Colonic Cell Proliferation and Aberrant Crypt Foci Formation Are Inhibited by Dairy Glycosphingolipids in 1,2-Dimethylhydrazine-Treated CF1 Mice

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ABSTRACT Dietary sphingomyelin (SM) inhibits early stages of colon cancer (appearance of aberrant crypt foci, ACF) and decreases the proportion of adenocarcinomas vs. adenomas in 1,2-dimethylhydrazine (DMH)-treated CF1 mice. To elucidate the structural specificity of this inhibition, the effects of the other major sphingolipids in milk (glycosphingolipids) were determined. Glucosylceramide (GluCer), lactosylceramide (LacCer) and ganglioside G_{D3} were fed individually to DMH-treated (six doses of 30 mg/kg body weight) female CF1 mice at 0.025 or 0.1 g/100 g of the diet for 4 wk. All reduced the number of ACF by >40% (P < 0.001), which is comparable to the reduction by SM in earlier studies. Immunohistochemical analysis of the colons revealed that sphingolipid feeding also reduced proliferation, with the most profound effect (up to 80%; P < 0.001) in the upper half of the crypts. Since the bioactive backbones of the glycosphingolipids (i.e., ceramide and other metabolites) are the likely mediators of these effects, the susceptibility of these complex sphingolipids to digestion in the colon was examined by incubating 500 μg of each sphingolipid with colonic segments from mice and analysis of substrate disappearance and product formation by tandem mass spectrometry. All of the sphingolipids (including SM) disappeared over time with a substantial portion appearing as ceramide. Partially hydrolyzed intermediates (such as GluCer from LacCer or G_{D3}) were not detected, which suggests that the cleavage involves colonic (or microflora) endoglycosidases. In summary, consumption of dairy SM and glycosphingolipids suppresses colonic cell proliferation and ACF formation in DMH-treated mice; hence, many categories of sphingolipids affect these key events in colon carcinogenesis.


KEY WORDS: proliferation • sphingomyelin • glycosphingolipids • colon cancer • mice

Complex sphingolipids are comprised of a sphingomyelin base backbone, an amide-linked fatty acid and a polar headgroup, as shown in Figure 1. The backbone moieties of sphingolipids [ceramide (Cer) and sphingosine] are potent inhibitors of cell growth, and induce differentiation and apoptosis (see reviews Spiegel and Merrill 1996, Merrill et al. 1997). Since these processes are deregulated in carcinogenesis and sphingolipids are abundant in some foods (Vesper et al. 1999), dietary sphingolipids are promising candidates for cancer chemoprevention.

Sphingomyelin (SM) feeding, in amounts that can be found in some foods, such as dairy products (0.025–0.1%) (Vesper et al. 1999), inhibits an early stage of colon carcinogenesis in mice (the formation of aberrant crypt foci, ACF) and decreases the proportion of tumors that appear as malignant adenocarcinomas vs. benign adenomas (Dillehay et al. 1994, Schmelz et al. 1996). However, it is not known if the other major category of sphingolipids (glycosphingolipids) has similar effects. To address this question, glucosylceramide (GluCer), lactosylceramide (LacCer) and ganglioside G_{D3} (G_{D3}) were fed to 1,2-dimethylhydrazine (DMH)-treated CF1 mice; all suppressed ACF formation and reduced proliferation in the colonic crypts. Thus, glycosphingolipids also can suppress key stages of colon carcinogenesis.

MATERIALS AND METHODS

Animals. Female CF1 mice were obtained from Charles River Laboratories (Portage, MI) at 5 wk of age. They were housed in microisolator cages (five mice per cage), and were maintained in a relative humidity of 50–60%, a temperature of 23°C and a 12-h light/dark cycle. The mice had free access to water and were weighed weekly and monitored closely for signs of illness. All protocols involving animals were approved by the Institutional Animal Care and Use Committee and conducted according to National Research Council Guidelines.

Sphingolipids. The glycosphingolipids (extracted from butter-milk) were purchased from Matreya (Pleasant Gap, PA). The purity was first determined by thin layer chromatography, and only one band on the plates was visible after exposure to iodine or ninhydrin when 100 μg were analyzed (developed in chloroform/methanol/ formic acid/water, 56:30:4:2, v/v/v/v). Next, mass spectrometric analyses (as described below) were employed to evaluate purity and provide detailed information about the structure of the sphingolipids.

Mass spectrometric analyses were performed on a Perkin Elmer Sciex (Norwalk, CT) API 3000 triple quadrupole mass spectrometer, equipped with a turbo-ionspray source. Solutions of sphingolipids...
mass range around the estimated molecular mass of each sphingolipid to enter into the first quadrupole.

The diets were mixed immediately before diet alone (control group) or supplemented with 0.025 or 0.1 g/100 g because these have replaced corn oil with soy oil, and soy is a rich essentially sphingolipid-free (Schmelz et al. 1996). The AIN 76A Feeds, Richmond, IN) (American Institute of Nutrition 1977) that is initiated, the mice were fed a semipurified AIN 76A diet (PMI Louis, MO) once a week for 6 wk. During this period of tumor initiation, the mice were fed a semipurified AIN 76A diet (PMI Feeds, Richmond, IN) (American Institute of Nutrition 1977) that is sphingolipid-free (Schmelz et al. 1996). The AIN 76A diet was selected over newer formulations (AIN 93G and 93M) because these have replaced corn oil with soy oil, and soy is a rich source of sphingolipids (Vesper et al. 1999).

One week after the last injection, the mice were fed the AIN 76A diet alone (control group) or supplemented with 0.025 or 0.1 g/100 g of GluCer, LacCer or GD3. The diets were mixed immediately before feeding period and kept in closed containers at 4°C. Sphingolipids are stable under these conditions. One group of mice was not injected with the carcinogen (untreated controls). These mice were fed the AIN 76A diet without sphingolipid supplements throughout the study.

The mice were killed by CO2 asphyxiation after 4 wk of feeding the experimental diets (5 wk after the last carcinogen injection). The abdominal cavity was opened, the colons were removed, rinsed with PBS, opened longitudinally and incubated with 3,3′-diaminobenzidine (Vector Laboratories, Burlingame, CA) for 10 min at room temperature. Endogenous alkaline phosphatase was blocked with 0.3% H2O2 in methanol for 30 min at room temperature. After treatment with a permeabilization solution (1 g/L Triton X-100 in 1 g/L of sodium citrate) for 2 min on ice, the sections were incubated with terminal deoxynucleotidyl transferase (which labels DNA strand breaks with fluorescein) for 60 min at 37°C. Incorporated fluorescein was detected by an anti-fluorescein antibody, conjugated with horseradish peroxidase (incubation for 30 min at 37°C). This complex was stained for light microscopic analysis with 5′-bromo-2′-deoxyuridine (BrdU)-staining of proliferating cells in situ. On the last day of the study, the mice were injected i.p. with a BrdU solution (10 μmol/L, 1 mL per 100 g body weight) (Boehringer Mannheim In Situ Cell Proliferation kit #1758756, Indianapolis, IN) to label proliferating cells. The mice were killed 2 h later by CO2 asphyxiation, and the tissue was processed as described above to determine the number of ACF. The colons were embedded in paraffin, and sections of 3–5 μm were cut longitudinally along the full length of the colon. The sections were deparaffinized with xylene and rehydrated through graded alcohol (100, 95, 80, 50, 30 and 0%). According to the manufacturers instructions, the sections were incubated with trypsin (1 g/L in PBS with 1 g/L of CaCl2) denaturated in 4 mol/L of HCl for 10 min and incubated with an alkaline phosphatase-conjugated anti-BrdU monoclonal antibody for 30 min at 37°C. This antibody complex was stained with fast red. The sections were covered with an aqueous mounting solution and analyzed by light microscopy at 10 or 40 times magnification; 50 fully visible colonic crypts per animal were scored in a blinded manner.

For comparison, colonic sections from tissue collected in an earlier study (feeding purified milk SM, and synthetic SM to DMH-treated CFI mice; Schmelz et al. 1997) were included in these immunohistochemical determinations of proliferation.

**Determination of apoptosis by TUNEL assay.** The number of apoptotic cells was evaluated by TUNEL assay (In Situ cell death detection kit, POD, Boehringer Mannheim). CFI mice were treated as described above and killed by CO2 asphyxiation. The colons were removed, cut into 3-mm segments, rinsed in PBS and fixed overnight in fresh 100 g/L of formalin. The colons were embedded in paraffin, sections of 3–5 μm were deparaffinized and rehydrated (as described above), and incubated with proteinase K (20 μg/mL in 10 mmol/L of Tris-HCl, pH 8.0) for 30 min at room temperature. Endogenous peroxidase was blocked with 0.3% H2O2 in methanol for 30 min at room temperature. After treatment with a permeabilization solution (1 g/L Triton X-100 in 1 g/L of sodium citrate) for 2 min on ice, the sections were incubated with terminal deoxynucleotidyl transferase (which labels DNA strand breaks with fluorescein) for 60 min at 37°C. Incorporated fluorescein was detected by an anti-fluorescein antibody, conjugated with horseradish peroxidase (incubation for 30 min at 37°C). This complex was stained for light microscopic analysis with 5′-bromo-2′-deoxyuridine (BrdU)-staining of proliferating cells in situ. For comparison, colonic sections from tissue collected in an earlier study (feeding purified milk SM, and synthetic SM to DMH-treated CFI mice; Schmelz et al. 1997) were included in these immunohistochemical determinations of proliferation.

**Analysis of glycosphingolipid hydrolysis.** Female CFI mice were killed by CO2 asphyxiation. The abdominal cavity was opened, the colons removed, rinsed with PBS, opened longitudinally and incubated in a mixed micelle solution for the indicated times. Mixed micelles were prepared by mixing 500 μg of the individual sphingolipid (in chloroform/methanol, 1:1; v/v) with an equal amount of phosphatidylcholine (from egg yolk), and sodium cholate (final concentration 20 mmol/L) (both from Sigma), evaporation of the solvents under nitrogen, addition of 3 mL of potassium phosphate buffer (20 mmol/L, pH 6.8) and sonication of the mixture until clear. After the indicated times, 100 μL aliquots were removed, the sphingolipids solubilized with 900 μL of methanol: chloroform (1:1; v/v), sonicated, and analyzed by mass spectrometry for the disappearance of the starting material, and the appearance of the metabolites (as described below).

**Mass spectrometric analyses of glycosphingolipid hydrolysis.** Multiple reaction monitoring scans were used to measure the abundances of undegraded SM, GluCer, LacCer and GD3, and the appearance of the metabolites: Cer, GluCer (as a metabolite of LacCer and GD3), and LacCer (as a metabolite of GD3). Aliquots of each sample (prepared as described above) were mixed with 580 μL of 5 mmol/L of ammonium acetate in methanol, and 10 μL of glacial acetic acid.
In each experiment, the mass spectrometer via a Rheodyne 8125 injector with a 20 µL injection loop at a flow rate of 50 µL min⁻¹. Cer, GluCer and LacCer were monitored in positive ion mode with the source needle at 5500 V, while the orifice voltage was 30 V. G23 was monitored in negative ion mode with the source needle at -4500 V and an orifice voltage of -50 V. Nitrogen was used as a nebulizing gas at a flow rate of 6 L min⁻¹ and at 50°C. The orifice voltage and the bath gas temperature were kept low to prevent decomposition of intact precursor ions before entry into Q1.

The starting sphingolipids were first examined by mass spectrometry (MS) to determine the major ceramide backbones, which were found to be comprised of sphingosine (d18:1) with the fatty acids 16:0, 18:0, 18:1, 20:0 and 24:0. Q1 was set to pass m/z 703.7, 787.9, 872.1, 886.2 for SM, m/z 700.7, 784.8, 798.8, 812.9 for GluCer, m/z 862.8, 947, 960.9, 975 for LacCer. Q3 was set to pass m/z 264.2 for the product ion from the sphingoid base (conjugated carbocation) for Cer, GluCer and LacCer. SM and GD3 were analyzed by setting Q3 to pass m/z 184.2 (the phosphocholine headgroup) and m/z 290.1 (the N-acetyl-neuraminic acid). Nitrogen was used to collisionally activate precursor ion decomposition in Q2, which was offset from Q1 by 264.2 m/z. The multiple reaction scan transitions were performed with a dwell time of 200 ms for SM, and Cer, and 50 ms for the glycosphingolipids.

The disappearance of the starting compounds (and the appearance of the metabolites) were expressed relative to the intensity of the starting compound at time zero. Because the appropriate internal standards are not available at this time, no further attempt was made to perform these analyses quantitatively. Each sphingolipid was analyzed in duplicate, with good agreement (i.e., <10% difference) in the results.

**Statistical analyses** All statistical analyses were executed using the Instat® software (Instat, San Diego, CA). The animal weight and number of ACF were evaluated by Student's t test (ANOVA analysis, followed by Bonferroni multiple comparison) and are shown as means ± SEM. The correlation of animal weight and number of ACF was evaluated by regression analysis. Differences were considered significant at P ≤ 0.05.

**RESULTS**

**Composition of milk glycosphingolipids.** The mass spectrometric analyses of the milk glycosphingolipids showed sphingosine as the most abundant sphingoid base backbone; sphinganine and 4-hydroxysphinganine (phytosphingosine) were not observed in large relative abundances (<1%). Hence, the glycosphingolipids were analyzed by tandem MS by selecting for m/z 264 (the sphingoid-derived carbocation referred to as "N" in Adams and Ann, 1993) as described under "METHODS". A precursor ion scan for Glu Cer is shown in Figure 2. The different m/z for the GluCer species are the result of variations in the amide-linked fatty acid chains: 16:0 (m/z 700.7), 22:0 (m/z 784.9), 23:0 (m/z 798.8), 24:0 carbons (m/z 812.9) and 24:1 (m/z 810.9).

The fatty acid composition of the ceramide backbones of the various glycosphingolipids are given in Table 1. Most of the fatty acids were saturated with the exception of a small amount of nervonic acid, 24:1. As seen in sphingolipids in general (and for the ceramides of milk SM, Schmelz et al. 1996), there was a bimodal grouping of ceramides with very-long-chain fatty acids (22 to 24 carbon atoms) and palmitic acid (16:0). G23 contained a higher proportion of 20:0 and 21:0 than the other glycolipids. The β-hydroxy group found in some sphingolipids (Vesper et al. 1999) was not detected in these glycosphingolipids.

**Inhibition of aberrant crypt foci formation.** When fed to DMH-treated CFI mice, all of the glycosphingolipids reduced significantly the number of ACF (Fig. 3). At 0.025 and 0.1% of the diet, the suppression was, respectively, 53 and 51% for GluCer (P < 0.001 and P < 0.01), 62 and 58% for LacCer (P < 0.001), and 42 and 54% for GD3 (P < 0.05 and P < 0.01). These results are comparable to the suppression of ACF by milk and synthetic SM (Dillehay et al. 1994, Schmelz et al. 1996, 1997). There was a trend (P = 0.38 and P = 0.25 for GluCer, P = 0.12 and P = 0.32 for LacCer and P = 0.12 and P = 0.33 for GD3) to a reduced size of ACF (number of aberrant crypts per focus); however, unlike earlier experiments (Schmelz et al. 1996), this did not reach statistical significance.

In the control group, the number of ACF correlated with the body weight (P < 0.05; r = 0.4684) (Fig. 4), but there was no correlation for the mice fed the glycosphingolipid or SM supplements (data not shown). This was not due to differences in weight because feeding sphingolipids had no significant effect on weight gain (data not shown).

**Determination of colonic proliferation.** DMH treatment caused a large increase in proliferation in the upper half of the colonic crypts, which was suppressed significantly (P < 0.001, for all but GluCer at 0.025%, P < 0.05) by feeding all of the glycosphingolipids (Fig. 5A). DMH treatment also increased the rate of proliferation in the lower half of the crypts, but by only 30% (P < 0.001). Dietary glycosphingolipids reduced proliferation in the lower half of the crypts (Fig. 5B), with the exception of 0.1% G3, which did not suppress cell proliferation. This suppression of proliferation is comparable to the effect of milk SM and synthetic SM (25 to 33% in the lower half, and 80% in the upper half) (Fig. 5A and B).

**Induction of apoptosis.** Exogenous addition of sphingolipids to cells in culture induces apoptosis (see review Merrill et al. 1997); therefore, the effect of a dietary sphingolipid (SM) on the number of apoptotic cells in the colonic epithelium was examined. There was no difference in the number of apoptotic cells per crypt in the control group (0.19 ± 0.03) or the groups fed milk SM or synthetic SM for 4 wk (0.21 ± 0.03, and 0.17 ± 0.03, respectively). Hence, the effects of dietary sphingolipids on apoptosis (if any) are not as easy to detect as the inhibition of proliferation.

**Hydrolysis of sphingolipids by colonic segments.** The proposed mechanism for the suppression of colonic cell proliferation and ACF formation by dietary sphingolipids is that they are hydrolyzed to ceramide and other "growth suppressive" metabolites (Schmelz et al. 1996, Vesper et al. 1999). To determine if the colon contains the requisite hydrolytic activi-
ty(ies), the sphingolipids were incubated with colonic segments from mice. As shown in Figure 6, all of the sphingolipids (including SM) disappeared during this incubation: by 2 h, only 50 to 70% of the starting compound remained; after 8 h, 25 to 40% remained. Over the same time course, increases in Cer were apparent (Fig. 6, open circles). However, increases in other intermediate metabolites (GluCer for LacCer, GluCer and LacCer for GD3) were not found, which suggests that hydrolysis of the metabolites is “coupled” (i.e., once hydrolysis begins, it continues rapidly until Cer is formed) or that the cleavage of these complex sphingolipids is effected by endoglycosidases (i.e., that cleave between the Cer backbone and the headgroups). There was no apparent preference for sphingolipids with a particular Cer backbone because all of the subspecies of each sphingolipid (i.e., with 16:0, 22:0, 23:0, 24:0 and 24:1) disappeared in parallel (data not shown). However, the Cer detected in all sphingolipid preparations after hydrolysis contained a higher proportion of the 16:0 fatty acid (Fig. 7), which suggests that the fate of the Cer may differ for various subspecies.

### TABLE 1

Composition of milk glycosphingolipid sphingoid base backbones and fatty acids as determined by mass spectrometry

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>16:0</th>
<th>18:0</th>
<th>20:0</th>
<th>21:0</th>
<th>22:0</th>
<th>23:0</th>
<th>24:0</th>
<th>24:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>%1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GluCer³</td>
<td>69.9</td>
<td>18.1</td>
<td>13.0</td>
<td>3.7</td>
<td>100.0</td>
<td>70.0</td>
<td>70.3</td>
<td>16.8</td>
</tr>
<tr>
<td>LacCer</td>
<td>50.9</td>
<td>4.8</td>
<td>5.1</td>
<td>3.7</td>
<td>94.1</td>
<td>100.0</td>
<td>57.2</td>
<td>5.4</td>
</tr>
<tr>
<td>GD3</td>
<td>54.8</td>
<td>13.6</td>
<td>41.7</td>
<td>40.1</td>
<td>100.0</td>
<td>75.0</td>
<td>63.2</td>
<td>ND²</td>
</tr>
</tbody>
</table>

1 Data are expressed as percentage of the most abundant peak of the spectrum.
2 Not determined.
3 Abbreviations used: GluCer, glucosylceramide; LacCer, lactosylceramide; GD3, ganglioside GD3.

### DISCUSSION

This study has established that GluCer, LacCer and GD3 at 0.025 to 0.1% of the diet inhibit ACF formation by 50 to 60%, which is comparable to the suppression that has been found with the same amounts of dairy and synthetic SM (Dillehay et al. 1994; Schmelz et al. 1996, 1997). It is not clear why the inhibition was not much different when the lower (0.025 g/100 g) and higher (0.1 g/100 g) amounts of sphingolipids were fed (It is possible, for example, that sphingolipids only “correct” the phenotype of cells with some (or multiple) genetic mutations produced by DMH.). It is possible, though, that at 0.025 g/100g, we have already reached the maximum response in this model. Nonetheless, these studies have proven that the effects of dietary sphingolipids on these early stages of colon carcinogenesis are not limited to compounds with a particular (phosphorylcholine) headgroup.

This headgroup independence is consistent with the hypothesis that the “active” component(s) are most likely the digestion products ceramide and/or sphingosine (Dillehay et al. 1994; Schmelz et al. 1996, 1997). This is difficult to prove in vivo, however, both of these metabolites inhibit proliferation of transformed colonic cells in culture (Schmelz et al. 1998). This study also established that all of these complex sphingolipids can be hydrolyzed to Cer by colonic enzymes of tissue (specific intracellular enzymes have been identified: Brady et al. 1965, Kobayashi and Suzuki 1981, Leese and Semenza 1973) or, perhaps, microbial origin (yet to be identified).
tified in regular intestinal microflora). Previous studies have also shown that Cer (as well as SM and GluCer) are digested, and the release metabolites such as sphingoid bases absorbed throughout the upper and lower intestinal tract of rodents (Nilsson 1968, 1969; Schmelz et al. 1994). Hence, ingestion of complex sphingolipids delivers these bioactive metabolites to colonic cells.

Sphingoid bases and ceramides are known from experiments with cells in culture to cause cell cycle arrest (Jayadev et al. 1995) by shifting retinoblastoma protein (Rb) to the dephospho- state (Chao et al. 1992, Lee et al. 1998, Pushkareva et al. 1995, Dbaibo et al. 1995), apparently due to up-regulation of endogenous inhibitors (p21CIP1/WAF1 and p27 Kip1) of cyclin-dependent protein kinases (Ciacci-Zanella et al. 1998). These effects may occur downstream of p53 (Dbaibo et al. 1998). This may be the mechanism for the lower proliferation of colonic crypt cells (and lower ACF formation) in mice fed sphingolipids.

Hyperproliferation is a common property of tumors and can have several causes: an increased rate of cell growth, a reduced rate of cell death (apoptosis) or a combination of both. In addition to inhibiting growth, sphingoid bases have been shown to be highly cytotoxic (Merrill et al. 1996, Stevens et al. 1990), and ceramides (and sphingoid bases) are potent inducers of apoptosis (Obeid et al. 1993, Ohra et al. 1995, Sakakura et al. 1998, Sweeney et al. 1996). For one of the compounds tested in this study (SM), immunohistochemical methods were used to determine if sphingolipid feeding altered both proliferation and the number of apoptotic cells in the colonic crypts. SM feeding for 4 wk did not increase the number of apoptotic cells, which suggests that the induction of apoptosis is not the predominant cause of reduced proliferation (and suppression of ACF formation) in this model. This question warrants further investigation because apoptotic cells of the colonic epithelium are short-lived and often difficult to detect.

FIGURE 5 Suppression of proliferation in the upper and lower half of colonic crypts of mice by dietary sphingolipids. CF1 mice were treated with 1,2-dimethylhydrazine and fed AIN 76A diet with or without sphingolipid supplements for 4 wk. The mice were injected with 5-2'-bromodeoxyuridine (BrdU) 2 h before they were killed, and colonic sections were stained with anti-BrdU antibody to detect proliferating cells. Values are means ± SEM (n = 200 crypts). Differences relative to the treated controls were significant at *P < 0.001.

FIGURE 6 Hydrolysis of complex sphingolipids. Sphingolipids were individually incubated as mixed micelles (with cholate and phosphatidylcholine) with colonic tissue and microflora of CF1 mice. The disappearance of the starting material (A), sphingomyelin (SM), (B) glucosylceramide (GluCer), (C), lactosylceramide (LacCer), (D), ganglioside G_{34} (G_{34}) and the appearance of the metabolites were monitored by mass spectrometry.

FIGURE 7 Composition of glucosylceramide (GluCer), and the metabolite ceramide (Cer). After incubation of GluCer in the preparation of colonic tissue and microflora, the fatty acid composition of the starting material and the metabolite Cer was determined by mass spectrometry. This composition is representative for all other complex sphingolipids also used in this study (sphingomyelin, lactosylceramide, ganglioside G_{34}).
It warrants mention that the amounts of sphingolipids used in these studies did not alter the overall fat (or energy) intake of the mice significantly, since high-fat diets (and high-energy intake) have been associated with a higher risk of cancer (Hocman 1988, Kumar et al. 1990, MacLennan 1997, Shivapurkar et al. 1992). This association might account, however, for the observation in this study that control mice with the higher body weights developed more ACF.

In summary, both the SM and glycosphingolipids of milk have been shown to suppress early stages of chemically induced colon cancer in female C57 mice. Milk contains 39 to 115 mg of SM per liter, which (based on nonaqueous mass) is close to amounts of SM (0.025 to 0.1 g/liter) that inhibit proliferation, ACF formation and carcinogenesis in this model (Dillehay et al. 1994, Schmelz et al. 1996 and this study). The amounts of glycosphingolipids in milk are also substantial (per liter: 6 to 11 mg of GlcCer, 6.5 to 15 mg of LacCer and ca. 11 mg of gangliosides) (Jensen 1995); hence, for the purpose of considering the effect of milk sphingolipids on colon carcinogenesis, the total content is 70 to 150 mg per liter. Whether this is effective in humans remains to be examined.

The inhibition of colon cancer by these glycosphingolipids also raises interesting possibilities with respect to other foods. Plants contain mostly ceramides (GlcCer), which could be effective inhibitors of colon cancer. Nonetheless, the glycosphingolipids of plants, fungi, yeast and many other organisms are composed of a variety of different sphingoid base backbones and fatty acids (Vesper et al. 1999), and little is known about the bioactivity of these species. An understanding of the impact of sphingolipid consumption by humans on colon cancer risk awaits additional studies of such structure/function relationships and the use of additional cancer models.

LITERATURE CITED