Sphingolipids in Food and the Emerging Importance of Sphingolipids to Nutrition

Hubert Vesper,1 Eva-Maria Schmelz, Mariana N. Nikolova-Karakashian,3 Dirck L. Dillehay,*† Daniel V. Lynch** and Alfred H. Merrill, Jr.4

Departments of Biochemistry and *Pathology, and 1Division of Animal Resources, Emory University, Atlanta, GA 30322–3050 and **Department of Biology, Williams College, Williamstown, MA 01267

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-aminoctadec-4-ene (sphingosine). The sphingolipids of mammalian tissues, lipoproteins, and milk include ceramides, sphingomyelins, cerebrosides, gangliosides and sulfatides; plants, fungi and yeast have mainly cerebroside 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 sphingoid bases) 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-sphingosine3 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 alky 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 (and 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.

1 Funded by the National Institutes of Health (GM46368) and NCI (CA61820) as well as by Dairy Management, Inc.
2 Current address: National Center of Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA 30341.
3 Current address: Department of Physiology, University of Kentucky, Lexington, KY 40236.
4 To whom correspondence should be addressed.

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

0022-3166/99 $3.00 © 1999 American Society for Nutritional Sciences.
Manuscript received 1 March 1999. Revision accepted 3 April 1999.
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

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6 Abbreviations used: Cer, ceramide; DMH, 1,2-dimethylhydrazine; Gal, galactose; GC, gas chromatography; Glc, glucose; GPI, glycosylphosphatidylinositol; HMG, β-hydroxy-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.

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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 GM3. Simple glycosphingolipids, such as GlcCer and LacCer, are often termed “cerebrosides,” whereas gangliosides specifically contain one or more N-acetylenuraminic acids (sialic acids).

**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 galactosylceramide (GalCer) in “microdomains” (other cerebrosides and SM can also form microdomains), and interactions between gangliosides (such as GM3) 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.
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 in-
direct 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 lacta-
tion, in the case of milk), losses during food preparation and

<table>
<thead>
<tr>
<th>Food sources</th>
<th>Sphingolipid content1</th>
<th>Food consumed per capita2</th>
<th>Sphingolipids consumed per capita</th>
<th>Reference</th>
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<tr>
<td>Dairy products3</td>
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<tr>
<td>Milk (3.5%)</td>
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<td>36</td>
<td>38,464</td>
<td>Zeisel et al. 1986; Newburg and Chaturvedi 1992; Jensen 1995</td>
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<td>Lowfat Milk (&lt;2%)</td>
<td>92</td>
<td>60</td>
<td>5764</td>
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<td>Cream (37%)</td>
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<td>1</td>
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<td>Cheese (29%)</td>
<td>1326</td>
<td>12</td>
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<td>Frozen dairy (11%)</td>
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<td>14</td>
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<td>Evaporated and condensed (9%)</td>
<td>412</td>
<td>4</td>
<td>1648</td>
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<td>Butter</td>
<td>460</td>
<td>2</td>
<td>920</td>
<td>Zeisel 1994</td>
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<td>Meat products and fish</td>
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<tr>
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<td>530</td>
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<td>14</td>
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<td>Bell peppers</td>
<td>36</td>
<td>3</td>
<td>108</td>
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<td>424</td>
<td>41</td>
<td>1722</td>
<td>Whitaker 1996, Zeisel 1994</td>
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<td>Potato</td>
<td>694</td>
<td>64</td>
<td>4116</td>
<td>Galliard 1968a, Zeisel 1994</td>
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<td>Sweet potato</td>
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<td>2</td>
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<tr>
<td>Spinach</td>
<td>675</td>
<td>0.3</td>
<td>20</td>
<td>Onnishi et al. 1983</td>
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<td>Soybeans</td>
<td>24105</td>
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<td></td>
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<tr>
<td>Cauliflower</td>
<td>1836</td>
<td>1</td>
<td>183</td>
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<td>Cucumber</td>
<td>276</td>
<td>5</td>
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<td>Zeisel 1994</td>
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<td>Lettuce</td>
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<td>10</td>
<td>500</td>
<td>Zeisel 1994</td>
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<tr>
<td>Other vegetables</td>
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<tr>
<td>Fruits</td>
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<td>Apples</td>
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<td>25</td>
<td>1725</td>
<td>Gaillard 1968b</td>
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<tr>
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<td>246</td>
<td>40</td>
<td>960</td>
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</tr>
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<td>3</td>
<td>234</td>
<td>Zeisel 1994</td>
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<td>Banana</td>
<td>205</td>
<td>13</td>
<td>260</td>
<td>Zeisel 1994</td>
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<tr>
<td>Other fruits</td>
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<tr>
<td>Cereals</td>
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<tr>
<td>Wheat flour</td>
<td>5765</td>
<td>66</td>
<td>38,016</td>
<td>Laine &amp; Renkomen 1974</td>
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<td>Total sphingolipid intake (µmol/y)</td>
<td>153,551</td>
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<td></td>
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<tr>
<td>Total sphingolipid intake (g, calculated as sphingomyelin)</td>
<td>116</td>
<td></td>
<td></td>
<td></td>
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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 Althouse 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 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.
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, foods that are particularly rich in sphingolipids.

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 (Merrill et al. 1995). Sphingomyelin is the major sphingolipid of LDL and

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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).

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8 The composition may depend on the source because we have recently analyzed soy cerebrosides and found one major GlcCer, with d18:2,4,8 and α-hydroxypalmitic acid (M. C. Sullards, D. V. Lynch, E. M. Schmelz, E. Wang, A. H. Merrill Jr. & J. Adams, unpublished data).
products, including “nonfat” dry milk (Jenson 1995).

than with the lipid droplet per se, a substantial portion remains in low fat dairy gosine might reduce the risk of colon cancer if, as shown in Therefore, digestion of sphingolipids to ceramide and 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 these cells, which appear to have defective regulation of sphingomyelinase.

**Intestinal enzymes and microflora**

**Colonic cells**

**FIGURE 3** A model for the suppression of colonic neoplasia by the uptake of sphingosine 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 may 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 sphingomyelin 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 C57BL/6 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-
Sphingolipids appear 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 GM1 is at least four- to eightfold more potent than sphingomyelin (Dillahay et al. 1994), and milk glucosylceramide, lactosylceramide and ganglioside G_{33} 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. 1994, Kim and Mason 1996, Pence et al. 1996, Potter et al. 1997). In vivo, diets supplemented with cholesterol (Geelen et al. 1992). -hydroxyl-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 (Kischner et al. 1997) via triggering of the sphingomyelin signal-
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 (Imazumi 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 3% 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, Venables et al. 1995). Therefore, modulation of sphingolipid metabolism by the diet could affect aging via this signaling pathway.

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

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10 This report described sphingomyelin hydrolysis to ceramide; in a recent collaboration (N. Augé, M. Nikolova-Karakashian, S. Carpenter, S. Parthasarathy, A. Ngère-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 fafa 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 Sweeley 1996); the most thoroughly characterized of these are the fumonisins, which are produced by Fusarium moniliforme and related fungi. Fumonisin A and B 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, fumonisin inhibits progression through the cell cycle (Candioloros et al. 1996). One of the other interesting inhibitors of sphingolipid metabolism is ISP1 (also called myricin), 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.


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 Guil lain-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.

**ACKNOWLEDGMENTS**

The authors are grateful to the many research collaborators who have contributed to studies summarized in this review, most notably Elaine Wang and Ronald T. Riley, and to Winnie Scherer for help in preparing the manuscript.

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