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# Orally administered sphingomyelin in bovine milk is incorporated into skin sphingolipids and is involved in the water-holding capacity of hairless mice

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ABSTRACT

*Background:* We previously reported that dietary sphingomyelin (SM) concentrate from bovine milk improves epidermal functions. SM is a known precursor of ceramide (Cer) in the stratum corneum (SC). Neither the uptake nor distribution of orally administered SM nor its effects on epidermal functions have been demonstrated.
*Objective:* We evaluated the effects of dietary SM on epidermal functions, and the distribution and fate of its radiolabeled metabolites in mice orally administered [4,5-$^3$H-sphinganyl] sphingomyelin ($^3$H-SM).
*Methods:* Bovine milk SM (98% purity) was administered orally to 13-week-old hairless mice at 142 mg/kg per day for eight weeks. Their SC hydration, transepidermal water loss (TEWL), and SC Cer content were measured. $^3$H-SM was then administered orally to 10-week-old hairless mice. Its distribution and metabolites in the skin were evaluated with whole-body autoradiography, liquid scintillation counting, and thin-layer chromatography.
*Results:* SC hydration in the SM-administered mice was higher than that in control mice, whereas their TEWL and Cer contents did not differ. Radioactivity was distributed extensively in the bodies of the experimental mice and decreased gradually with time. In contrast, the radioactivity in the SC remained constant after its administration, and radiolabeled SM and Cer were detected in the skin. This suggests that dietary SM is transferred to the skin and then converted to Cer in the SC.
*Conclusions:* Orally administered SM is incorporated into skin SM and converted to SC Cer, which is involved in the water-holding capacity of the SC.


## 1. Introduction

The epidermis has a considerable water-holding capacity and a permeability barrier function, which protect the organism from dehydration, infection by pathogens, and environmental stresses, such as chemicals and physical damage. The stratum corneum (SC), the outermost layer of the epidermis, is composed of corneocytes and an intercellular lipid matrix. Ceramide (Cer), which comprises approximately 50% of these intercellular lipids, plays an important role in retaining epidermal water and in the epidermal permeability barrier function, in combination with cholesterol and free fatty acids [1–3]. The Cer in the SC consists of a heterogeneous family of at least seven molecular groups (Cer1–7), which vary in their long-chain sphingoid base structures, their chain lengths, and the α-hydroxylation of their constituent amide-linked fatty acids [4–6]. SC Cer1–7 are generated from glucosylceramide (GlcCer), whereas Cer2 and Cer5 are also generated from sphingomyelin (SM). Thus, SM and GlcCer are important precursors of SC Cer [7,8], and are synthesized by keratinocytes and stored in epidermal lamellar bodies. At the transition from the stratum granulosum to the SC, the lamellar bodies fuse with the plasma membrane of the uppermost granular cells and extrude their contents into the intercellular spaces of the SC. Hydrolytic enzymes, such as β-glucocerebrosidase and acid sphingomyelinase, then convert the secreted GlcCer and SM, respectively, into SC Cer [9,10].

Recently, several studies have reported that dietary constituents play a beneficial role in epidermal functions [11–14]. In our previous study, we also demonstrated that the oral administration of an SM concentrate prepared from bovine milk to hairless mice increased the hydration of their SC and the SC Cer content and reduced their transepidermal water loss (TEWL) [15,16]. Moreover, the daily intake of SM concentrate improves the water-holding capacity of the

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human epidermis [17]. These results indicate that the dietary SM concentrate improves the water-holding capacity and permeability barrier function of the epidermis. However, the SM concentrate contains other components, so it is unclear whether these effects on epidermal functions are attributable to SM, or whether orally administered SM is distributed to the skin and converted to Cer.

In this study, we examined the effects of dietary SM on epidermal functions and its distribution and the distribution of its metabolites in the skin of hairless mice orally administered $^{3}$H-radiolabeled SM.

## 2. Materials and methods

### 2.1. Chemicals

SM from bovine milk (>98% purity) was purchased from Nof Corporation (Tokyo, Japan). Radiolabeled [4,5-$^{3}$H-sphinganyl] sphingomyelin ($^{3}$H-SM; Fig. 1) was synthesized from SM by Blychem Ltd (Billingham, UK). The radiolabeled compound had a specific activity of 17 Ci/mmol and a radiochemical purity of >99% according to thin-layer chromatography (TLC). The SM concentrate (Milk Ceramide MC-5; Megmilk Snow Brand Co., Ltd, Tokyo, Japan) was prepared from bovine milk, according to a previous report [15]. The SM concentrate contained 6.9% SM and other components, including proteins, phospholipids, triglycerides, and carbohydrates (Table 1). Cer standards were obtained from Larodan Fine Chemicals (Malmö, Sweden), and contained non-OH Cer and α-OH Cer from bovine brain. Other high-grade reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

### 2.2. Animals

Male hairless HR-1 mice, aged 10–14 weeks and weighing 22–32 g, were purchased from Japan SLC (Hamamatsu, Japan) [16,17]. The mice were housed in individual plastic cages in a temperature- and humidity-controlled room (23 °C and 50 ± 5% relative humidity), on a 12 h light/dark cycle. The mice were given free access to food and distilled water. All procedures for animal care and use complied with the regulations established by the NIH Guide for the Care and Use of Laboratory Animals and the Experimental Animal Care and Use Committee of Fukuoka University.

### 2.3. Effects of orally administered SM on epidermal functions

Twelve-week-old male HR-1 mice were used in this study. After a one-week adaptation period, the mice were separated into three

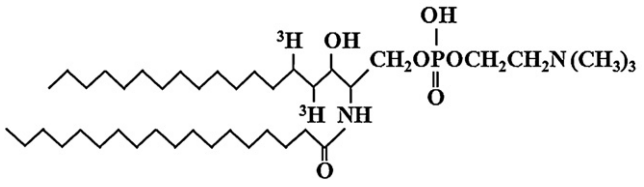


**Fig. 1.** Structure of $^{3}$H-SM. Radioactive labeling was at the C4 and C5 positions of the sphingoid moiety.

**Table 1**
Composition of SM concentrate.

| Component | Content (%) |
|---|---|
| Moisture | 1.2 |
| Protein | 22.9 |
| Fat | 57.2 |
| Ash | 6.7 |
| Carbohydrate | 12.0 |
| SM | 6.9 |

**Table 2**
Compositions of experimental diets.

| Component[a] | Content (%) | | |
|---|---|---|---|
| | Control | SM-supplemented | SM-concentrate supplemented |
| Casein | 20.0 | 20.0 | 17.6 |
| Corn oil | 5.0 | 5.0 | – |
| Olive oil | 5.0 | 5.0 | – |
| Safflower oil | – | – | 4.2 |
| SM | – | 0.1 | – |
| SM concentrate | – | – | 9.8 |
| DL-Methionine | 0.3 | 0.3 | 0.3 |
| Mineral mixture | 3.5 | 3.5 | 3.5 |
| Vitamin mixture | 1.0 | 1.0 | 1.0 |
| Cellulose | 5.0 | 5.0 | 5.0 |
| Cornstarch | 15.0 | 15.0 | 15.0 |
| Sucrose | 45.2 | 45.1 | 43.6 |
| SM | – | 0.1 | 0.7 |
| Linoleic acid | 3.1 | 3.1 | 3.1 |
| Linolenic acid | 0.1 | 0.1 | 0.1 |

[a] The experimental diets were modified from AIN-76 (American Institute of Nutrition, 1977), supplemented with olive oil or safflower oil.

experimental groups: the control group ($n$ = 10), the SM-supplemented group ($n$ = 7), and the SM-concentrate-supplemented group (as the positive control; $n$ = 10). Each mouse had a similar mean body weight. All mice were given free access to one of the three experimental diets and distilled water for eight weeks. The experimental diets were modified from the AIN-76 diet (Table 2). The amounts of linoleic acid (3.1%) and linolenic acid (0.1%) were adjusted to ensure the same concentrations in the three diets with the addition of safflower oil and/or olive oil, because they are known to affect the permeability barrier function of the epidermis [18,19]. In the SM-concentrate-supplemented group (positive control), the lipids in the diet were partly replaced by the SM concentrate. The SM contents in the SM-supplemented and SM-concentrate-supplemented diets were 0.1% (w/v) and 0.7% (w/v), respectively. The mouse body weights were recorded once a week and their food intake was monitored daily. The water-holding capacity and permeability barrier function of the epidermis were evaluated by assessing the hydration of the SC and TEWL using a Corneometer CM825 and Tewameter TM300 (Courage and Khazaka Electronics, Cologne, Germany), respectively, at four and eight weeks. After the eight-week feeding period, the mice were fasted for 18 h and sacrificed with diethyl ether. The whole skin was stripped from each mouse with scissors, and the SC sheet was isolated by incubating the skin in 5% (w/v) trypsin solution in phosphate-buffered saline (pH 7.3) for 1 h at 37 °C. The SC sheets were dried overnight in a vacuum oven and weighed, and then stored at −80 °C until further analysis. The Cer content of the SC was quantified by high-performance liquid chromatography (HPLC), as described in Section 2.8.

### 2.4. Whole-body autoradiography

$^{3}$H-SM (18.5 MBq) was diluted in 0.2 mL of 15% (w/v) SM concentrate and administered orally to 10-week-old male HR-1 mice that had been fasted for 18 h. The mice were housed singly in metabolic cages (Metabolica, Sugiyama-gen Co., Tokyo, Japan) after the administration of $^{3}$H-SM. The mice were sacrificed 24 h after the administration of $^{3}$H-SM, rapidly frozen at −80 °C, and embedded in carboxymethylcellulose gel. Serial sections (30 μm thick) through the sagittal plane of each mouse were made with the tape-sectioning method using a Cryo Polycut cryostat (Reichert-Jung, Nussloch, Germany) at −20 °C. The sections on the adhesive tape (Yu-Ki Ban, Nitto Medical Co., Ltd, Osaka, Japan)