

Intestinal absorption of dietary maize glucosylceramide in lymphatic duct cannulated rats

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Abstract Sphingolipids are ubiquitous in all eukaryotic organisms. Various physiological functions of dietary sphingolipids, such as preventing colon cancer and improving the skin barrier function, have been recently reported. One of the common sphingolipids used as a foodstuff is glucosylceramide from plant sources, which is composed of sphingoid bases distinct from those of mammals. However, the fate of dietary sphingolipids derived from plants is still not understood. In this study, we investigated the absorption of maize glucosylceramide in the rat intestine using a lipid absorption assay of lymph from the thoracic duct. The free and complex forms of *trans*-4, *cis*-8-sphingadienine, the predominant sphingoid base of maize glucosylceramide, were found in the lymph after administration of maize glucosylceramide. This plant type of sphingoid base was detected in the ceramide fraction and N-palmitoyl-4,8-sphingadienine (C16:0-d18:2) and N-tricosanoyl-4,8-sphingadienine (C23:0-d18:2) were identified by LC-MS/MS. The cumulative recovery of 4,8-sphingadienine in the lymph was very low. These results indicate that dietary glucosylceramide originating from higher plants is slightly absorbed in the intestine and is incorporated into ceramide structures in the intestinal cells. However, it appears that the intact form of sphingoid bases is not reutilized well in the tissues.—Sugawara, T., T. Tsuduki, S. Yano, M. Hirose, J. Duan, K. Aida, I. Ikeda, and T. Hirata. **Intestinal absorption of dietary maize glucosylceramide in lymphatic duct cannulated rats.** *J. Lipid Res.* 2010. 51: 1761–1769.

Supplementary key words ceramide • sphingadienine • sphingosine

Sphingolipids are ubiquitous in all eukaryotic organisms and constitute a family of compounds that have a sphingoid base (long-chain base) with an amide-linked fatty acid and a polar head group. It is well known that there are diverse structures of sphingoid bases in nature (Fig. 1) (1, 2). The most common sphingoid base of

mammalian sphingolipids is sphingosine (*trans*-4-sphingosine, d18:1^{4t}). Smaller amounts of other sphingoid bases, such as sphinganine (dihydrosphingosine, d18:0) and phytosphingosine (4-hydroxysphinganine, t18:0), are encountered frequently. In higher plants, the structures of sphingoid bases are more complicated than in mammals, because they can be desaturated at the C8-position by a Δ 8-sphingolipid desaturase, yielding *cis*- and *trans*- isomers of Δ 8-unsaturated sphingoid bases (d18:2^{4t,8c(t)}) (3, 4). 9-Methyl-*trans*-4, *trans*-8-sphingadienine (d19:2^{4t,8t}) is a typical structure found in yeasts (5). Sphingolipids of marine invertebrates have unique triene types of sphingoid bases with a conjugated diene such as 2-amino-4,8,10-octatriene-1,3-diol (d18:3^{4,8,10}) and 2-amino-9-methyl-4,8,10-octatriene-1,3-diol (d19:3^{4,8,10}) (6). Therefore, sphingolipids having various structures of sphingoid bases are ingested daily from foodstuffs (7–9).

Dietary sphingolipids have gained attention for their potential to protect against inflammation and cancers in the gut (10–13). One plausible mechanism for these effects may be via the hydrolysis of complex sphingolipids to bioactive ceramides and sphingosine, because those breakdown products are known to play important roles as intracellular mediators (14, 15). We previously demonstrated that dietary maize and yeast sphingolipids with sphingoid bases distinct from those of mammals are able to prevent the formation of aberrant crypt foci in 1,2-dimethylhydrazine-treated mice (16, 17). We further showed that sphingoid bases prepared from various dietary sources can induce apoptosis in cancer cells (18–20).

In early studies, Nilsson (21–23) investigated the digestion and intestinal absorption of sphingolipids containing sphingosine and sphinganine. Dietary sphingolipids can be hydrolyzed to their components, such as sphingoid bases, fatty acids, and the polar head group, by intestinal enzymes and are then taken up by mucosal cells (24, 25). A large portion of sphingosine absorbed

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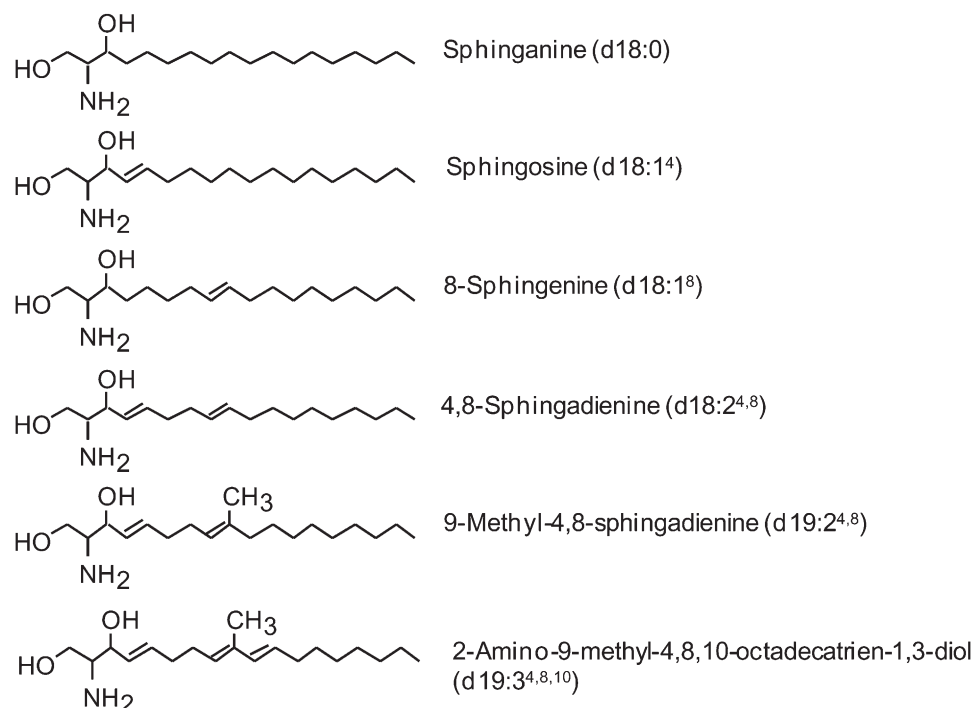


Fig. 1. Structures of naturally occurring sphingoid bases. Names and shorthand designations are as described by Karlsson (1). The 8-position of the double bond occurs in the *cis* and *trans* configurations.

by the intestine is metabolized to fatty acids and a small part is resynthesized to complex sphingolipids (21, 23). We previously investigated the digestion and absorption of plant-derived sphingolipids in the rat digestive tract and by Caco-2 human intestinal cells (26, 27). Our study demonstrated that the digestibility of glucosylceramide from maize is similar to that of mammalian origin, but the metabolic fate of plant-derived sphingoid bases within enterocytes differs from that of sphingosine. Sphingoid bases, except for sphingosine, appear to be transported out of cells across the apical membranes of enterocytes by P-glycoprotein after absorption, and consequently the intestinal uptake is poor. However, the exact fate of dietary plant-derived sphingolipids is still not well understood. In this study, we investigated the absorption of dietary plant-derived glucosylceramide in the rat intestine using a lipid absorption assay of lymph from the thoracic duct.

MATERIALS AND METHODS

Materials

D-Glucosyl- β -1,1'-N-palmitoyl-D-erythro-sphingosine (C16 glucosylceramide) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Sodium taurocholate, sphinganine, and sphingosine were purchased from Sigma Chemical Co. (St. Louis, MO). o-Phthalaldehyde (OPA) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Other chemicals and solvents were of reagent grade.

The maize glucosylceramide was kindly donated by Nippon Flour Mills Co. Ltd. (Atsugi, Japan), and its purity was 96% determined by HPLC equipped with an evaporative light-scattering detector, as described previously (8). The composition of

sphingoid bases in maize glucosylceramide was determined by oxidation with sodium periodate and subsequent gas liquid chromatography of the resultant fatty aldehydes (28). The constituent sphingoid bases were 54.9% 4*t*,8*c*-sphingadienine (d18:2^{4*t*,8*c*}), 11.2% 4*t*,8*t*-sphingadienine (d18:2^{4*t*,8*t*}), 9.2% 4-hydroxy-8*c*-sphingenine (t18:1^{8*c*}), 4.4% 4-hydroxy-8*t*-sphingenine (t18:1^{8*t*}), and others. 4*t*,8*c*-Sphingadienine was isolated from maize glucosylceramide as described previously (26, 27).

Cannulation of the thoracic ducts of rats

This study was conducted in conformity with the policies and procedures detailed in the Animal Experiment Guidelines of Tohoku University. Surgeries and maintenance of rats and all other procedures were as described previously (29). Male Sprague-Dawley rats (9 weeks old) were obtained from Japan SLC (Hamamatsu, Japan) and were housed in stainless steel wire-mesh cages in a room kept at $23 \pm 1^\circ\text{C}$ with a 12 h-light:dark cycle. Each experimental or control group had five rats. After acclimatization for 1 week with MF Standard Rodent Chow (Oriental Yeast, Tokyo, Japan) and distilled water (free access), the rats were anesthetized and a cannula (SV35, Dural Plastics) was inserted into their left thoracic channel to collect lymphatic fluid, and a catheter (SP-55, Dural Plastics) was inserted into their stomach. After surgery, each rat was placed in a restraining cage in a warm recovery room. A physiological solution containing 139 mmol/l glucose and 85 mmol/l NaCl was infused continuously overnight at a rate of 3 ml/h through the stomach cannula, and the same solution was also provided as drinking water. On the next morning, after collection of lymph for 2 h as a blank control, the rats were infused with 3 ml of an emulsion as a single bolus through the stomach catheter. Test emulsions containing 200 mg triolein, 5 mg maize glucosylceramide or glucosyl-N-palmitoylsphingosine, 50 mg fatty acid-free albumin, and 200 mg sodium taurocholate were prepared by ultrasonication. Control experiments were done using an emulsion without glucosylceramide. After infusion of those emulsions into the rats,

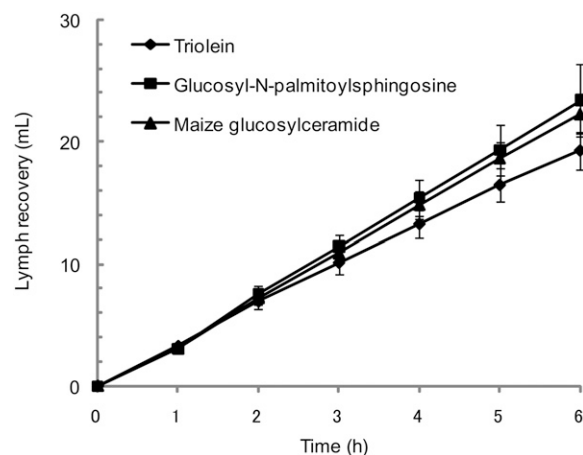


Fig. 2. Cumulative recovery of lymph fluid from the thoracic ducts of rats. After infusion of test emulsions containing glucosyl-N-palmitoylsphingosine or maize glucosylceramide, the lymph flow was measured. Control experiments used emulsions without glucosylceramide (triolein only). Data are reported as means \pm SEM ($n = 5$).

the infusion of the glucose:NaCl solution was continued. Lymph was collected in an EDTA-containing tube for analysis during the following intervals after the test-oil infusion: -2 to 0 , $0-1$, $1-2$, $2-3$, $3-4$, $4-5$, and $5-6$ h. After the lymph flow was measured, lymph was stored at -30°C until analyzed.

Lipid extraction

Lipids were extracted from each sample of lymph with chloroform-methanol (2:1, v/v). After evaporation of the collected chloroform phase, the residue was saponified with 0.4 M KOH in methanol at 38°C for 2 h to remove glycerolipids (30). The alkali-stable fraction was recovered with chloroform and then washed with water. The chloroform phase was evaporated to dryness, dissolved in chloroform-methanol (2:1, v/v), and then subjected to HPLC analysis for quantification of free sphingoid bases (free sphingoid base fraction). A portion of the alkali-stable fraction of lymph extract was degraded with aqueous methan-

olic 1 M HCl for 18 h at 70°C (31). The free sphingoid bases liberated from the complex sphingolipids were then subjected to HPLC analysis for quantification of total sphingolipids (total sphingoid base fraction).

HPLC analyses

The OPA derivatives of the free forms of sphingoid bases were analyzed using a reverse-phase HPLC system equipped with a fluorescence detector (32). Each dry sample extract was dissolved in methanol and was then mixed with OPA reagent (methanol-OPA reagent, 4:1, v/v), which was freshly prepared daily by dissolving 5 mg OPA and 50 μL 2-mercaptoethanol in 0.1 ml ethanol and adjusting the volume to 10 ml with a borate buffer (pH adjusted to 10.5 with 1 M KOH). After the solution had been incubated for 30 min at room temperature, aliquots were injected into the HPLC system, which consisted of an LC-10AD pump and an RF-10AXL fluorescence detector (Shimadzu Co., Kyoto, Japan). Sphingoid bases were separated on a $4.6 \times 250\text{ mm}$ TSK gel ODS-80Ts (Tosoh, Tokyo, Japan). The column was eluted using a binary gradient consisting of acetonitrile (A) and water (B). The gradient profile was as follows: $0-15\text{ min}$, 45–20% B linear; $15-30\text{ min}$, 20% B; $30-40\text{ min}$, 20–0% B linear; $40-60\text{ min}$, 0% B. The flow rate was 1.0 ml/min and the column temperature was maintained at 40°C . OPA derivatives were detected with an excitation wavelength of 334 nm and an emission wavelength of 440 nm. The sphingoid bases were quantified from their peak area by comparison with standard curves (26, 27). After the column eluent passed through the fluorescence detector, it was analyzed on a LCMS-2010EV mass spectrometer (Shimadzu) equipped with an electrospray ionization interface for further identification of peak components.

LC-MS/MS analyses

For the identification of complex sphingolipids consisting of sphingadienine, the alkaline stable fraction of each lymph extract was further analyzed using a Prominence HPLC system coupled to LCMS-IT-TOF equipped with an electrospray ionization interface (Shimadzu) (33). A TSK gel super ODS column ($2.0 \times 50\text{ mm}$, Tosoh) was eluted with methanol-water (95:5, v/v) containing 5 mM ammonium acetate at a flow rate of 0.2 ml/min . The MS was

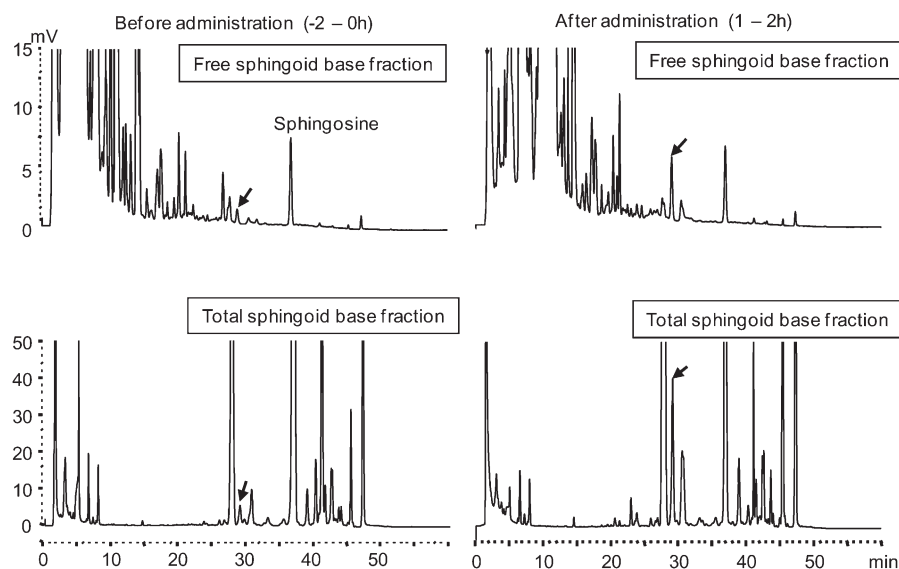


Fig. 3. HPLC chromatograms of free and total sphingoid base fractions extracted from rat lymph before and after administration of maize glucosylceramide. The peaks corresponding to 4,8c-sphingadienine are indicated by arrows.

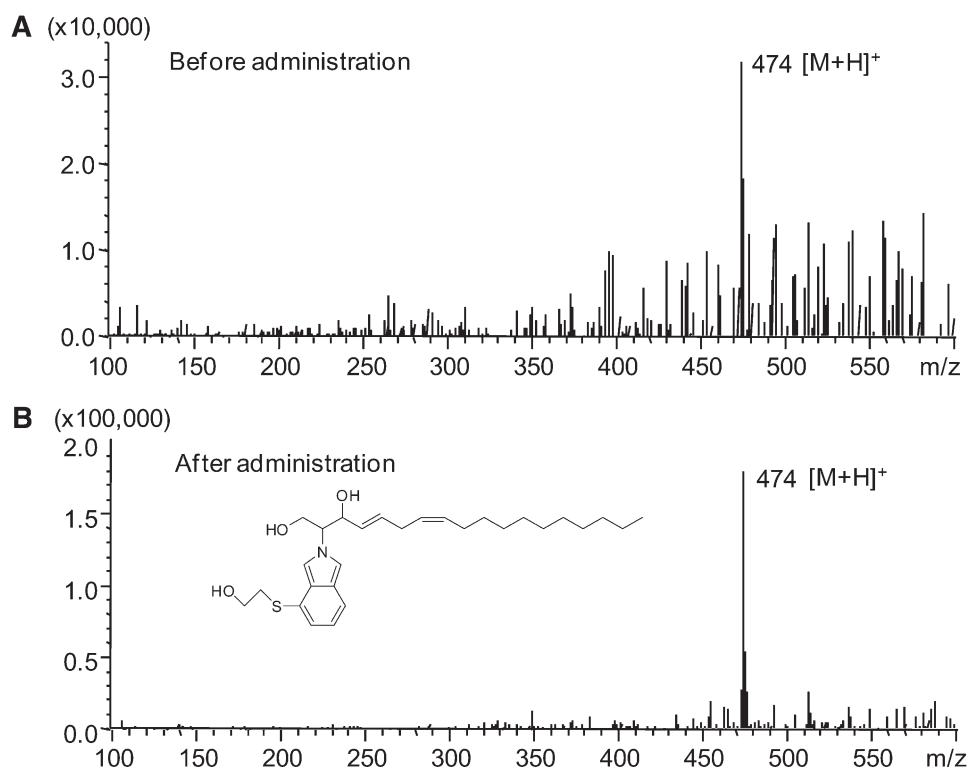


Fig. 4. Mass spectra of LC/MS peaks corresponding to an OPA derivative of 4*t*, 8*c*-sphingadienine in total sphingoid base fractions extracted from rat lymph before (A) and after (B) administration of maize glucosylceramide.

operated with the following conditions: probe voltage of 4.50 kV, CDL temperature of 200°C, block heater temperature of 200°C, nebulizer gas flow of 1.5 L/min, ion accumulation time of 10 msec, MS range of m/z 500–700, MS^2 range of m/z 200–300, and CID parameters as follows: energy, 60%; collision gas, 60%.

Statistical analysis

Data are reported as means \pm SEM, $n = 5$. Statistical analyses were made by one-way ANOVA or Student's *t*-test.

RESULTS

There was no difference in the lymph output among rats infused with each emulsion, confirming that the surgery and maintenance of rats had been carried out appropriately (**Fig. 2**). To quantify the absorption of dietary sphingolipids via the lymph, the OPA derivatives of sphingoid bases in lymph extracts were analyzed using an HPLC system with a fluorescence detector (**Fig. 3**). After administration of maize glucosylceramide (1–2 h fraction), 4*t*, 8*c*-sphingadienine, a dominant constituent of maize glucosylceramide, clearly appeared in the alkali stable fraction (free sphingoid base fraction) of the lymph extract. The peak ascribed to 4*t*, 8*c*-sphingadienine was prominently increased in the lymph extract by hydrolysis with aqueous methanolic 1 M HCl (total sphingoid base fraction). These results demonstrate that the intact form of 4*t*, 8*c*-sphingadienine is absorbed from the digestive tract into the lymph. A small peak at the same retention time as 4*t*, 8*c*-sphingadienine that might be due to

the chow provided was detected in lymph collected from –2 h to 0 h (i.e., before administration of the glucosylceramide). Baseline values of 4*t*, 8*c*-sphingadienine in the free and total fractions were 4.1 ± 0.6 and 56.4 ± 15.1 pmol/ml, respectively. The recovery of sphingoid bases after administration was corrected by the baseline value individually. The peak components detected by the fluorescence detector were further identified by LC-MS. Both

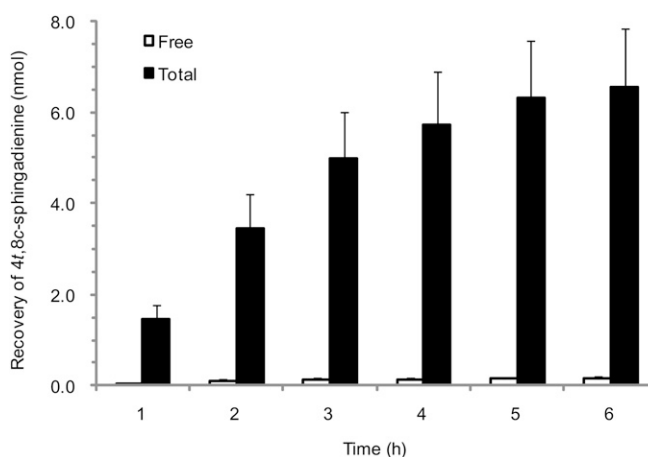


Fig. 5. Cumulative recovery of 4*t*, 8*c*-sphingadienine in the thoracic duct lymph of rats infused with an emulsion containing maize glucosylceramide. The calculated dosage of glucosylceramide consisting of 4*t*, 8*c*-sphingadienine was $3.74 \mu\text{mol/rat}$. The recovery was corrected by the baseline value (–2 to 0 h fraction) individually. Data are reported as means \pm SEM ($n = 5$).

peaks in the chromatogram of the lymph extracts before and after administration with the same retention time as 4*t*,8*c*-sphingadienine showed a molecular ion of 4,8-sphingadienine OPA derivative $[M+H]^+$ at m/z 474 (Fig. 4).

The lymphatic recovery of 4*t*,8*c*-sphingadienine from the dietary maize-derived glucosylceramide is shown in Fig. 5. The recovery of sphingadienine from the total sphingoid base fraction, which contains hydrolysates of complex sphingolipids such as glucosylceramide and ceramide, is more abundant than that from the free form

fraction in the lymph after administration. The result indicates that 4*t*,8*c*-sphingadienine, one of the principal components of maize glucosylceramide, can be absorbed via the lymph duct and is mainly incorporated into lymphatic complex sphingolipids.

To identify complex sphingolipids consisting of 4,8-sphingadienine in the lymph after administration of maize glucosylceramide, the alkaline-stable fraction of lymph extract (total sphingoid base fraction) was analyzed to obtain structural information using LCMS-IT-TOF. Two

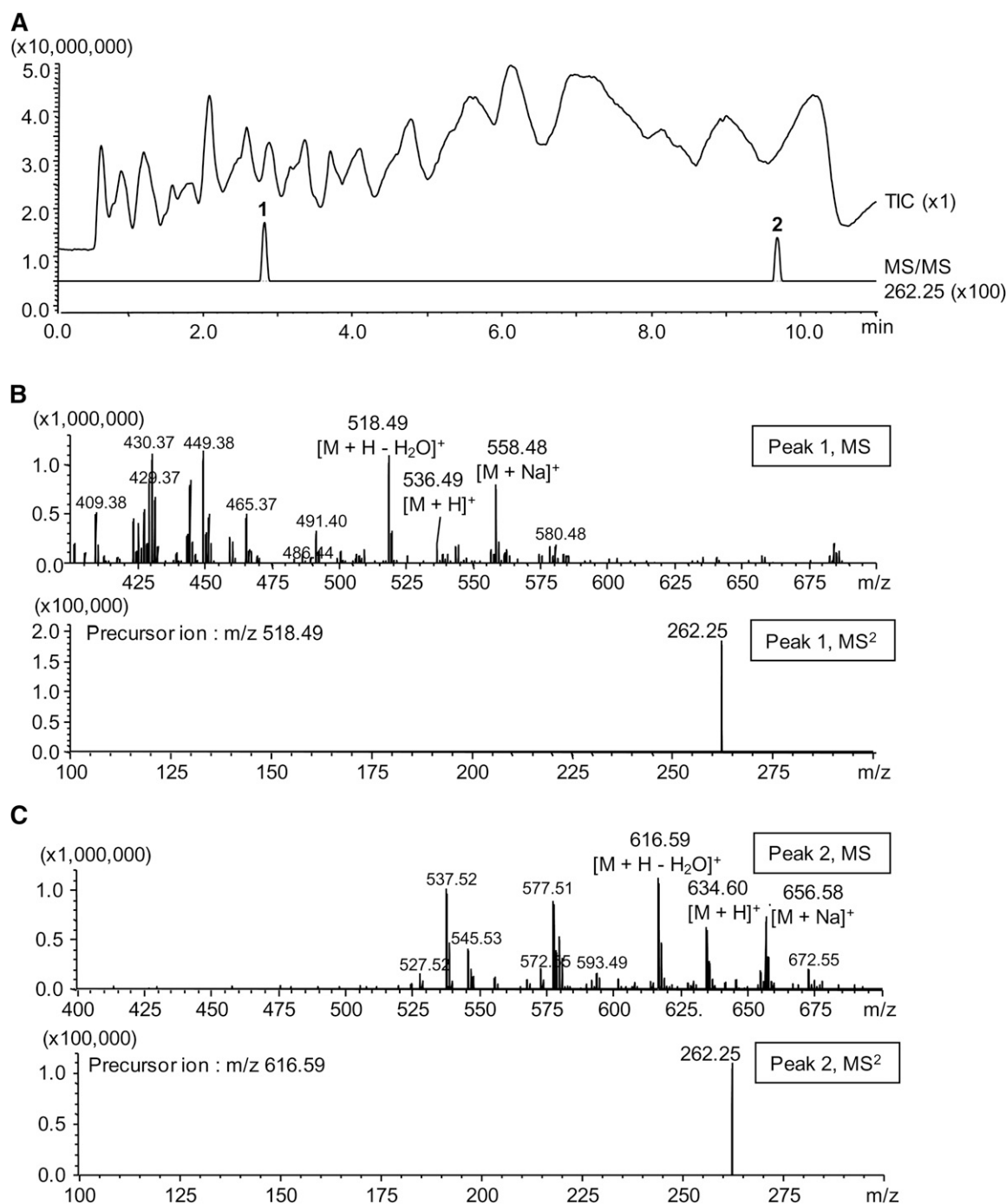


Fig. 6. Identification of ceramide structures consisting of 4,8-sphingadienine in the thoracic duct lymph of rats infused with maize glucosylceramide as analyzed by LC-MS/MS. TIC and MS/MS chromatograms of the alkaline-stable fraction extracted from rat lymph after administration (1–2 h) (A). MS and MS/MS spectra of the components of peaks 1 (B) and 2 (C).

types of ceramides consisting of 4,8-sphingadienine, i.e., N-palmitoyl-4,8-sphingadienine (C16:0-d18:2) and N-tricosanoyl-4,8-sphingadienine (C23:0-d18:2), were identified by auto MS/MS detection mode (Fig. 6). Sphingolipids containing sphingadienine showed a characteristic product ion at m/z 262.25 (33). As the precursor ion of m/z 262.25, $[M+H-18]^+$ ions at m/z 518.49 and 616.59 were detected (Fig. 6B, C). C16:0-d18:2, which had a precursor ion $[M+H-18]^+$ at m/z 518.49, was detected in the lymph before and after the administration, but C23:0-d18:2, which had a precursor ion $[M+H-18]^+$ at m/z 616.59, was detected only after administration in the MS/MS chromatogram of selected ion at m/z 262.25 (Fig. 7). The ion intensity corresponding to C16:0-d18:2 was increased 2–3 times by administration of the maize glucosylceramide. Fatty acids of maize glucosylceramide are mostly the 2-hydroxy type (16), but ceramides consisting of 4,8-sphingadienine found in the lymph have nonhydroxy fatty acids (C16 and C23). This result indicates that 4,8-sphingadienine produced by the digestion of maize glucosylceramide in the intestinal tract is metabolized to ceramide in intestinal cells during absorption.

Focusing on the difference of sphingoid base structure, the absorption of plant-derived sphingoid bases were compared with those of an authentic glucosyl-N-palmitoylsphingosine used as a model for glucosylceramide of mammalian origin. Sphingosine in the free and total sphingoid base fractions of lymph extract before administration (-2 h to 0 h) was 19.8 ± 4.7 and $4,635 \pm 595$ pmol/ml, respectively. Therefore, the recovery of sphingosine

in lymph $0-6$ h after administration was corrected by the amount of sphingosine in the lymph collected from -2 h to 0 h individually. The recovery of sphingosine in the lymph of rats infused with emulsion containing triolein only were increased in a time-dependent manner, because chylomicrons contain endogenous sphingomyelin and ceramide (34). The lymphatic recovery of sphingosine in glucosyl-N-palmitoylsphingosine-treated rats was significantly higher than in rats infused with triolein (Fig. 8). Figure 9 shows the calculated recovery rate of sphingoid bases from dietary glucosylceramides. The recovery of sphingosine after administration of glucosyl-N-palmitoylsphingosine was corrected by the mean value of sphingosine in rats infused with triolein (Fig. 8). The cumulative recovery of total sphingosine were significantly higher than that of 4,8-sphingadienine at 1 h and at 2 h (Fig. 9B). However, there was no significant difference between the groups 3 h after administration. Up to 6 h after the treatment, the cumulative recovery of 4,8-sphingadienine from maize glucosylceramide and sphingosine from glucosyl-N-palmitoylsphingosine was $0.18 \pm 0.04\%$ and $0.55 \pm 0.22\%$, respectively.

DISCUSSION

In the present study, we investigated the absorption in the rat intestine of dietary glucosylceramide derived from maize compared with glucosyl-N-palmitoylsphingosine. We demonstrate that the intact form of 4,8-sphingadienine,

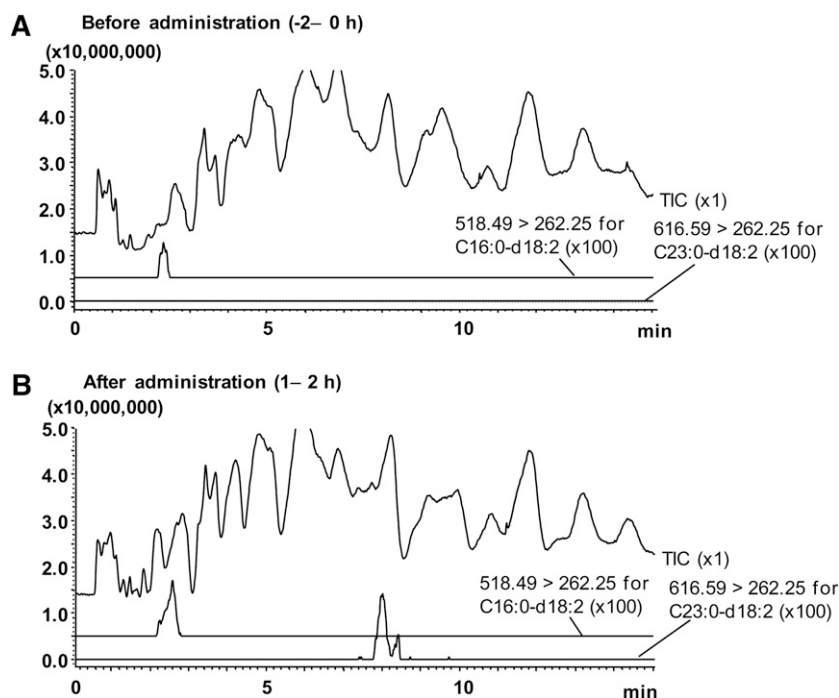


Fig. 7. LC-MS/MS analysis of N-palmitoyl-4,8-sphingadienine (C16:0-d18:2) and N-tricosanoyl-4,8-sphingadienine (C23:0-d18:2) in rat lymph before (A) and after (B) administration of maize glucosylceramide. C16:0-d18:2, which had a precursor ion $[M+H-18]^+$ at m/z 518.49, was detected in MS/MS chromatogram of selected ion at m/z 262.25. C23:0-d18:2, which had a precursor ion $[M+H-18]^+$ at m/z 616.59, was detected in the MS/MS chromatogram of selected ion at m/z 262.25.

the predominant structure of sphingoid bases in higher plants, can be absorbed into the lymph by intestinal cells after administration of maize glucosylceramide, but the recovery is extremely low. A large portion of the 4,8-sphingadienine absorbed by intestinal cells is a constituent of complex sphingolipids, including at least ceramide, while the smaller portion is in the free form in the lymph. The cumulative recovery of 4,8-sphingadienine is relatively lower than that of sphingosine after administration of glucosyl-N-palmitoylsphingosine.

Dietary sphingolipids having sphingosine can be degraded in the intestine (21–25). Several reports have indicated that alkaline sphingomyelinase is present in the intestinal mucosa (35–37) and that the glucosylceramidase activity in the intestine is due to lactase-phlorizin hydrolase (38, 39). Neutral ceramidase encoded by *Asah2* is also present in the small intestine (40). We have reported that the hydrolytic activity in the small intestine toward glucosylceramide and ceramide derived from maize is consistent with the findings of such activity toward mammalian-derived sphingolipids (26). Plant-derived sphingolipids might be hydrolyzed on the surfaces of intestinal villi, because free sphingoid bases and ceramide appear in the small intestinal lumen after ingestion. Plant-derived glucosylceramide would be hydrolyzed to free sphingoid bases in the digestive tract before absorption into the lymph. The low lymphatic recovery of sphingadienine (0.18%) can be attributed to the relatively low capacity for hydrolysis of sphingolipids in the intestine.

We have reported that the uptake of sphingosine is significantly higher than the uptake of other sphingoid bases (including 4,8-sphingadienine) in differentiated Caco-2 cells (as a model for intestinal cells) and that P-glycoprotein probably contributes to this selective absorption of sphingosine (27). P-glycoprotein, encoded by the *MDR1* gene, transports a wide variety of hydrophobic compounds, including drugs, natural products, toxicants, and peptides, and contributes to the barrier function of the gut (41). In this study, the cumulative recovery of 4,8-sphingadienine in the lymph is significantly lower than that of sphingosine until 2 h after the administration of glucosylceramide. It is difficult to quantify the exact amount of sphingosine due to the diet in the lymph, because chylomicrons secreted from intestinal cells contain endogenous sphingolipids (34). However, endogenous sphingosine might not affect the recovery of sphingosine from the diet within short time periods after administration (Fig. 8). Therefore, our observations in this study appear to support an important role for P-glycoprotein in the efflux of 4,8-sphingadienine, but not sphingosine, across the apical membranes of enterocytes after absorption.

In this study, the total recovery of sphingosine in an intact form after administration of glucosyl-N-palmitoylsphingosine was lower than reported in other studies, which used labeled sphingolipids (42), because most of the dietary sphingosine is converted to palmitic acid in the intestinal mucosa and is incorporated into chylomicron

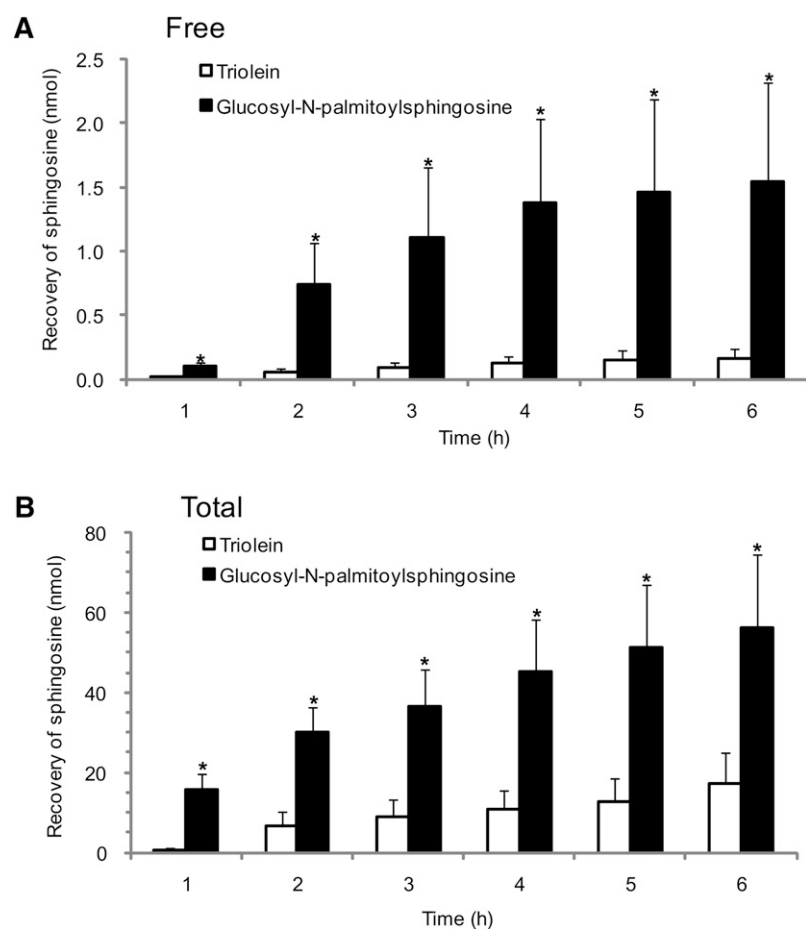


Fig. 8. Cumulative recovery of sphingosine in the thoracic duct lymph of rats infused with an emulsion containing glucosyl-N-palmitoylsphingosine. The dosage of glucosyl-N-palmitoylsphingosine was 7.14 $\mu\text{mol}/\text{rat}$. Control experiments used emulsions without glucosylceramide (triolein only). The recovery of sphingosine in the lymph collected from -2 h to 0 h individually. A: Free sphingoid base fraction. B: Total sphingoid base fraction. Data are reported as means \pm SEM ($n = 5$). *Significantly different from triolein only at each time point, $P < 0.05$.

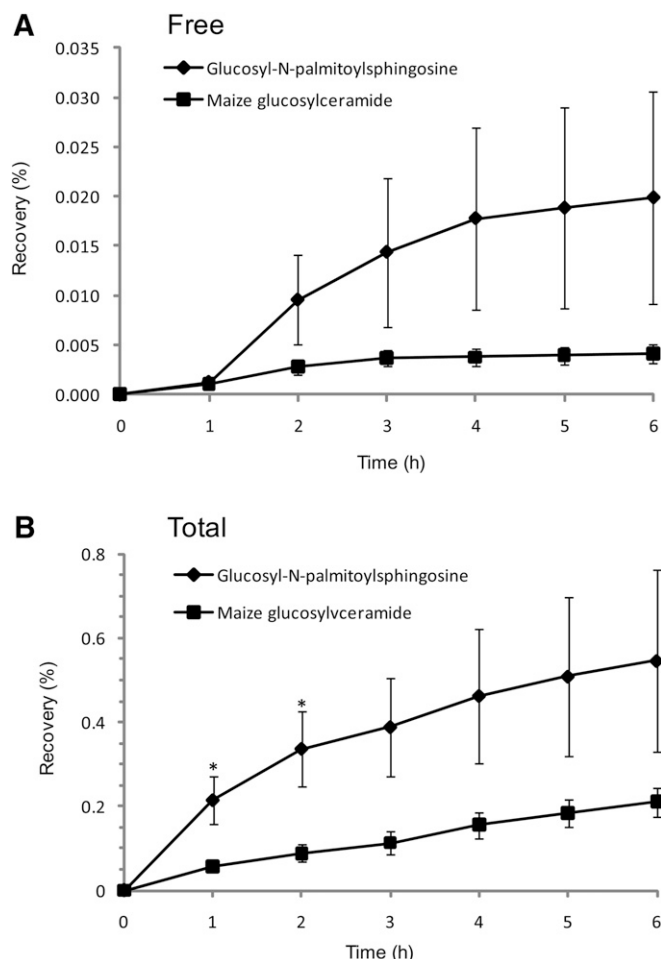


Fig. 9. Calculated recovery rates of sphingosine from dietary glucosyl-N-palmitoylsphingosine and sphingadienine from dietary maize glucosylceramides. The recovery of sphingosine after administration of glucosyl-N-palmitoylsphingosine was corrected by the mean value of sphingosine in rats infused with triolein at each time point. Data are reported as means \pm SEM ($n = 5$). *Significantly different from maize glucosylceramide at each time point, $P < 0.05$.

triacylglycerol (21, 23). Phosphorylation of the primary hydroxyl group of sphingoid bases is the penultimate step in the degradation of sphingolipid (43). The phosphate esters formed are subsequently cleaved by sphingosine phosphate lyase into ethanolamine phosphate and the corresponding aldehyde after which the long-chain aldehyde is further converted to fatty acids (44). Our results show the recovery of the intact form of 4,8-sphingadienine but not the complete recovery of sphingoid bases, including their catabolites absorbed via the lymph. Clarification of the catabolism of various sphingoid bases is important to understanding the metabolism and biological function of dietary sphingolipids from various foodstuffs.

Although fatty acids of maize-derived glucosylceramide are mostly 2-hydroxy fatty acids (16), ceramides consisting of 4,8-sphingadienine found in the lymph after administration of maize glucosylceramide have nonhydroxy fatty acids (C16 and C23). This result indicates that the 4,8-sphingadienine produced by the digestion of maize glucosylceramide in the intestinal tract is metabolized to ceramide

in intestinal cells during absorption. Interestingly, Mao et al. (45) surmised that phosphorylation followed by dephosphorylation is required for the incorporation of exogenous sphingoid bases into complex sphingolipids. Considering our results, some 4,8-sphingadienine might be phosphorylated by sphingosine kinase after uptake by intestinal cells. However, there are no data about the phosphorylation in mammals of sphingoid bases that originated from plants.

It is well known that ceramides in the stratum corneum are required for the epidermal permeability barrier. There have been several reports concerning the improvement of skin barrier function by dietary sphingolipids prepared from higher plants (46–49). However, the structures of sphingoid bases of ceramide in the skin are quite different from those of plant-derived sphingolipids. In addition, the present study shows that the recovery of dietary sphingolipid prepared from higher plants is extremely low. We are interested in elucidating the mechanism for the effect of dietary plant-derived sphingolipids on skin barrier function.

In conclusion, we demonstrated that dietary glucosylceramide derived from higher plants is only slightly absorbed by the intestine and is incorporated into ceramide structures in intestinal cells. It appears that the intact form of sphingoid bases is hardly reutilized in tissues, because the recovery is extremely low. Our findings provide an important insight into the intestinal absorption of dietary sphingolipids derived from plant sources.

REFERENCES

- Karlsson, K. A. 1970. Sphingolipid long chain bases. *Lipids*. **5**: 878–891.
- Sperling, P., and E. Heinz. 2003. Plant sphingolipids: structural diversity, biosynthesis, first gene and functions. *Biochim. Biophys. Acta*. **1632**: 1–15.
- Sperling, P., U. Zähringer, and E. Heinz. 1998. A sphingolipid desaturase from higher plants. *J. Biol. Chem.* **273**: 28590–28596.
- Sperling, P., B. Libish, U. Zähringer, J. N. Napier, and E. Heinz. 2001. Functional identification of a Δ^8 -sphingolipid desaturase from *Borago officinalis*. *Arch. Biochem. Biophys.* **388**: 293–298.
- Takakuwa, N., M. Kinoshita, Y. Oda, and M. Ohnishi. 2002. Existence of cerebroside in *Saccharomyces kluyveri* and its related species. *FEMS Yeast Res.* **2**: 533–538.
- Ohashi, Y., T. Tanaka, S. Akashi, S. Morimoto, Y. Kishimoto, and Y. Nagai. 2000. Squid nerve sphingomyelin containing an unusual sphingoid base. *J. Lipid Res.* **41**: 1118–1124.
- Vesper, H., E. M. Schmelz, M. N. Nikolova-Karakashian, D. L. Dillehay, D. V. Lynch, and A. H. Merrill, Jr. 1999. Sphingolipids in food and the emerging importance of sphingolipids to nutrition. *J. Nutr.* **129**: 1239–1250.
- Sugawara, T., and T. Miyazawa. 1999. Separation and determination of glycolipids from edible plant sources by high-performance liquid chromatography and evaporative light-scattering detection. *Lipids*. **34**: 1231–1237.
- Yunoki, K., T. Ogawa, J. Ono, R. Miyashita, K. Aida, Y. Oda, and M. Ohnishi. 2008. Analysis of sphingolipid classes and their contents in meals. *Biosci. Biotechnol. Biochem.* **72**: 222–225.
- Duan, R. D., and Å. Nilsson. 2009. Metabolism of sphingolipids in the gut and its relation to inflammation and cancer development. *Prog. Lipid Res.* **48**: 62–72.
- Schmelz, E. M. 2004. Sphingolipids in the chemoprevention of colon cancer. *Front. Biosci.* **9**: 2632–2639.
- Schmelz, E. M., M. C. Sullards, D. L. Dillehay, and A. H. Merrill, Jr. 2000. Colonic cell proliferation and aberrant crypt formation are inhibited by dietary glycosphingolipids in 1,2-dimethylhydrazine-treated CF1 mice. *J. Nutr.* **130**: 522–527.

13. Schmelz, E. M., P. C. Roberts, E. M. Kustin, L. A. Lemonnier, M. C. Sullards, D. L. Dillehay, and A. H. Merrill, Jr. 2001. Modulation of intracellular β -catenin localization and intestinal tumorigenesis in vivo and in vitro by sphingolipids. *Cancer Res.* **61**: 6723–6729.
14. Hannun, Y. A., and L. M. Obeid. 2008. Principles of bioactive lipid signaling: lessons from sphingolipids. *Nat. Rev. Mol. Cell Biol.* **9**: 139–150.
15. Cuvillier, O. 2002. Sphingosine in apoptosis signaling. *Biochim. Biophys. Acta.* **1585**: 153–162.
16. Aida, K., M. Kinoshita, M. Tanji, T. Sugawara, M. Tamura, J. Ono, N. Ueno, and M. Ohnishi. 2005. Prevention of aberrant crypt foci formation by dietary maize and yeast cerebrosides in 1,2-dimethylhydrazine-treated mice. *J. Oleo Sci.* **54**: 45–49.
17. Kinoshita, M., K. Aida, Y. Tokuji, T. Sugawara, and M. Ohnishi. 2009. Effects of dietary plant cerebroside on gene expression in the large intestine of 1,2-dimethylhydrazine (DMH)-treated mice determined by DNA microarray analysis. *J. Food Lipids.* **16**: 200–208.
18. Sugawara, T., M. Kinoshita, M. Ohnishi, and T. Miyazawa. 2002. Apoptosis induction by wheat-flour sphingoid bases in DLD-1 human colon cancer cells. *Biosci. Biotechnol. Biochem.* **66**: 2228–2231.
19. Aida, K., M. Kinoshita, T. Sugawara, J. Ono, T. Miyazawa, and M. Ohnishi. 2004. Apoptosis induction by plant and fungus sphingoid bases in human colon cancer cells. *J. Oleo Sci.* **53**: 503–510.
20. Sugawara, T., N. Zaima, A. Yamamoto, S. Sakai, R. Noguchi, and T. Hirata. 2006. Isolation of sphingoid bases of sea cucumber cerebrosides and their cytotoxicity against human colon cancer cells. *Biosci. Biotechnol. Biochem.* **70**: 2906–2912.
21. Nilsson, Å. 1968. Metabolism of sphingomyelin in the intestinal tract of the rat. *Biochim. Biophys. Acta.* **164**: 575–584.
22. Nilsson, Å. 1969. The presence of sphingomyelin- and ceramide-cleaving enzymes in the small intestinal tract. *Biochim. Biophys. Acta.* **176**: 339–347.
23. Nilsson, Å. 1969. Metabolism of cerebroside in the intestinal tract of the rat. *Biochim. Biophys. Acta.* **187**: 113–121.
24. Schmelz, E. M., K. J. Crall, R. Larocque, D. L. Dillehay, and A. H. Merrill, Jr. 1994. Uptake and metabolism of sphingolipids in isolated intestinal loops of mice. *J. Nutr.* **124**: 702–712.
25. Nyberg, L., Å. Nilsson, P. Lundgren, and R. D. Duan. 1997. Localization and capacity of sphingomyelin digestion in the rat intestinal tract. *J. Nutr. Biochem.* **8**: 112–118.
26. Sugawara, T., M. Kinoshita, M. Ohnishi, J. Nagata, and M. Saito. 2003. Digestion of maize sphingolipids in rats and uptake of sphingadienine by Caco-2 cells. *J. Nutr.* **133**: 2777–2782.
27. Sugawara, T., M. Kinoshita, M. Ohnishi, T. Tsuzuki, T. Miyazawa, J. Nagata, T. Hirata, and M. Saito. 2004. Efflux of sphingoid bases by P-glycoprotein in human intestinal Caco-2 cells. *Biosci. Biotechnol. Biochem.* **68**: 2541–2546.
28. Ohnishi, M., S. Ito, and Y. Fujino. 1983. Characterization of sphingolipids in spinach leaves. *Biochim. Biophys. Acta.* **752**: 416–422.
29. Tsuzuki, T., Y. Kawakami, R. Abe, K. Nakagawa, K. Koba, J. Imamura, T. Iwata, I. Ikeda, and T. Miyazawa. 2006. Conjugated linolenic acid is slowly absorbed in rat intestine, but quickly converted to conjugated linoleic acid. *J. Nutr.* **136**: 2153–2159.
30. Fujino, Y., and M. Ohnishi. 1983. Sphingolipids in wheat grain. *J. Cereal Sci.* **1**: 159–168.
31. Gaver, R. C., and C. C. Sweely. 1965. Methods for methanolysis of sphingolipids and direct determination of long-chain bases by gas chromatography. *J. Am. Oil Chem. Soc.* **42**: 294–298.
32. Merrill, A. H., Jr., E. Wang, R. E. Mullins, W. C. L. Jamison, S. Nimkar, and D. C. Liotta. 1988. Quantitation of free sphingosine in liver by high-performance liquid chromatography. *Anal. Biochem.* **171**: 373–381.
33. Sugawara, T., K. Aida, J. Duan, and T. Hirata. 2010. Analysis of glucosylceramides from various sources by liquid chromatography-ion trap mass spectrometry. *J. Oleo Sci.* In **59**: 387–394.
34. Merrill, A. H., Jr., S. Lingrell, E. Wang, M. Nikolova-Karakashian, T. R. Vales, and D. E. Vance. 1995. Sphingolipid biosynthesis *de novo* by rat hepatocytes in culture: ceramide and sphingomyelin are associated with, but not required for, very low density lipoprotein secretion. *J. Biol. Chem.* **270**: 13834–13841.
35. Nilsson, Å., and R. D. Duan. 2006. Absorption and lipoprotein transport of sphingomyelin. *J. Lipid Res.* **47**: 154–171.
36. Duan, R. D., T. Bergman, N. Xu, J. Wu, Y. Cheng, J. Duan, S. Nelander, C. Palmberg, and Å. Nilsson. 2003. Identification of human intestinal alkaline sphingomyelinase as a novel ecto-enzyme related to the nucleotide phosphodiesterase family. *J. Biol. Chem.* **278**: 38528–38536.
37. Wu, J., Y. Cheng, C. Palmberg, T. Bergman, Å. Nilsson, and R. D. Duan. 2005. Cloning of alkaline sphingomyelinase from rat intestinal mucosa and adjusting of the hypothetical protein XP_221184 in GenBank. *Biochim. Biophys. Acta.* **1687**: 94–102.
38. Leese, H. J., and G. Semenza. 1973. On the identity between the small intestinal enzymes phlorizin hydrolase and glucosylceramidase. *J. Biol. Chem.* **248**: 8170–8173.
39. Büller, H. A., A. G. Van Wassenae, S. Raghavan, R. K. Montgomery, M. A. Sybicki, and R. J. Grand. 1989. New insights into lactase and glucosylceramidase activities of rat lactase-phlorizin hydrolase. *Am. J. Physiol.* **257**: G616–G623.
40. Kono, M., J. L. Dreier, J. M. Ellis, M. L. Allende, D. N. Kalkofen, K. M. Sanders, J. Bielawski, A. Bielawska, Y. A. Hannun, and R. L. Proia. Neutral ceramidase encoded by the asah2 gene is essential for the intestinal degradation of sphingolipids. *J. Biol. Chem.* **281**: 7324–7331.
41. Fricker, G., and D. S. Miller. 2002. Relevance of multidrug resistance proteins for intestinal drug absorption in vitro and in vivo. *Pharmacol. Toxicol.* **90**: 5–13.
42. Ueda, O., M. Hasegawa, and S. Kitamura. 2009. Distribution in skin of ceramide after oral administration to rats. *Drug Metab. Pharmacokinet.* **24**: 180–184.
43. Macecka, M., S. G. Payne, S. Milstein, and S. Spiegel. 2002. Sphingosine kinase, sphingosine 1-phosphate, and apoptosis. *Biochim. Biophys. Acta.* **1585**: 193–201.
44. Van Veldhoven, P. P., and G. P. Mannaerts. 1993. Sphingosine-phosphate lyase. *Adv. Lipid Res.* **26**: 69–98.
45. Mao, C., M. Wedleigh, G. M. Jenkins, Y. A. Hannun, and L. M. Obeid. 1997. Identification and characterization of *Saccharomyces cerevisiae* dihydrosphingosine-1-phosphate phosphatase. *J. Biol. Chem.* **272**: 28690–28694.
46. Miyazaki, K., N. Shiono, H. Shirai, M. Dombo, and H. Kimata. 2005. Reduction of transepidermal water loss by oral intake of glucosylceramides in patients with atopic eczema. *Allergy.* **60**: 1454–1455.
47. Kimata, H. 2006. Improvement of atopic dermatitis and reduction of skin allergic responses by oral intake of konjac ceramide. *Pediatr. Dermatol.* **23**: 386–389.
48. Tsuji, K., S. Mitsutake, J. Ishikawa, Y. Takagi, M. Akiyama, H. Shimizu, T. Tomiyama, and Y. Igarashi. 2006. Dietary glucosylceramide improves skin barrier function in hairless mice. *J. Dermatol. Sci.* **44**: 101–107.
49. Uchiyama, T., Y. Nakano, O. Ueda, H. Mori, M. Nakashima, A. Noda, C. Ishizaki, and M. Mizoguchi. 2008. Oral intake of glucosylceramide improves relatively higher level of transepidermal water loss in mice and healthy human subjects. *J. Health Sci.* **54**: 559–566.