source because we have recently analyzed soy cerebrosides and found one major GlcCer, with d18:2$^{\Delta 4,8}$ and α-hydroxypalmitic acid (h16:0) (M. C. Sullards, D. V. Lynch, E. M. Schmelz, E. Wang, A. H. Merrill. Jr. & J. Adams, unpublished data).

HDL, whereas VLDL contain mainly ceramide. Small amounts of free sphingoid bases are present in blood (Wang et al. 1992), associated with albumin and circulating cells (both erythrocytes and leukocytes) (Wilson et al. 1988); sphingosine 1-phosphate is also found in plasma and serum, but appears to be derived from platelets (Yatomi et al. 1995). The latter finding is intriguing because endothelial cells have a high affinity receptor (Edg-1) for sphingosine 1-phosphate (Van Brocklyn et al. 1998).

Cellular metabolism of sphingolipids and regulation of sphingolipid biosynthesis by diet. An in-depth discussion of cellular sphingolipid metabolism lies beyond the scope of this review, but can be found elsewhere (Merrill and Sweeley 1996, Merrill et al. 1997). All organs appear to be capable of de novo sphingolipid biosynthesis (Merrill et al. 1985, Nagiec et al. 1996), and there is no evidence that consumption of dietary sphingolipids is required for growth under normal conditions. Nonetheless, exogenous sphingolipids are required for the growth of mammalian cells with defects in serine palmitoyltransferase (Hanada et al. 1992), the initial enzyme of sphingolipid biosynthesis, which establishes that sphingolipids are necessary for normal cell function.

De novo sphingolipid synthesis is subject to some degree of feedback regulation. Incorporation of radiolabeled serine into sphingolipids is partially suppressed by LDL (Chatterjee 1998, Verdery and Theolis 1984) or sphingoid bases (Merrill 1983, van Echten et al. 1990) at the level of serine palmitoyltransferase expression (Mandon et al. 1991) and involving sphingoid base 1-phosphates (van Echten-Deckert et al. 1997). Therefore, it is possible that the sphingoid base backbones that are recovered from dietary sphingolipids affect tissue sphingolipid biosynthesis.

Dietary sphingolipids and cancer

Sphingosine and ceramide affect cell growth, differentiation and apoptosis in most types of cells that have been studied in culture (Hannun & Obeid 1995, Jayadev et al. 1995, Sweeney et al. 1998). This raises the possibility that release of these compounds during digestion of dietary sphingolipids may alter the behavior of normal or transformed cells, especially of the intestine. No deleterious effects have yet been noted in several sphingolipid feeding studies (Dillehay et al. 1994, Imaizumi et al. 1992); the latter study involved sphingolipid feeding at 1% of the diet for two generations.

Effects of sphingolipids on colon carcinogenesis. Normal intestinal cells undergo rapid turnover, except in cancer in which there is loss of normal growth arrest and apoptosis. Therefore, digestion of sphingolipids to ceramide and sphingosine might reduce the risk of colon cancer if, as shown in **Figure 3**, uptake of these compounds induces growth arrest, differentiation and/or apoptosis (perhaps by by-passing a defect in sphingomyelinase that was noted by Dudeja et al. 1986 to be one of the earliest biochemical changes detected in colon cancer). To test this hypothesis, sphingomyelin was purified from powdered milk[9] and fed to female CF1 mice that had been treated with 1,2-dimethylhydrazine (DMH) to induce colon tumors (Dillehay et al. 1994). The controls were fed a standard AIN76A diet, which is composed of defined ingredients that contain very low amounts of sphingolipids. Sphingomyelin supplementation at 0.1% of the diet (wt/wt) had no effect on weight gain of the animals, but reduced the number

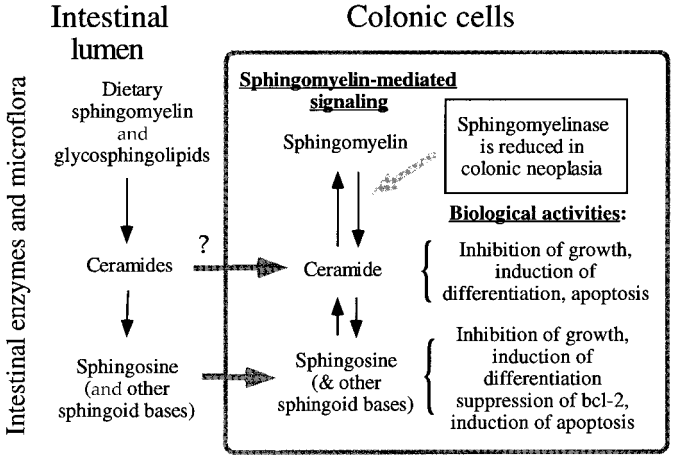

FIGURE 3 A model for the suppression of colonic neoplasia by the uptake of sphingoid bases (and possibly ceramides) derived from the digestion of dietary sphingolipids. Sphingomyelin and at least some categories of glycosphingolipids are hydrolyzed throughout the intestine, including the colon, to ceramide and backbone sphingoid bases, which are taken up by the cells. Colonic cells degrade the sphingoid base (not shown) or resynthesize more complex sphingolipids (ceramides, sphingomyelin and glycosphingolipids). Sphingoid bases and ceramides can also inhibit cell growth and induce apoptosis in transformed cells, which appear to have defective regulation of sphingomyelinase(s).

of aberrant colonic crypt foci (an early marker of colon carcinogenesis) by ~70% and, with longer feeding, reduced the number of adenocarcinomas (the latter was only marginally significant, $P = 0.08$, perhaps due to the small number of animals used in this study).

In a larger follow-up investigation (Schmelz et al. 1996), sphingomyelin caused a comparable reduction in aberrant colonic crypt foci and the number of crypts per focus, but after 40 wk there was no difference in tumor number. Nonetheless, all of the tumors of the mice fed the standard diet (without sphingolipid supplementation) were malignant adenocarcinomas, whereas there was a significant shift in tumor type from adenocarcinomas to the more benign adenomas in mice fed 0.025% ($P = 0.075$) or 0.05% sphingomyelin ($P = 0.043$). The shift in tumor type suggests that sphingomyelin feeding suppresses the conversion of adenomas to adenocarcinomas, although this is only one of several possible mechanisms. Perhaps as importantly, the amounts that had a detectable effect (0.025–0.5% of the diet) are close to the estimated consumption in the United States (0.01–0.02% of the diet). Therefore, if "mice and men" are similar with respect to sphingolipids and colon carcinogenesis, modest increases in consumption as part of sphingolipid-rich foods or supplements might further reduce the risk of colon cancer.

Structure-function relationships between sphingolipids and their effects on colon carcinogenesis. As already noted, the sphingolipids of food vary in both the lipid backbones and headgroups. To evaluate whether the sphingosine backbone is required, *N*-palmitoylsphingomyelins with sphingosine or sphinganine as the backbone were synthesized and fed to DMH-treated CF1 mice (Schmelz et al. 1997). Dihydrosphingomyelin (with sphinganine) was more effective than sphingomyelins (with the sphingosine backbone) in the reduction in aberrant crypt formation. These findings are noteworthy because ceramide signaling usually requires the 4,5-*trans*-double bond (Bielawska et al. 1993); therefore, the inhibition of aberrant colonic crypt formation by dietary (dihydro)sphingo-

[9] Because sphingolipids are associated with the globule membrane rather than with the lipid droplet per se, a substantial portion remains in low fat dairy products, including "nonfat" dry milk (Jenson 1995).

myelins appears to be due to the free sphingoid base (sphingosine or sphinganine) rather than ceramide.

The efficacy of glycosphingolipids in reducing the formation of adenocarcinomas has not yet been determined. However, ganglioside G_{M1} is at least four- to eightfold more potent than sphingomyelin (Dillehay et al. 1994), and milk glucosylceramide, lactosylceramide and ganglioside G_{D3} are comparable to sphingomyelin (Schmelz et al., unpublished observations) in suppressing aberrant colonic crypt formation. Thus, both sphingomyelin(s) and glycosphingolipids affect this early stage of colon carcinogenesis.

Sphingolipids and human colon cancer. Neither human clinical trials nor epidemiologic studies have yet evaluated whether sphingolipids influence human colon cancer. Nonetheless, sphingosine and ceramide induced apoptosis in a human adenocarcinoma cell line, HT29 cells (Schmelz et al. 1998), and we have recently found that sphingolipids reduce tumor number in Min mice (Schmelz et al. unpublished observations), which have a genetic defect similar to that found in human familial adenomatous polyposis (which arises from a defective APC gene). Mutation of the APC gene is also found in up to 60% of sporadic human colon cancers (Powell et al. 1992). In addition, sphingomyelinase activity is decreased in human colorectal carcinoma (Hertervig et al. 1997), as has been seen in colon carcinogenesis in rodents (Dudeja et al. 1986). On the basis of these findings, it is plausible that dietary sphingolipids influence human colon cancer risk.

Studies of anticancer activity in other cell types. Sphingolipids are growth inhibitory and cytotoxic for numerous transformed cell lines in culture (Merrill et al. 1996, Stevens et al. 1990), and inhibit the transformation of C3H10T1/2 cells by both γ-irradiation (Borek et al. 1991) and chemical carcinogens (Borek and Merrill 1993) with phorbol esters as the promoter. Sphingoid bases and their analogs inhibit the growth and metastasis of human and mouse tumor cells in athymic and euthymic mice (Endo et al. 1991, Sadahira et al. 1992), inhibit the induction of ornithine decarboxylase in mouse skin (Enkvetchakul et al. 1989), and increase skin cancer–free survival under some application protocols (Birt et al. 1998). Therefore, dietary sphingolipids may affect cancers at sites other than the colon. It should be borne in mind that sphingosine is mitogenic in a few instances (Zhang et al. 1990), apparently via its conversion to sphingosine 1-phosphate (Zhang et al. 1991); therefore, effects in vivo should be evaluated carefully and thoroughly.

Epidemiologic relationships between diet and cancer in view of sphingolipids. The risk of colon cancer has been associated with diet; however, identification of the responsible factors remains controversial (Kim and Mason 1996). Because the sphingolipid content of food has not been considered in any of these analyses, this might explain some of this confusion. Some foods that are rich in sphingolipids (such as dairy products and soy) have received attention from cancer researchers for some time. For example, dairy products reduce the incidence of aberrant crypts (Abdelali et al. 1995, Nelson et al. 1987) in rats, reduce aberrant colonic epithelial cell proliferation and restore a more normal differentiation profile in humans (Holt et al. 1998) and are correlated with a reduced risk of human colon cancer (Glinghammar et al. 1997, Van der Meer et al. 1997). These effects may reflect the calcium and vitamin D in dairy products; however, case-control and cohort studies concerning calcium intake and colon carcinogenesis have been inconclusive (Giovannucci and Willet 1994, Kim and Mason 1996, Pence et al. 1996, Potter et al. 1993). It is possible that the presence of sphingolipids may help explain some of the benefits of dairy products and other foods.

Other potential relationships between diet, sphingolipids and disease

Sphingolipids may have roles in other diseases and aging through the effects of dietary sphingolipids per se and through the effects of other components of the diet on sphingolipid metabolism and cell regulation. Some of the underlying mechanisms that could account for such associations are shown in **Figure 4**.

Sphingomyelin and cholesterol associations. Associations between sphingomyelin and cholesterol have intrigued researchers for decades (Barenholz and Thompson 1980, Ikonen 1997, Merrill and Jones 1990, Slotte and Bierman 1988, Vandenheuvel 1965). At least one molecular explanation for the cellular association of these lipids is their colocalization in "microdomains" such as caveolae (Harder and Simons 1997) that are thought to be enriched in membrane receptors and transporters, especially those linked to the plasma membrane via glycosylphosphatidylinositol (GPI)-anchors (Bilderback et al. 1997, Fiedler et al. 1994). Depletion of either sphingomyelin or cholesterol disrupts these microdomains and the functioning of proteins associated with them, as has been seen in the loss of folate transport in Caco-2 cells treated with inhibitors of cholesterol or sphingolipid biosynthesis (Stevens and Tang 1997).

Sphingomyelin affects many aspects of cholesterol transport and metabolism (and vice versa), as indicated in Figure 4, including the following: cholestrol efflux from cells (Jian et al. 1997, Yancey et al. 1995, Zhao et al. 1996); the conversion of cholesterol to bile acids, cholesterol esters and other metabolites (Boldin and Jonas 1996, Rye et al. 1996, Subbaiah and Liu 1993; the regulation of β-hydroxyl-β-methyl glutarate (HMG)-CoA reductase activity (Gupta and Rudney 1991); and, proteolysis of sterol regulatory element binding proteins (Scheek et al. 1997). Induction of sphingomyelin turnover as part of cell signaling (in response to TNF-α) increases cholesterol esterification (Chatterjee 1994), which provides a relatively unexplored link between cell signaling events and cholesterol homeostasis.

Cholesterol and other lipids can also alter sphingomyelin metabolism (Leppimaki et al. 1998). An inhibitor of cholesterol synthesis, 25-hydroxycholesterol, stimulates sphingomyelin synthesis in Chinese hamster ovary cells (Ridgway 1995). In vivo, diets supplemented with cholesterol (Geelen et al. 1995, Nikolova-Karakashian et al. 1992) affect tissue sphingomyelin content and metabolism. Feeding of different oils to experimental animals (Bettger et al. 1996) influences the fatty acid composition of sphingomyelin; and essential fatty acid deficiency reduces the formation of the skin ceramides (Wertz 1992).

These interactions suggest that sphingomyelin may influence atherosclerosis, either directly or by affecting other risk factors such as cholesterol. Additional observations that also support this possibility are as follows: *1*) sphingomyelin affects LDL binding and utilization by cells in culture (Chatterjee 1993); *2*) hydrolysis of LDL sphingomyelin by an extracellular sphingomyelinase that is enriched in atherosclerotic lesions alters the aggregation state of the particle and promotes foam cell formation by macrophages (Marathe et al. 1998, Schissel et al. 1996a and 1996b); *3*) oxidized lipoproteins have been reported to stimulate the growth of vascular smooth muscle cells (Augé et al. 1996) and human blood monocytes (Kinscherf et al. 1997) via triggering of the sphingomyelin signal-

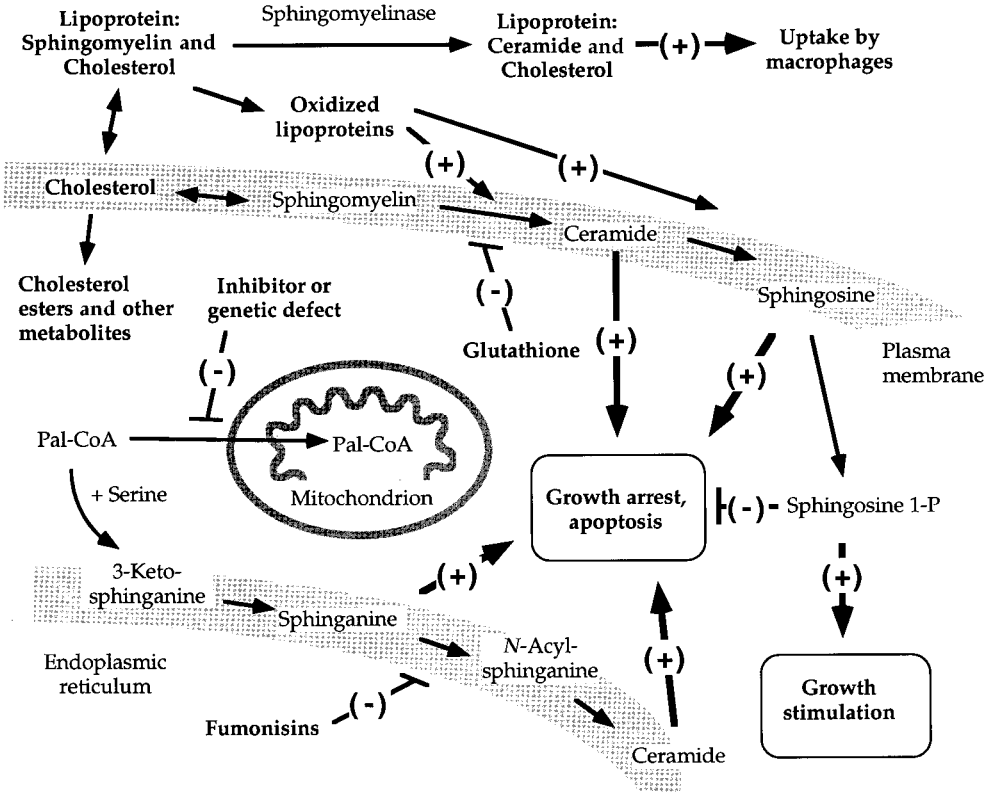

FIGURE 4 An overview of some of the interrelationships among sphingolipid metabolism, factors that can modulate sphingolipid metabolism and cell regulation. These include the following: *1*) the influence of sphingomyelin on lipoprotein structure as well as the involvement of sphingolipid signaling pathway(s) in the cellular effects of oxidized lipoproteins; *2*) associations between sphingomyelin and cholesterol in cell membranes, which affect membrane structure and function, the efflux and metabolism of cholesterol and sphingomyelin signaling pathway(s); *3*) the triggering of sphingomyelin hydrolysis by depletion of cytosolic glutathione, an inhibitor of neutral sphingomyelinase(s); and *4*) perturbation of sphingolipid biosynthesis by precursors (e.g., palmitoyl-CoA or serine) or inhibitors (such as fumonisins) of key enzymes of this pathway. The bioactive sphingolipid metabolites are ceramide, sphingosine, and sphingosine 1-phosphate and analogs of these compounds (such as sphinganine), which alter diverse cell behaviors, including the stimulation (+) or inhibition (−) of cell growth, differentiation and apoptosis.

ing pathway;[10] *4*) there is an elevation of sphingomyelin in aortic lesions in which this lipid can account for 70% of the total phospholipid (Barenholz and Gatt 1982); a substantial portion of the sphingomyelin found in arteries and atherosclerotic lesions appears to arise from synthesis in the arterial tissue accompanied by decreased turnover (Eisenberg et al. 1969, Zilversmit et al. 1961); and 5) the ratio of sphingomyelin to phosphatidylcholine increases fivefold in VLDL from hypercholesterolemic rabbits (Rodriguez et al. 1976). There are also interesting associations between glycosphingolipids and atherosclerosis (see Chatterjee 1998, Prokavoza and Bergelson 1994).

Short-term (Imaizumi et al. 1992) and long-term (Kobayashi et al. 1997) feeding experiments with rats have indicated that sphingolipids reduce plasma cholesterol, a risk factor for atherosclerosis. Plasma total cholesterol was 30% lower for rats fed semipurified diets supplemented with a mixture of sphingomyelin and glycosphingolipids (1% of the total diet) plus 4% soybean oil for up to two generations, compared with rats fed 5% soybean oil (plasma triacylglycerols were not different). Unfortunately, the supplement contained additional components (including cholesterol) that may have also contributed to these results. More in vivo studies of this association are clearly warranted.

Sphingolipid signaling may play a role in some of the progressive loss of cell function that accompanies aging. Changes in sphingomyelin content with aging have been seen in many tissues, including calf liver (Jenkins and Kramer 1988), rat brush border membranes (Levi et al. 1989), human aorta (Eisenberg et al. 1969) and heart myocytes (Yechiel and Barenholz 1986). As noted earlier in this review, ceramide can inhibit cell growth and induce apoptosis (Hannun and Obeid 1995), and has been implicated as a mediator of senescence in a cell culture model for aging (Lee and Obeid 1997, Venable et al. 1995). Therefore, modulation of sphingolipid metabolism by the diet could affect aging via this signaling pathway(s).

Sphingolipid signaling is likely to be involved in the mechanism of action of a substantial number of other components of the diet. A growing list of nutritional factors can modulate this signaling pathway by affecting sphingomyelinase activity, such as 1α,25-dihydroxycholecalciferol (Okazaki et al. 1989 and 1990), unsaturated fatty acids (Robinson et al. 1997) and cellular levels of glutathione (Liu and Hannun 1997). Dietary (n-3) polyunsaturated fatty acids (PUFA) have been reported to suppress the formation of ceramide (and diacylglycerol) (Jolly et al. 1997). Furthermore, sphingolipid signaling pathways are involved in the regulation of important enzymes, such as some isoforms of cytochrome P450 (Merrill et al. 1999, Nikolova-Karakashian et al. 1997).

"Bioactive" sphingolipid metabolites (e.g., sphinganine or ceramide) can be produced by aberrant induction of sphingolipid biosynthesis (Fig. 4), as has been shown in the toxicity of palmitate for cells in culture when uptake by mitochondria is blocked genetically or by inhibitors (Paumen et al. 1997). The toxicity was attributed to sphingolipid biosynthesis because it was selective for palmitic acid (Paumen et al. 1997) (serine palmitoyltransferase activity is highly dependent on cellular levels of serine and fatty acyl-CoA, with a high degree of selectivity for palmitoyl-CoA; Merrill et al. 1988) and was prevented by inhibition of serine palmitoyltransferase. Zucker diabetic fatty (ZDF) rats exhibit loss of β cells by apoptosis and

[10] This report described sphingomyelin hydrolysis to ceramide; in a recent collaboration (N. Augé, M. Nikolova-Karakashian, S. Carpentier, S. Parthasarathy, A. Nègre-Salvayre, R. Salvayre, A. H. Merrill, Jr. & T. Levade, J. Biol. Chem., in press), we have also found activation of sphingosine kinase, which is consistent with sphingosine 1-phosphate mediating the growth stimulation (ceramide formation may play a role in the toxicity of oxidized lipoproteins).

have been shown to have elevated ceramide; incubation of islets from prediabetic and diabetic ZDF rats with fatty acids increased ceramide and apoptosis (Shimabukuro et al. 1998b). Therefore, these authors concluded that β cell apoptosis is induced by de novo ceramide formation. Overexpression of serine palmitoyltransferase can also induce apoptosis, as has recently been reported for obese prediabetic *fa/fa* rats (Shimabukuro et al. 1998a) and associated with induction of apoptosis in pancreatic β cells. These studies suggest that perturbation of intermediary metabolism (perhaps by many means) affects sphingolipid biosynthesis; when intermediates of this pathway accumulate, there can be profound effects on cell behavior.

The implications for diabetes are especially provocative because other interrelationships between sphingolipids and diabetes have been noted as follows: free sphingoid bases inhibit insulin-induced glucose uptake and oxidation by adipose cells (Robertson et al. 1989); ceramide down-regulates GLUT4 gene transcription in 3T3-L1 adipocytes (Long and Pekala 1996); and sphingolipids may alter insulin action at the level of the cell membrane (Candiloros et al. 1996).

Perturbation of sphingolipid metabolism is the mechanism of action of mycotoxins and other fungal secondary metabolites. A number of microorganisms produce secondary metabolites that disrupt sphingolipid metabolism (Merrill and Sweeley 1996); the most thoroughly characterized of these are the fumonisins, which are produced by *Fusarium moniliforme* and related fungi. Fumonisins are common contaminants of maize and other foods and cause equine leukoencephalomalacia, porcine pulmonary edema and various other diseases of animals, including humans (Marasas 1995). Fumonisins inhibit ceramide synthase (Wang et al. 1991), which results in accumulation of sphinganine (and sometimes sphingosine) and reduced formation of complex sphingolipids. As a consequence of disruption of sphingolipid metabolism, fumonisins inhibit progression through the cell cycle (Ciacci-Zanella et al. 1998, Lee et al. 1998) and induce apoptosis (Riley et al. 1996, Schmelz et al. 1998). Elevations in sphinganine can be detected in blood and urine of animals that consume fumonisins and can be used as a biomarker for exposure (Riley et al. 1994, Wang et al. 1992).

One of the other interesting inhibitors of sphingolipid metabolism is ISP1 (also called myriocin), a potent inhibitor of serine palmitoyltransferase (Miyake et al. 1994). Long-term treatment with ISP1 can be toxic. However, by preventing the accumulation of sphingoid bases and ceramides, ISP1 protects cells (Schmelz et al. 1998) and animals (Riley et al. 1999) from fumonisin toxicity. Thus, naturally occurring inhibitors of sphingolipid metabolism can have both toxic and protective effects, depending on the context in which they are encountered.

The presence of sphingolipids in food may protect against bacteria toxins and infection. Many microorganisms, microbial toxins and viruses bind to cells via sphingolipids. These include cholera toxin (ganglioside G_{M1}) (Thompson and Schengrund 1998), verotoxin (globosides) (Bast et al. 1997, Farkas-Himsley et al. 1995), Shiga-like toxin 2e (globotriaosylceramide, G_{b3}) (Jacewicz et al. 1995, Keusch et al. 1995), and *Clostridium botulinum* type B neurotoxin (to synaptotagmin II associated with gangliosides G_{T1b}/G_{D1a}) (Nishiki et al. 1996). Furthermore, many bacteria utilize sphingolipids to adhere to cells, e.g., *Escherichia coli* (galactosylceramide) (Blomberg et al. 1993, Khan et al. 1996, Payne et al. 1993), *Hemophilus influenza* (gangliotetraosylceramide and gangliotriosylceramide) (Hartmann and Lingwood 1997), *Helicobacter pylori* (gangliotetraosylceramide, gangliotriaosylceramide, sulfatides and G_{M3}) (Huesca et al. 1996, Kamisago et al. 1996, Simon et al. 1997, Wadstrom et al. 1997), *Borrelia burgdorferi* (galactocerebroside; Virulent strain 297: glucosylceramide, lactosylceramide and galactosylgloboside) (Garcia Monco et al. 1992, Kaneda et al. 1997), and *Pseudomonas aeruginosa* and *Candida albicans* (asialo-G_{M1}) (Yu et al. 1994). Virus binding can be mediated via sphingolipids, including HIV-1 gp120 (galactosylceramide) (Fantini et al. 1997), Sendai virus (ganglioside G_{D1a}) (Epand et al. 1995) and influenza viruses (gangliosides, sulfatides and polyglycosylceramides) (Fakih et al. 1997, Matrosovich et al. 1996 and 1997, Sato et al. 1996, Suzuki et al. 1996).

Synthetic sphingolipids are effective in inhibiting the binding of bacteria and viruses (Fantini et al. 1997); therefore, it is plausible that sphingolipids in food also compete for cellular binding sites and facilitate the elimination of pathologic organisms from the intestine. Glycosphingolipids have been hypothesized to be one of the nonimmunoglobulin compounds in human milk that confer protection against pathogens (Newburg and Chaturvedi 1992, Zopf 1996). Rueda et al. (1998) recently reported that preterm newborn infants given an adapted milk formula supplemented with gangliosides (1.43 mg/100 kcal) had significantly fewer *E. coli* in feces (and higher fecal bifidobacterial counts) than infants fed the control formula. Interestingly, sphingolipids help protect plants against necrotic lesions induced by parasitic fungi (Lhomme et al. 1990).

Unfortunately, some glycosphingolipids also appear to be participants in disease induced by microorganisms. A fraction of the persons infected with *Campylobacter jejuni* develop Guillain-Barre or Miller Fisher syndrome, which appears to involve development of cross-reactive antibodies against gangliosides and *C. jejuni* lipopolysaccharides (Jacobs et al. 1997).

SUMMARY AND PERSPECTIVES FOR THE FUTURE

Dietary sphingolipids do not contribute much to daily energy needs of animals, nor do they appear to be "essential" nutrients, although this has not yet been explored in special circumstances or disease. Nonetheless, given their potent biological activities and widespread occurrence in food, it is likely that sphingolipids can be categorized as "functional" components of food. At present, the diseases for which there is the most evidence for a beneficial effect of dietary sphingolipids are atherosclerosis and colon cancer; however, these associations are based on few studies, and there is clearly a need for follow-up investigations with laboratory animals and humans. Considering the number and complexity of the biological processes that are affected by this category of compounds, much work remains to be done before the nutritional significance of sphingolipids will be fully known.

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