Improvement of skin condition by oral supplementation with sphingomyelin-containing milk phospholipids in a double-blind, placebo-controlled, randomized trial

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ABSTRACT

Sphingomyelin (SM), an essential phospholipid for the skin, is contained largely in the milk fat globule membrane surrounding milk fat, concentrated fractions of which are also generated concurrently during the manufacture of dairy products. Such an SM-containing milk phospholipid concentrate (SM-MPC) is useful for investigating the benefits of dietary SM. Here, we examined the effect of consuming SM-MPC on the condition of skin in a double-blind, placebo-controlled, randomized trial. Ninety-six healthy subjects aged 20 to 39 yr with low skin hydration were randomly assigned to 3 groups: a high-SM group supplemented with SM-MPC at a dose equivalent to 10 mg/d of SM, a low-SM group supplemented with SM-MPC equivalent to 5 mg/d of SM, and a placebo group fed a vehicle composed of olive oil and beeswax. During daily supplementation for 12 wk, parameters related to the condition of skin were evaluated at baseline and every 3 wk. Skin hydration at the heel was significantly increased at wk 9 and 12 in the low-SM group compared with the placebo group. Skin elasticity in the region below the eye was significantly increased at wk 9 in the high-SM group versus placebo. Questionnaire-based subjective perceptions of skin conditions were significantly improved for facial skin moisture at wk 3 and 12, and in the wrinkle around the eyes at wk 9 and 12 in the high-SM group versus placebo. Our results indicate that constant and long-term supplementation with SM-MPC is capable of improving the general condition of skin.

Key words: sphingomyelin, phospholipid, milk fat globule membrane, skin

INTRODUCTION

Milk fat is often used as a source of sphingomyelin (SM) in the study of the physiological importance of

dietary SM (Parodi, 1997; Graves et al., 2007). Dietary SM is also known as a factor for increasing ceramide levels in the body (Schmelz, 2000; Ohlsson et al., 2010), and ceramides are involved in maintaining epidermal hydration and the barrier function of skin (Brod, 1991; Imokawa et al., 1994). Dietary SM is hydrolyzed to ceramides and sphingosine in the small intestine following ingestion, of which a portion is reused for sphingolipid synthesis (Schmelz et al., 1994). Sphingolipids can upregulate the expression of enzymes for ceramide synthesis (Duan et al., 2012). It was reported that hairless mice orally administered with radiolabeled SM had detectable levels of radiolabel in the skin in the form of reconstructed SM and ceramides (Haruta-Ono et al., 2012a).

Sphingomyelin, as well as other phospholipids, is abundantly contained in the milk fat globule membrane that surrounds milk fat globules and prevents them from aggregating (Jensen et al., 1991; Spitsberg, 2005). Accordingly, milk fat globule membrane-enriched fractions can provide a more concentrated source of SM than unprocessed milk fat. Such an SM-milk phospholipid concentrate (SM-MPC) is also useful for investigating the physiological effects of dietary SM (Küllenberg et al., 2012; Contarini and Povolito, 2013).

Oral administration of SM-MPC to hairless mice was reported to increase the ceramide content of skin, which is associated with elevated hydration and reduced transepidermal water loss (Haruta et al., 2008; Haruta-Ono et al., 2012b). The effect of consuming SM-MPC on the general condition of skin was preliminarily tested in healthy volunteers, and the results indicated possible improvements in skin hydration and subjective perceptions of the general condition of facial skin (Haruta et al., 2009). However, these potentially beneficial effects of dietary SM on skin have not been rigorously examined by human trials.

The present study investigated the effect of consuming SM-MPC on skin hydration, transepidermal water loss, sebum production, skin elasticity, and subjects' perceptions of the condition of their skin in subjects with low skin hydration, via a double-blind, placebo-controlled, randomized trial.

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Table 1. Baseline characteristics of subjects

Item	Treatment group ¹			<i>P</i> -value (ANOVA)
	Placebo	Low SM	High SM	
Subjects completing the study (no.)	25	29	30	
Male	14	15	16	
Female	11	14	14	
Age ² (yr)	28.70 ± 0.26	28.00 ± 1.13	28.70 ± 1.28	0.452
Anthropometric measures ²				
Height (cm)	164.07 ± 0.33	164.74 ± 1.84	164.07 ± 1.67	0.933
BW (kg)	62.28 ± 2.66	58.77 ± 2.13	59.79 ± 2.64	0.609
BMI ³ (kg/m ²)	22.75 ± 0.72	21.51 ± 0.52	22.01 ± 0.73	0.440

¹Low SM group was supplemented with sphingomyelin milk phospholipid concentrate (SM-MPC) equivalent to 5 mg/d of sphingomyelin; high SM group was supplemented with SM-MPC equivalent to 10 mg/d of sphingomyelin; placebo group received vehicle only (230 mg of olive oil and 35 mg of beeswax).

²Values are the mean ± standard error.

³Body mass index.

MATERIALS AND METHODS

Subjects

A total of 314 healthy adults aged 20 to 39 yr were assessed for their eligibility to participate in this study. The exclusion criteria were (1) having serious disorders, including internal organ diseases, diabetes, or hypersensitivity to dairy products; (2) being pregnant or breast feeding; (3) consuming >5 servings of dairy products a week; (4) having skin problems in the areas to be tested; and (5) using anti-aging skin care products on the areas to be tested >3 times per week or regularly taking skin care-related supplements. Candidates were screened for skin hydration in the region below the eye using a corneometer, and those who had a value of <55 arbitrary units (AU), indicating low skin hydra-

tion, were included. Ninety-six subjects (50 men and 46 women) were enrolled and randomized to SM-MPC treatment or placebo, and 84 subjects (25 subjects in the placebo group, 29 in the low-SM group, and 30 in the high-SM group) completed the study (Figure 1). No adverse reactions were reported. No significant differences were observed in any baseline characteristics of subjects among groups (Table 1).

Study Design

A double-blind, placebo-controlled, randomized trial was conducted. The study protocol was in accordance with the principles of the amended Declaration of Helsinki, and was approved by the Ethics Review Board of Kensyokai Medical Corporation (Osaka, Japan). Written informed consent was obtained from all partici-

Figure 1. Flow diagram outlining the recruitment, randomization, and study completion of study participants supplemented with low or high levels of sphingomyelin (SM) or supplemented with placebo.

pants. The study was conducted by a contract research organization (Total Technological Consultant, Tokyo, Japan) between December 2013 and February 2014 at Dermis Research Center (Osaka, Japan).

Test Food

Commercially produced Milk Ceramide MC-5 (Megmilk Snow Brand, Tokyo, Japan) prepared from buttermilk (Haruta et al., 2008) was used as the SM-MPC and was composed of 1.1% water, 20.5% protein, 57.9% fat (including 5.9% SM), 5.3% ash, and 15.2% carbohydrate (wt/wt). Soft gel capsules for supplementation contained an amount of SM-MPC equivalent to either 1.67 or 3.33 mg of SM, so that consuming 3 capsules per day provided either 5 or 10 mg of SM per day, respectively. The capsules also contained olive oil and beeswax as a vehicle. Placebo capsules contained only the vehicle (230 mg of olive oil and 35 mg of beeswax). Olive oil was used to compensate for differences in SM-MPC content. The placebo and test capsules were visually identical. The capsules were manufactured by Catalent Japan (Shizuoka, Japan).

Study Schedule and Protocol

A total of 96 subjects were randomized to 3 groups: high SM (supplemented with SM-MPC equivalent to 10 mg/d of SM), low SM (supplemented with SM-MPC equivalent to 5 mg/d of SM), and placebo groups. The supplementation period was 12 wk, and the condition of skin was evaluated at baseline and every 3 wk.

During the study period, subjects were prohibited from changing their living and dietary habits, particularly their dairy product intake and use of cosmetic items. Subjects recorded their consumption of dairy products and functional foods, their physical condition, and any nonroutine use of cosmetics or special activities (e.g., excessive exercise or sunburn). In addition, subjects were prohibited from bathing in a hot spring during the week before each evaluation, forbidden from shaving hair on the area to be evaluated during the 3 d before each evaluation, and asked to fast for 4 h before each evaluation. Each subject attended at the same time of day (± 3 h) for each evaluation.

Instrumental Measurement of Parameters for Assessment of Skin Condition

Skin hydration was assessed using a Corneometer CM 825 (Courage and Khazaka, Cologne, Germany). The corneometer measurement probe was applied to

the skin below the left eye, on the forearm, and on the heel. Data were given in arbitrary units.

Skin elasticity was assessed using a Cutometer MPA580 (Courage and Khazaka). A 2-mm measuring probe generating a negative pressure of 45 kPa was applied below the left eye. Variables of skin elasticity were calculated as an R2 value (ratio of total recovery to total elasticity), which represents gross elasticity, according to the manufacturer's instruction. Values closer to 1 indicate greater elasticity.

Transepidermal water loss values of the skin below the left eye and on the forearm were determined using a Tewameter TM 300 (Courage and Khazaka), and that of the heel was evaluated using a Vapo Scan AS-VT100RS (Asahi Biomed, Tokyo, Japan). Sebum production of the skin below the right eye was evaluated using a Sebumeter SM810 (Courage and Khazaka).

Assessment of Subjects' Perceptions of Their Skin Condition

Subjects' perceptions of the condition of their skin were evaluated using a questionnaire with a 7-point scale: worsened [highly (−3), moderately (−2), slightly (−1), not changed (0)], and improved [slightly (+1), moderately (+2), highly (+3)]. The items queried were 8 items relating to facial skin (moisture, wrinkling around the eyes, flakiness, tension, sagging, smoothness, fine texture, and makeup conditions), 4 items relating to arm skin (moisture, smoothness, and cracking and flakiness of elbow skin), and 4 items relating to leg skin (moisture, smoothness, and cracking and flakiness of heel skin). The questionnaire was completed by subjects at baseline and every 3 wk during the study.

Statistical Analysis

Data values were expressed as mean $\pm$ standard error; ANOVA was used to evaluate differences among groups at baseline. Dunnett's test was used to evaluate differences between each SM-supplemented group and the placebo group. Holm's test was used to assess the significance of changes from baseline. Statistical tests were 2-tailed, and a *P*-value of 0.05 to 0.1 was regarded as a statistical tendency, whereas a *P*-value of <0.05 was considered significant.

RESULTS

Instrumentally Measured Parameters of Skin Condition

Baseline skin hydration, sebum production, and transepidermal water loss at each skin region tested

Figure 2. Changes in skin hydration. Skin hydration at the heel region was measured using a Corneometer CM 825 (Courage and Khazaka, Cologne, Germany) and represented in arbitrary units (AU) as changes in the value from baseline. The low SM group (gray triangles) was supplemented with sphingomyelin milk phospholipid concentrate (SM-MPC) equivalent to 5 mg/d of sphingomyelin; the high SM group (closed diamonds) was supplemented with SM-MPC equivalent to 10 mg/d of sphingomyelin; and the placebo group (open circles) received vehicle only (230 mg of olive oil and 35 mg of beeswax). Values are means $\pm$ standard error. * = significant difference versus the placebo group by Dunnett's test ($P < 0.05$); § = significant difference from baseline by Holm's test ($P < 0.05$).

(below the eye, the heel, and the forearm) did not differ significantly among the groups. Levels of skin hydration at the heel region were significantly higher in the low-SM group than in the placebo group at wk 9 ($P = 0.020$) and 12 ($P = 0.018$), and significantly increased from baseline at wk 12 in both the SM-supplemented groups ($P = 0.025$ for the high-SM group and $P = 0.011$ for the low-SM group; Figure 2). Transepidermal water loss and sebum production showed no significant changes among the groups at any time point or region (data not shown).

Skin Elasticity

Skin elasticity in the region below the eye was significantly elevated in the high-SM group at wk 9 ($P = 0.016$) compared with that of the placebo group. Although the low-SM and placebo groups showed significant decreases in elasticity from baseline at wk 3, the high-SM group showed no significant reduction (Figure 3).

Figure 3. Changes in skin elasticity. Skin elasticity at the region below the left eye was measured using a Cutometer MPA 580 (Courage and Khazaka, Cologne, Germany) and represented as an R2 value (ratio of total recovery to total elongation). The low SM group (gray triangles) was supplemented with sphingomyelin milk phospholipid concentrate (SM-MPC) equivalent to 5 mg/d of sphingomyelin; the high SM group (closed diamonds) was supplemented with SM-MPC equivalent to 10 mg/d of sphingomyelin; and the placebo group (open circles) received vehicle only (230 mg of olive oil and 35 mg of beeswax). Values are means $\pm$ standard error. * = significant difference versus the placebo group by Dunnett's test ($P < 0.05$); § = significant difference from baseline by Holm's test ($P < 0.05$).

Subjects' Perceptions of Skin Condition

Figure 4 shows the changes from baseline in the questionnaire scores for each item assessing subjects' perceptions of the condition of their skin of the face (A) and arm (B). Increases in each score from baseline corresponded to improvements in the condition of skin.

Questionnaire scores for the moisture of facial skin showed a significantly greater increase from baseline at wk 3 ($P = 0.035$) and 12 ($P = 0.030$), and a tendency toward a greater increase from baseline at wk 6 ($P = 0.069$) in the high-SM group than in the placebo group.

The score for wrinkling around the eyes tended to show a greater increase from baseline at wk 6 ($P = 0.081$) and showed a significantly greater increase from baseline at wk 9 ($P = 0.040$) and 12 ($P = 0.039$) in the high-SM group than in the placebo group. The score for the flakiness of facial skin tended to show a greater increase from baseline at wk 9 ($P = 0.083$) in the high-SM group than in the placebo group. The score for the tension of facial skin tended to show a greater increase

Figure 4. Subjects' perceptions of the condition of their facial (A) and arm (B) skin. Changes from baseline in the scores for items relating to the condition of skin are shown. Each item was evaluated using a subject-completed questionnaire with a 7-point scale. The low SM group (gray triangles) was supplemented with sphingomyelin milk phospholipid concentrate (SM-MPC) equivalent to 5 mg/d of sphingomyelin; the high SM group (closed diamonds) was supplemented with SM-MPC equivalent to 10 mg/d of sphingomyelin; and the placebo group (open circles) received vehicle only (230 mg of olive oil and 35 mg of beeswax). Values are means $\pm$ standard error. * = significant differences ($P < 0.05$) and † = statistical tendency ($P = 0.05$ – 0.1) toward a difference versus the placebo group by Dunnett's test.

from baseline at wk 6 ($P = 0.099$), and the score assessing the sagging of facial skin tended to show a greater increase from baseline at wk 9 ($P = 0.094$) in the low-SM group than in the placebo group. No significant differences were observed for other scores, including

smoothness, fine texture, or makeup conditions (data not shown).

On the arm, the scores for cracking ($P = 0.070$) and flakiness ($P = 0.069$) of elbow skin tended to show a greater increase from baseline at wk 12 in the high-

SM group compared with that observed in the placebo group.

DISCUSSION

The present study demonstrated that dietary supplementation with SM-MPC, a concentrate of milk phospholipids including SM, improved several parameters related to the condition of skin in humans. Although a large number of benefits of consuming milk phospholipids have been investigated (Dewettinck et al., 2008), only a limited number of studies have examined skin condition, for which SM is considered a key epidermal phospholipid (Imokawa and Ishida, 2014).

Our results showed a significant increase in the hydration levels of the heel skin following oral supplementation with SM-MPC. Maintaining adequate hydration levels is considered to be of primary importance for the softness and smoothness of the skin (Boelsma et al., 2003). Dryness of heel skin is a commonly observed condition; however, the heel's relatively thick stratum corneum (Ya-Xian et al., 1999) limits transdermal absorption of moisturizing creams (Feldmann and Maibach, 1967), which are often used to ease the problem. We suggest that dietary SM could be an alternative strategy to alleviate dryness for skin regions with poor transdermal permeability such as the heel.

This increase in the hydration levels of the heel skin was significant in the low-SM group but not significant in the high-SM group compared with the placebo group; however, the high-SM group showed a significant increase at wk 12 from baseline (Figure 2). We consider that this lack of clear dose-dependency might be attributable to variations in the amount of SM in the daily diet of subjects. Yunoki et al. (2008) reported that sphingolipid intake of normal Japanese subjects varied from 45 to 292 mg/d and calculated their SM intake to be 14.5 to 220.3 mg/d. Thus, the supplementation levels of SM applied in the present study, 5 and 10 mg of SM/d, were relatively low compared with the reported lowest dietary intake (14.5 mg of SM/d), although still accounting for as much as 34.5 and 69.0% of the value, respectively; and were considerably low compared with the highest value (220.3 mg of SM/d), accounting for only 2.3 and 4.6%, respectively. Because no strict dietary restrictions were applied in the present study, the variation in the amount of SM in the daily diet of subjects may have incidentally affected the significance of the results. Furthermore, taking into account the fact that significant improvements in several other conditions of skin such as facial skin elasticity, moisture, and wrinkling were obviously observed, we consider that SM-MPC supplementation had a certain contribution to heel skin hydration as well. Potentially,

the supplementation levels of this study could be effective when subjects consume a lower amount of SM from the daily diet or with long-term supplementation of SM-MPC.

Several dietary factors have been reported to improve skin elasticity by oral supplementation in women with, for example, a pine bark extract containing a variety of polyphenols, which had antioxidant effects (Marini et al., 2012); glucosyl-ceramide prepared from a beet extract (Hori et al., 2010); an oil formulation rich in fish oil (Segger et al., 2008); and soy isoflavone (Izumi et al., 2007). In some of those studies, enhanced production of extracellular matrix constituents was suggested as a possible mechanism for the improvement in skin elasticity, with the *de novo* synthesis of hyaluronic acid being induced by the pine bark extract (Marini et al., 2012) and fibronectin synthesis being upregulated by glucosyl-ceramide from the beet extract (Hori et al., 2010). The present study showed a significant improvement in skin elasticity with oral administration of SM-MPC. Based on the fact that ingested SM can be transformed into ceramides (Haruta et al., 2008; Haruta-Ono et al., 2012b) and that SM can enhance the synthesis of ceramides (Duan et al., 2012), ceramides metabolically converted from ingested SM or newly synthesized in an SM-induced manner might enhance the production of extracellular matrix, and thereby lead to improved skin elasticity.

The questionnaire-based evaluation revealed that SM-MPC supplementation significantly improved subjects' perception of facial skin moisture as well as wrinkling around the eyes. On the other hand, the instrumentally measured parameters showed that SM-MPC supplementation significantly improved skin elasticity below the eye (Figure 3), but did not significantly improve skin hydration below the eye (data not shown). Thus, there appears to be no close correlation between similar subjective and objective measures relating to hydration of the face. We believe that subjects' perceptions of the condition of facial skin might reflect the conditions of facial skin as a whole, not limited to the region below the eye. It is conceivable that multiple physiological factors, including skin hydration, skin elasticity, and possibly extracellular matrix production in the skin, which would be enhanced by dietary SM, collectively contributed to the subjectively perceived improvement in the condition of skin.

CONCLUSIONS

Oral supplementation with SM-MPC significantly improved hydration of the skin at the heel and elasticity of the skin below the eye, as well as subjects' perceptions of the condition of their skin, including the

moisture of facial skin and wrinkling around the eyes. These findings suggest that long-term supplementation with SM-MPC could contribute to the improvement of the general condition of skin.

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