Importance of Sphingolipids and Inhibitors of Sphingolipid Metabolism as Components of Animal Diets\textsuperscript{1,2}

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ABSTRACT Sphingolipids are highly bioactive compounds that participate in the regulation of cell growth, differentiation, diverse cell functions, and apoptosis. They are present in both plant and animal foods in appreciable amounts, but little is known about their nutritional significance. Recent studies have shown that feeding sphingomyelin to female CF1 mice treated with a colon carcinogen (1,2-dimethylhydrazine) reduced the number of aberrant colonic crypt foci; longer-term feeding also affected the appearance of colonic adenocarcinomas. Therefore, dietary sphingolipids should be considered in studies of the relationships between diet and cancer. Sphingolipids have also surfaced as important factors in understanding the mechanism of action of a recently discovered family of mycotoxins, termed fumonisins. Fumonisins are produced by fungi commonly found on maize and a few related foods, and their consumption can result in equine leukoencephalomalacia, porcine pulmonary edema and a number of other diseases of veterinary animals and, perhaps, humans. A cellular target of fumonisins is the enzyme ceramide synthase, and disruption of sphingolipid metabolism by fumonisins has been established by studies with both cells in culture and animals that have consumed these toxic mycotoxins. These findings underscore the ways in which sphingolipids and agents that affect sphingolipid utilization should be given consideration in selecting animal diets for nutritional and toxicological studies. J. Nutr. 127: 830S–833S, 1997.

KEY WORDS: • sphingolipids • ceramide • mycotoxins • cancer

Sphingolipids are the most structurally diverse class of membrane lipids, being composed of over 70 long-chain (sphingoid) bases, dozens of amide-linked fatty acids, and more than 300 headgroups. The structures of representative sphingolipids are shown in Figure 1 (for a more detailed introduction to sphingolipids, see Merrill and Sweeley 1996). Sphingolipids are present in all eukaryotic and some prokaryotic organisms, but there have been relatively few analyses of the amounts and types in food. Fruits and vegetables contain from 0.1 μmol/g fresh weight (for apples) (Hitchcock and Nichols 1971) to approximately 2 μmol/g dry weight (for soybeans) (Ohnishi and Fujino 1982); most of the sphingolipids in plants are cerebroside, whereas ceramide phosphoinositol is the predominant compound in yeast. Foods of animal origin contain sphingomyelin and glycosphingolipids with both simple and complex carbohydrate headgroups; typical amounts range from 0.3 to 0.5 μmol/ g for pork, beef and chicken (Blank et al. 1992) to approximately 1 μmol/g for milk, butter and cheese (Jensen and Newburg 1995). Sphingolipids are not “essential” nutrients and provide few calories; nonetheless, recent studies have uncovered ways in which they are nutritionally significant as highly bioactive components of food, and a number of mycotoxins of agricultural importance act via disruption of sphingolipid metabolism.

FUNCTIONS OF SPHINGOLIPIDS

Sphingolipids are both structural and functional lipids. Their physical properties (such as high phase transition temperatures) affect the properties of membranes and lipoproteins, and they are critical components of the water barrier of skin. They serve as ligands for extracellular matrix proteins and receptors on neighboring cells, as well as for enteric bacteria and viruses. Sphingolipids are also important in cell regulation as modulators of growth factor receptors and as second messengers for a growing list of agonists (including tumor necrosis factor-α, interleukin-1β, nerve growth factor and 1α,25-dihydroxyvitamin D3). Much recent work has focused on these functions of the sphingolipid backbones (sphingosine, ceramide and sphingosine 1-phosphate) as second messengers be-

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SPHINGOLIPIDS AND ANALOGS IN ANIMAL DIETS

FACTORS THAT ALTER SPHINGOLIPID METABOLISM: FUMONISINS AND OTHER MICROBIAL INHIBITORS OF SPHINGOLIPID METABOLISM

Fumonisins are mycotoxins produced by Fusarium moniliforme and related fungi that are prevalent on corn, sorghum, millet and other agricultural products (Benaidenhou et al. 1988). They cause a number of diseases of animals, including equine leukoencephalomalacia, porcine pulmonary edema, renal toxicity, hepatotoxicity and hepatocarcinogenicity in rats, and consumption of contaminated maize has been correlated with esophageal cancer in areas of southern Africa and China (for a review see Riley et al. 1994).

The enzyme ceramide synthase is a major cellular target for fumonisins (Wang et al. 1991), and fumonisins both block the biosynthesis of complex sphingolipids and cause accumulation of sphinganine—a compound that is toxic to many cells and may account for many of the cellular effects of these mycotoxins (Merrill et al. 1996, Riley et al. 1996). Studies of various animals (including ponies, pigs and rats) have established that fumonisins also disrupt sphingolipid metabolism in vivo and that an elevation of sphinganine causes they seem to be key players in the regulation of cell growth, differentiation and apoptosis. For a brief overview of sphingolipid metabolism and some of the signaling pathways, see Figure 2.

DIETARY SPHINGOLIPIDS AND COLON CARCINOGENESIS

Dietary sphingolipids are hydrolyzed throughout the small and large intestine to the same bioactive compounds (e.g., sphingosine and ceramide) that are used as second messengers by cells (Fig. 2) (Schmelz et al. 1994). These compounds might be harmful to the gastrointestinal tract; however, a recent study in which mice were fed sphingomyelin observed no deleterious effects on the gastrointestinal tract or on weight gain (Dillehay et al. 1994). Instead, the feeding of sphingomyelin to female CF1 mice that had been treated with 1,2-dimethylhydrazine to induce colon tumors caused a significant reduction in the appearance of aberrant colonic crypts, an early marker of colon carcinogenesis, and decreased the appearance of adenocarcinomas (Dillehay et al. 1994). The levels of sphingomyelin that were used in these studies (0.025–0.1% of the diet, wt/wt) were comparable to that of the source from which the sphingomyelin was isolated (milk) (Zeisel et al. 1986); therefore, the findings indicate that dietary sphingolipids could play a role in colon carcinogenesis. If so, sphingolipids might account for some of the current confusion regarding the agents in foods that account for the relationships between diet and cancer.

It warrants comment that one might raise a similar concern about studies with experimental animals because many defined diets contain "unnaturally" low amounts of sphingolipids. For example, the AIN-76A diet contains very little sphingolipid (i.e., <0.005%) because the components (casein, corn oil, sucrose, starch, etc.) are essentially sphingolipid free (Dillehay et al. 1994). This is probably the case also for other diets composed of purified protein, carbohydrates and many plant oils as the source of fat because sphingolipids seem to be present mainly in cell membranes rather than oils. However, there have been no systematic studies of the amounts of sphingolipids (ceramides) in oils other than corn oil; therefore, this question cannot be answered with currently available information.

**FIGURE 1** Basic structures of sphingolipids. The lipid backbone (ceramide) is composed of a long-chain (sphingoid) base (the most common of which is sphingosine) and an amide-linked, long-chain fatty acid. More complex sphingolipids have a headgroup at the position shown for the examples: sphingomyelin, galactosylceramide, lactosylceramide (GalGlucCer), and ganglioside G_{M3} (N-acetyleneuraminic acid-galactosyl-glucosyl-ceramide).

*Sphinganine lacks the 4,5-trans double bond

**FIGURE 2** An abbreviated metabolic pathway for cellular sphingolipids and examples of cellular systems that are modulated by these bioactive compounds. The enzymes that are inhibited by naturally occurring compounds are so indicated; also shown are the hydrolytic reactions that occur in the intestine during the digestion of dietary sphingolipids, and the products that may be responsible for the inhibition of colon carcinogenesis by sphingomyelin.

Inhibited by sphingofungins, lipoxymycin, and ISP-1/myricin

Inhibited by fumonisins, Alternaria toxic, and australifungins

Likely mediators of the inhibition of colon carcinogenesis by sphingomyelin
levels in such diets. Figure 3 shows the results of a pilot feeding study in which five rats (female Sprague-Dawley–derived adult rats) were fed several standard diets for 2–3 wk. Urine was then collected and analyzed for sphinganine and sphingosine by high performance liquid chromatography (as in Riley et al. 1993). Linear regression analysis of the ratio vs. the fumonisin content of the diets revealed a statistically significant correlation, even though the fumonisin levels were fairly low (i.e., <4 μg/g). These findings suggest that the presence of fumonisins in natural composition diets should be considered in studies in which the results might be affected by the presence of these mycotoxins. Because one of the effects of fumonisins is to alter immune function (Martinova and Merrill 1995), this might be a particular concern in studies with transgenic and/or immunocompromised animals.

The list of microbial agents that affect sphingolipid metabolism is expanding and now includes other inhibitors of ceramide synthase (i.e., alternaria toxin and australifungins) (Mandala et al. 1995, Merrill et al. 1992) and inhibitors of sphinganine N-acyltransferase from Sporomiella australis. J. Antibiot. 48: 1099–1105. The importance of these potentially food-borne inhibitors of sphingolipid metabolism in animals has yet to be evaluated.

OTHER POTENTIAL RELATIONSHIPS BETWEEN SPHINGOLIPIDS AND ANIMAL DIETS

There are a few additional ways that the sphingolipids in food have been suggested to be nutritionally relevant: the role of sphingomyelin as a source of dietary choline has been addressed in a number of studies (Wurtman 1979, Zeisel et al. 1986); a sphingolipid mixture has been fed to rats and noted to alter serum lipid levels (Imaizumi et al. 1992); and glycolipids that are bound by enteric toxins (and bacteria and viruses) may promote their passage through the intestinal tract (Laegrid et al. 1986). It is likely that many more functions would be known if these compounds were not so difficult to obtain in the amounts necessary for nutritional studies.

SUMMARY AND PERSPECTIVES FOR THE FUTURE

The selection of diets for experimental animals should take into account the fact that sphingolipids are usually found in food and that, although their roles in normal physiology and disease are only beginning to emerge, they have potent biological activities that are likely to make them relevant to nutrition. In addition, now that we know that diets are often contaminated with agents that alter sphingolipid metabolism, this should be factored into the interpretation of long-term feeding studies.

It is possible that some of the difficulties in extrapolating nutritional findings with model systems to more complex diets have been due to lack of consideration of the roles played by dietary sphingolipids. One is lead to wonder whether, in addition to colon cancer, there are other diseases (of the gastrointestinal tract, cancers at other sites, hyperlipidemias, etc.) that are affected by sphingolipids.

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